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Poster

203. Oligodendrocyte Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 203.01/A1

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH R01 NS088529

Title: Investigating the role of MAPK and AKT pathways downstream of Shp2 signaling in oligodendrocyte progenitor cell generation in the telencephalon

Authors: *R. R. WACLAW[1], V. KOHLI[1], D. NARDINI[1], B. DASGUPTA[2], L. EHRMAN[1]; [1]Cancer Biol. and Neural Tumors Program, Exp. Hematology and Cancer Biol., [2]Div. of Oncology, Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstract: Shp2 (PTPN11) is a non-receptor protein tyrosine phosphatase that has been implicated in several signaling pathways including the MAPK and AKT pathways. Multiple lines of evidence suggest that Shp2 plays a key role in oligodendrocyte development. However, the downstream mechanisms of Shp2 signaling that drive these crucial functions in oligodendrocytes remain largely unknown. We are using mouse genetics to understand the role of Shp2 signaling during oligodendrocyte progenitor cell (OPC) development in the ventral telencephalon. Loss of Shp2 protein in the *Olig2* lineage (*Shp2* cKO generated with $Olig2^{cre/+}$) results in severe reductions of OPC markers (Pdgfra, Olig1, and Olig2) in the parenchyma of the ventral telencephalon. Interestingly, the expression of progenitor cell markers in the medial ganglionic eminence (MGE) and preoptic area (POA) (Nkx2.1, Olig2, Zfp536, and Prox1) are unchanged in *Shp2* cKOs, suggesting a specific role for Shp2 in the generation of parenchyma OPCs. To understand the role of MAPK signaling downstream of Shp2, we generated Shp2 cKOs that also express a cre inducible constitutively active MEK1 from the *Rosa26* gene. Increasing MAPK signaling after loss of Shp2 improves OPC marker expression in the ventral telencephalon but the distribution of OPCs remained abnormal with accumulation of OPC marker positive cells near the MGE/POA. To understand the role of AKT signaling downstream of Shp2, we deleted the AKT negative regulator, PTEN in *Shp2* cKOs (*Shp2;PTEN* double cKO) . The loss of PTEN results in a hyperactivation of the AKT pathway in both control and *Shp2* ckO embryos but did not improve OPC marker expression in the Shp2 cKO ventral telencephalon. Our results suggest that the MAPK pathway and not the AKT pathway play a major role in the OPC phenotype in Shp2 cKOs. However, since Shp2 cKO are not completely rescued after increasing MEK1 activity, Shp2 likely regulates OPC development in a MAPK-dependent and -independent manner. Future experiments are focused on determining the relationship of these two pathways downstream of abnormal Shp2 signaling via RASopathy mutations and identifying additional pathways influenced by altered Shp2 protein function.

Disclosures: **R.R. Waclaw:** None. **V. Kohli:** None. **D. Nardini:** None. **B. Dasgupta:** None. **L. Ehrman:** None.

Poster

203. Oligodendrocyte Differentiation

Location: Hall A

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Topic: A.01. Neurogenesis and Gliogenesis

Support: R01NS072427

R01NS075243

RG3978

Title: Stage-dependent functions of the wnt effector tcf7l2 mediated by interaction with distinct partners control oligodendrocyte lineage progression

Authors: ***C. ZHAO**[1], Y. DENG[1], W. LIU[3], Q. LU[2];
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Abstract: Constitutive activation of Wnt/β-catenin signaling inhibits oligodendrocyte myelination. However, specific deletion in oligodendrocyte lineage shows that Tcf7l2/Tcf4, a DNA-binding transcriptional partner of β-catenin, is required for oligodendrocyte differentiation. How Tcf7l2 modifies β-catenin signaling over development to facilitate myelination processes remains elusive. Here we show that Tcf7l2 interacts with distinct co-regulators other than β-catenin during oligodendrocyte lineage progression. Integrative analysis of genomic occupancy and gene expression profiling reveals that Tcf7l2 interacts sequentially with a competitive inhibitor of β-catenin binding, Kaiso/Zbtb33, and a differentiation-promoting factor, Sox10, to repress wnt gene eanpression and initiate and sustain myelin gene expression, respectively. We further reveal that Tcf7l2 cooperates with Sox10 to activate the genes encoding enzymes for cholesterol biosynthesis by directly targeting their proximal promoters/enhancers. Mice lacking the Tcf7l2 DNA-binding domain exhibit defects in myelination and remyelination after demyelination. Cholesterol supplementation can partially rescue differentiation defects of Tcf7l2-mutant oligodendrocyte precursors both *in vitro* and *in vivo*. Together, our studies identify Tcf7l2-regulated transcriptional switches that promote oligodendrocyte lineage differentiation, indicating a crucial role of Tcf7l2 for metabolic control of CNS myelination.

Disclosures: **C. Zhao:** None. **Y. Deng:** None. **W. Liu:** None. **Q. Lu:** None.

Poster

203. Oligodendrocyte Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 203.03/A3

Topic: A.01. Neurogenesis and Gliogenesis

Title: Topographical organisation of oligodendrocytes in the mouse corpus callosum - establishment, regeneration and modulation by electrical activity

Authors: P. T. RÖTH[1], S. MITEW[2], Y. L. XING[1], J. S. STRATTON[1], B. H. CHUANG[1], R. B. TRIPATHI[3], I. GOBIUS[4], W. D. RICHARDSON[3], L. J. RICHARDS[4], B. EMERY[5], T. J. KILPATRICK[2], *T. D. MERSON[1];
[1]Florey Inst. of Neurosci. and Mental Hlth., University of Melbourne, Australia; [2]Dept. of Anat. and Neurosci., Univ. of Melbourne, Parkville, Australia; [3]Wolfson Inst. for Biomed. Res., University College London, United Kingdom; [4]Queensland Brain Inst., The University of Queensland, Australia; [5]Jungers Ctr. for Neurosciences Res., Oregan Heath & Science University, OR

Abstract: Rapid electrical conduction in the vertebrate central nervous system (CNS) relies upon myelination of particular subsets of axons. However, the underlying mechanisms that orchestrate this process remain poorly defined. We have investigated how myelinating oligodendrocytes establish their unique topographical arrangement within white matter during early postnatal brain development. Linear arrays of glial cell somata occur in all white matter tracts throughout the CNS. These structures are characterised by the tandem alignment of glial cell bodies along the axonal axis. We demonstrate that linear arrays of cells in the corpus callosum appear before the onset of myelination of this white matter tract. Throughout postnatal ontogeny they continue to be generated and increase in size. Oligodendroglial lineage cells represent between 60-70% of all cells within linear arrays, irrespective of postnatal age, and the proportion of mature oligodendrocytes increases with age. By using Cre-lox fate mapping in transgenic mice, we demonstrate that different oligodendrocyte populations from the medial ganglionic eminence and the anterior entopeduncular area are responsible for forming intermixed as well as homogeneous linear arrays. Additional characterisation using hemizygous female mice carrying an X-linked β-galactosidase gene revealed that clonal expansion also contributes to the formation of linear arrays. Increasing the activity of subsets of transcallosal axons that project from the developing somatosensory cortex increased the generation of linear arrays. By contrast, cuprizone challenge in adult mice abolished linear arrays in the caudal corpus callosum and arrays were only partially regenerated by 7 weeks after cuprizone withdrawal. Collectively, our data provide new insights into the cellular dynamics underlying the generation of oligodendrocyte topography during normal development and ageing. Modulating electrical activity or inducing de/remyelination alters the juxtapositioning of oligodendrocyte cell bodies within white matter and could have important implications for how myelin internodes are organised in space.

Disclosures: **P.T. Röth:** None. **S. Mitew:** None. **Y.L. Xing:** None. **J.S. Stratton:** None. **B.H. Chuang:** None. **R.B. Tripathi:** None. **I. Gobius:** None. **W.D. Richardson:** None. **L.J. Richards:** None. **B. Emery:** None. **T.J. Kilpatrick:** None. **T.D. Merson:** None.

Poster

203. Oligodendrocyte Differentiation

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Topic: A.01. Neurogenesis and Gliogenesis

Support: RO1 MH083517

National MS Society RG4558A8/2

RO1 MH098742

T32NS007180-29

T32NS007180

Title: Oligodendrocyte differentiation deficits induced by oxidative stress are not rescued by monomethyl fumarate (mmf), the active metabolite of an ms therapeutic

Authors: *B. K. JENSEN[1], K. L. JORDAN-SCIUTTO[2], J. B. GRINSPAN[3];
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Abstract: Precipitating events leading to autoimmune targeting of myelin in MS patients are not understood. Despite this unknown etiology, consistent pathological observations are chronic oxidative stress and inflammation, which are the main focus of therapies. The drug Tecfidera (BG-12) is widely thought to act as an antioxidant, and has been shown to reduce oxidative stress through transcription factor Nrf2-mediated activation of the endogenous antioxidant response (EAR) and upregulation of target genes in neurons and macrophages. However, the mechanism by which this drug reduces MS relapse rates has not been established. Tecfidera is composed of dimethyl fumarate, which is rapidly metabolized to monomethyl fumarate (MMF). The effects of MMF on the affected cell population of oligodendrocytes (OLs) have not been examined. Effective MS therapy will require both prevention of further myelin loss and promotion of re-myelination through differentiation and maturation of brain-resident OL precursor cells (OPCs). We have previously demonstrated that oxidative stress can halt OL differentiation. We have also shown that MMF can attenuate oxidative stress-induced neurotoxicity through Nrf2-mediated EAR upregulation. The goal of the present study was to investigate whether MMF can reduce oxidative stress, upregulate the EAR, and reverse stress-induced deficits in OL differentiation. Using our primary culture system, we have shown that oxidant tert-Butyl hydroperoxide induces

reactive oxygen species (ROS) accumulation in both OPCs and mature OLs. Oxidant treatment over a 72hr differentiation period led to significant reduction in maturation by immunofluorescent staining of stage-specific markers, and reduction in myelin proteins by immunoblotting. MMF applied to cultures prior to insult significantly reduced ROS accumulation induced by multiple oxidants. Unexpectedly, this did not coincide with upregulation of EAR target genes. MMF treatment also did not rescue maturation defects induced by oxidants, as comparable significant decreases were evident in oxidant alone and oxidant+ MMF conditions. Our findings suggest that the EAR is not activated under conditions of oxidative stress in OLs to restore redox homeostasis. Additionally, while MMF prevents ROS accumulation, it also does so without EAR pathway activation and is not effective at rescuing oxidative-stress induced differentiation deficits. Therefore, it is likely that the therapeutic effect of MMF in MS patients is due to alternate anti-inflammatory pathway actions, or through upregulation of EAR proteins in other cell types which act cell non-autonomously to provide protection to mature OLs.

Disclosures: **B.K. Jensen:** None. **K.L. Jordan-Sciutto:** None. **J.B. Grinspan:** None.

Poster

203. Oligodendrocyte Differentiation

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH grant 41958

Myelin Repair Foundation grant 31677

Title: Phosphorylation state of ZFP191 regulates maturation of late-stage oligodendrocytes

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Abstract: Myelin is a multilayered membrane that wraps axons. It provides protection to the axons and allows for the rapid conduction of action potentials along the nerves. In the brain and spinal cord (central nervous system), myelin is formed by oligodendrocytes. Previous work from our laboratory demonstrated that ZFP191 is critical for CNS myelination: *Zfp191* null mutants are severely hypomyelinated despite the presence of normal numbers of mature oligodendrocytes (Howng et al., 2010 Genes Dev 24(3):301-311). Our current results demonstrate that the phosphorylation state of ZFP191 changes as oligodendrocytes mature from their progenitors. In oligodendrocyte progenitor cells, the majority of the protein is phosphorylated, but as the cells differentiate to mature myelinating oligodendrocytes, the non-phosphorylated form of ZFP191 accumulates. We have also identified the DNA motif to which ZFP191 binds, and strikingly, we

have detected binding sites in proximity to several genes that are crucial for maturation of oligodendrocytes. Moreover, our data shows that the changes in ZFP191 phosphorylation state results in changes in ZFP191 DNA binding capability: non-phosphorylated ZFP191 has a greater capacity to bind its target DNA sequence than does the phosphorylated form. Based on these finding we have used site direct mutagenesis to demonstrate that only a mutant that resembles the non-phosphorylated form of ZFP191 was able induce oligodendrocytes maturation. Based on our results we suggest that changes in the phosphorylation state of ZFP191 control the maturation of oligodendrocyte lineage cells and the potential of oligodendrocytes to myelinate axons. Importantly, this provides a new potential target for intervention to enhance myelination and remyelination.

Disclosures: **B. Elbaz:** None. **J.D. Aaker:** None. **B. Popko:** None.

Poster

203. Oligodendrocyte Differentiation

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Topic: A.01. Neurogenesis and Gliogenesis

Support: MEXT/JSPS KAKENHI 15K18330 (M.N)

MEXT/JSPS KAKENHI 25122704 (Y.I)

Title: FGF-2 signal promotes the proliferation of cerebellar progenitor cells and their oligodendrocytic differentiation at early postnatal stage

Authors: *M. NARUSE, K. SHIBASAKI, Y. ISHIZAKI;
Dept. of Mol. and Cell. Neurobiology,, Gunma Univ. Grad. Sch. of Med., Gunma, Japan

Abstract: The origin and the developmental regulation of cerebellar oligodendrocytes are largely unknown, and several origins of cerebellar oligodendrocytes at embryonic stages were suggested. It is also known that neural stem cells exist in the white matter of postnatal cerebellum, though it is unclear whether these neural stem cells generate oligodendrocytes at postnatal stages. We showed previously that CD44 is expressed by cerebellar cells including neural stem cells at around postnatal day 3. CD44-positive cells prepared from postnatal day 3 cerebellum gave rise to neurospheres, while CD44-negative cells prepared from the same cerebellum did not. These neurospheres differentiated mainly into oligodendrocytes and astrocytes, suggesting that CD44-positive cells might generate oligodendrocytes in postnatal cerebellum. We cultured CD44-positive cells from postnatal day 3 cerebellum in the presence of several signaling molecules known as mitogens or inductive differentiation factors for oligodendrocyte progenitor cells. Of these, only FGF-2 promoted survival and proliferation of CD44-positive cells, and these cells differentiated into O4+ oligodendrocytes. Furthermore, we

examined the effect of FGF-2 on cerebellar oligodendrocyte development *ex vivo*. FGF-2 enhanced proliferation of oligodendrocyte progenitor cells and increased the number of O4+ and CC1+ oligodendrocytes in slice culture. These results suggest that CD44-positive cells might be a source of cerebellar oligodendrocytes and that FGF-2 plays important roles in their development at early postnatal stage.

Disclosures: **M. Naruse:** None. **K. Shibasaki:** None. **Y. Ishizaki:** None.

Poster

203. Oligodendrocyte Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 203.07/A7

Topic: A.01. Neurogenesis and Gliogenesis

Title: *In vivo* role of p38alpha mitogen activated protein kinase in oligodendrocyte development and myelination

Authors: *S. CHUNG[1], S. BISWAS[2], J. SOHN[3], P. JIANG[2], C. CHEN[2], F. CHMILEWSKY[1], W. AYAZ[1], H. MARZBAN[4], W. DENG[2];
[1]Oral Biol., Univ. of Illinois At Chicago, Chicago, IL; [2]Biochem. and Mol. Med., Univ. of California, Davis, Sacramento, CA; [3]Inst. for Pediatric Regenerative Medicine, Shriners Hosp. for Children, Sacramento, CA; [4]Univ. of Manitoba, Winnipeg, AB, Canada

Abstract: The mitogen-activated protein kinases (MAPK) belong to the family of serine/threonine protein kinases that allow cells to respond to stimuli received from their extracellular environment including mitogens as well as to intracellular stress. Previous *in vitro* studies using p38 inhibitors suggested that p38α MAPK is required for myelination and oligodendrocyte (OL) differentiation. The proper development of OL and myelination is essential for maintaining the efficiency and speed of electrical nerve impulse. In an effort to identify the specific roles of p38α in myelination during early postnatal development, we have bred p38α-floxed mice with NG2 or PLP-cre mice to generate homozygous conditional NG2/Plp-specific p38α conditional knockout (CKO) mice for the first time. Our main finding was that, although a myelination phenotype was not evident at a gross level, there were several myelination defects at the ultra-structural level. Specifically, myelin bundles in the corpus callosum failed to develop normally, and there was a delayed onset of myelination in the corpus callosum. These defects could be partly due to a delay in OL differentiation during postnatal development since OL progenitor cell (OPC) proliferation remained normal in these knockout mice. This was supported by our observation that gene expression levels of several critical transcription factors of OPC maturation such as Olig1, Zfp488, and the OPC marker NG2 were significantly downregulated during early neonatal development in these knockout mice. Additionally, similar to previous reports, an inherent myelination defect was apparent in the

primary OPCs isolated from p38α CKO mouse brains. These OPCs failed to synthesize MBP when differentiated *in vitro*. These data indicate that p38α MAPK is as an important regulator of positive effectors of OL differentiation and myelination progression. Taken together, present study suggests p38α MAPK as a key regulator in OL development and myelination process in the CNS.

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Poster

203. Oligodendrocyte Differentiation

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH R01NS073425

Title: The role of histone methyltransferase Ezh2 in oligodendrocyte progenitor cell proliferation and differentiation

Authors: *K. PATEL[1], J. LIU[2], S. MOYON[2], P. CASACCIA[2], A. NISHIYAMA[1]; [1]Dept. of Physiol. and Neurobio., Univ. of Connecticut, Storrs, CT; [2]Dept. of Neurosci. and Dept. of Genet., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Oligodendrocyte progenitor cells (OPCs, NG2 cells, polydendrocytes) continue to proliferate and differentiate into adulthood. The mechanisms underlying the decision to proliferate and self-renew or differentiate into mature oligodendrocytes are not completely understood. Histone methylation is a posttranslational modification that has been shown to control cell fate by regulating transcription in various stem and progenitor cell types. We examined the role of trimethylation of lysine 27 on histone H3 (H3K27me3) in regulating OPC proliferation and differentiation. Comparison of the intensity of immunofluorescence labeling for H3K27me3 in oligodendrocyte lineage cells revealed that H3K27me3 in premyelinating oligodendrocytes is 5.4-fold higher than that in OPCs and 4.6-fold higher than that in mature oligodendrocytes, suggesting a role for H3K27me3 in cell cycle exit or the maturation of oligodendrocytes. This was further examined in Ezh2 conditional knockout mice (cko) provided by Dr. Yukiko Gotoh (Tokyo, Japan). The H3K27me3 modification is catalyzed by Ezh2, a component of the polycomb repressive complex 2 that induces chromatin compaction, thereby repressing transcription of target genes. When Ezh2 was deleted in OPCs perinatally, the proportion of Ezh2-deleted OPCs in the corpus callosum that incorporated the thymidine analog EDU (5-ethynyl-2'-deoxyuridine) was 10-fold lower than the proportion of Ezh2+ OPCs in control mice. Additionally 5 days after Cre induction, 1% of the Ezh2-deleted cells in the corpus

callosum of cko mice had differentiated into CC1+ oligodendrocytes, while 51% of induced cells were CC1+ in control mice. At 11 days after Cre induction, 52.1% of Ezh2-deleted cells had become CC1+, which was significantly lower than in the control mice where 72.2% of the induced cells had become CC1+. These data suggest that Ezh2 regulates both OPC proliferation and the timing of oligodendrocyte differentiation. We are currently quantifying the effects of Ezh2 deletion on the repair of acutely demyelinated lesions and analyzing the Ezh2 target genes affected by Ezh2 deletion.

Disclosures: K. Patel: None. **J. Liu:** None. **S. Moyon:** None. **P. Casaccia:** None. **A. Nishiyama:** None.

Poster

203. Oligodendrocyte Differentiation

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Topic: A.01. Neurogenesis and Gliogenesis

Support: TIRR Mission Connect

Gillson Longenbaugh Foundation

Chinese Scholar Council

John S. Dunn Research Foundation

Title: Signaling pathways underlying Neurobasal-A medium-promoted oligodendrocyte differentiation from human neural stem cells

Authors: *P. WU[1], L. WANG[1,2], J. GAO[1], Y. HAO[1,3], T. DUNN[1], E. MCGRATH[1], J. ALLENDE LABASTIDA[1], S.-Q. FENG[3], S.-Y. LIU[2];
[1]Dept Neurosci/Cell Biol, UTMB, Galveston, TX; [2]Sun Yat-sen Univ., Guangzhou, China; [3]Tianjin Med. Univ. Gen. Hosp., Tianjin, China

Abstract: Remyelination, important for neurotrauma and demyelination diseases, may restore myelin to denuded axons through the generation of oligodendrocytes (OL) from oligodendrocyte progenitor cells (OPCs). To obtain more OL lineage cells, different strategies for induction of OL lineage differentiation from neural stem cells (NSCs) have been reported previously. However, molecular mechanisms underlying NSC differentiation into OPCs are complex and still remain largely unknown. Recently we found that the Neurobasal medium (NB), but not the regular NSC cultivating medium [Dulbecco's Modified Eagle Medium with F12 (DF)], induced human fetal forebrain-derived NSCs toward the $O4^+$ OPC differentiation, To investigate the underlying mechanisms, we used several methods such as real time RT-PCR (RT^2-PCR), Western Blotting, and immunofluorescence staining. RT^2-PCR confirmed that the levels of the

OPC-related transcription factors Olig2 and Nkx2.2 were significantly different between NB and DF groups. An initial protein phosphorylation array screening revealed NB-mediated activation of several signaling molecules, including Akt, extracellular signal-regulated kinases (ERK1/2), and c-Src. Western blot analyses further confirmed that phosphorylation levels of ERK1/2 and Akt were increased during NB cultivation and sustained throughout the subsequent FGF priming procedure to promote OPC differentiation. On the other hand, c-Src phosphorylation was increased only temporarily during NB culturing. Inhibitors of PI3K/Akt (LY294002), ERK1/2 (U0126), and c-Src (Saracatinib) were confirmed for their activities to reduce protein phosphorylation. Interestingly, c-Src inhibition within a specific time window during priming increased the O4-positive OPCs, whereas inhibition of Akt and ERK1/2 decreased OPCs. This study suggested that sustained activation of ERK and PI3K/Akt pathways were required for NB-mediated oligodendrocyte differentiation, whereas c-Src activation was restricted in a narrow time window for induction of hNSC differentiation into the oligodendrocyte lineage.

Disclosures: **P. Wu:** None. **L. Wang:** None. **J. Gao:** None. **Y. Hao:** None. **T. Dunn:** None. **E. McGrath:** None. **J. Allende Labastida:** None. **S. Feng:** None. **S. Liu:** None.

Poster

203. Oligodendrocyte Differentiation

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Topic: A.01. Neurogenesis and Gliogenesis

Support: Ministry of Science and Technology, Taiwan (MOST 103-2321-B-006 -010)

Title: Mir-212 regulates glial differentiation and maturation

Authors: *S.-F. TZENG;
Natl. Cheng Kung Univ., Tainan City, Taiwan

Abstract: Spinal cord injury (SCI) causes the death of spinal neural cells, the degeneration of spinal fibers degeneration and gliosis as well as demyelination. Change of gene expression by deregulated microRNAs (mir) is an emerging mechanism involved in neurodegeneration and neuroinflammation. Mir-212 has been reported to be highly expressed in neurons and involved in regulation of neuronal survival and BDNF expression. The role of mir-212 role in CNS glia is poorly understood. Our recent study showed that differential change in mir-212 level was detected during brain development. Moreover, mir-212 was reduced at the lesion site at 1 week and 1 month after moderate and severe SCI. In addition, *in situ* miRNA hybridization combined with immunohistochemistry indicated that mir-212 was not only highly expressed in spinal neurons, but also detected in oligodendrocytes and astrocytes in the white matter of the spinal cord. The downregulation of mir-212 level induced by interferon-γ (IFN-γ) were observed in

cultured astrocytes and oligodendrocytes derived from glial progenitor cells (GPCs). Furthermore, we found that the lentivirus-mediated overexpression of mir-212 suppressed the differentiation and maturation of oligodendrocytes derived from GPCs by examining the reduction in the complexity of cell processes and myelin sheath formation. The process number of astrocytes derived from GPCs was also decreased by mir-212 overexpression. The expression of inhibitor of DNA binding protein 2 (ID2), a potential mir-212 targets, was inhibited in GPCs and derived cells by mir-212 overexpression. Myelin basic protein that has been known to be regulated by ID2 through olig1/2 action was also downregulated in GPC-derived oligodendrocytes with mir-212 overexpression. Based on our findings, we suggest that mir-212 expression can be regulated by inflammatory cytokines, such as IFN-γ; moreover, mir-212 is required for the differentiation and maturation of oligodendrocytes from GPCs through the inhibition of the expression of their target molecules, such as ID2.

Disclosures: S. Tzeng: None.

Poster

203. Oligodendrocyte Differentiation

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH/NINDS R01NS083947

Title: Ampa receptor inhibition promotes myelination in preterm rabbits with intraventricular hemorrhage

Authors: *P. BALLABH, P. DOHARE, G. VINUKONDA;
Pediatrics, Cell Bio & Anat., New York Med. Coll, Valhalla, NY

Abstract: Intraventricular hemorrhage (IVH) is the most common neurological disorder of premature infants and is an important cause of white matter injury in this population. Glutamate excito-toxicity, mediated by AMPA type glutamate receptor, damages oligodendrocytes in animal models of neonatal hypoxia-ischemia and multiple sclerosis. Accordingly, inhibition of AMPA receptors enhances remyelination in adult models of brain injury. JAK-STAT signaling are important mediators of inflammation and STAT3 inhibition represses inflammation as well as offers neuroprotection. Therefore, we hypothesized that IVH would result in distinct changes in the expression of AMPA receptors and that AMPA receptor inhibition might promote myelination and clinical recovery in preterm rabbit pups with IVH. We also postulated that AMPA receptor inhibition would reduce inflammation and oligodendrocyte cell death, and that the effect would be mediated by Stat-3 signaling. To test our hypotheses, we employed preterm rabbit (E29, Term=32d) model of glycerol-induced IVH and autopsy samples from preterm

infants (23-26 gestational weeks). To inhibit AMPA receptors, rabbit pups with IVH were treated with intramuscular NBQX (15 mg/kg twice a day, i.p.) for 5 days; and intramuscular AG490 was used to inhibit JAK-STAT signaling. We found that GluR1-4 expression was comparable between human infants and rabbits with and without IVH on immunohistochemistry and Western blot analyses. NBQX treatment significantly increased myelin basic protein (MBP) expression in pups with IVH compared to vehicle controls on both Western blot analyses and stereological quantification of immunostained sections at P14. Moreover, NBQX treatment significantly increased myelin associated glycoprotein (MAG) and CNPase levels, but did not affect the GFAP levels in pups with IVH. The mRNA levels of TNFα, IL1β, IL-6, Leukemia inhibiting factor, Ciliary neurotrophic factor, density of TUNEL+Olig2+ oligodendrocytes, Iba1+ microglia cells, and phophoStat3 levels were significantly elevated in rabbits with IVH compared to controls without IVH and substantially reduced in NBQX-treated pups with IVH compared to vehicle controls at P 3 ($P < 0.05$, n=5 each). STAT3 inhibition inhibition reduced the density of Iba1+ and TUNEL+ cells at P3. Our data suggested that NBQX treatment suppressed oligodendrocyte death and inflammation in pups with IVH, which was mediated by STAT-3 signaling. More importantly, NBQX therapy enhanced myelination and clinical recovery in rabbit pups with IVH. We speculate that AMPA receptor inhibition might improve the outcome of survivors with IVH.

Disclosures: P. Ballabh: None. **P. Dohare:** None. **G. Vinukonda:** None.

Poster

203. Oligodendrocyte Differentiation

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R01-NS056427

NMSS RG 4706A4/2

NMSS RG 3954A1/2

Title: Sox17-mediated oligodendrocyte regeneration and protection involves coordinated stage-specific alterations in Hedgehog and Wnt signaling

Authors: *L.-J. CHEW[1], X. MING[1], J. DUPREE[2], V. GALLO[1];
[1]Ctr. Neurosci Res., Children's Res. Inst., Washington, DC; [2]Virginia Commonwealth Univ., Richmond, VA

Abstract: We have shown that Sox17 overexpression in CNP-Sox17 transgenic mice promotes postnatal oligodendrocyte (OL) development and prevents OL loss after focal lysolecithin (Lyso)

demyelination. Our present studies indicate that oligodendrocyte regeneration and attenuated cell death by Sox17 overexpression in the adult white matter (WM) are mediated by alterations in canonical Wnt and Hedgehog signaling. In addition to increased nascent generation of OLs, Sox17 reduced microglial and astrocyte activation compared with WT, and attenuated the loss of O4+ cells following cuprizone demyelination. Despite a larger NG2+ OPC population in the intact CNPSox17 mouse, demyelination did not induce an increase in NG2+ OPCs greater than that in WT; instead it increased O4 and CC1 cells, indicating enhanced differentiation. Sox17 also inhibited b-catenin activation by LYSO, evidenced by unchanged levels of activated b-catenin (ABC). Stereotaxic injections of the b-cat antagonist CCT036477 (CCT) into the adult corpus callosum showed that while ABC+ cells were reduced in both strains, CCT specifically increased O4+ cells only in WT, while it decreased NG2+ and CC1+ cells without affecting O4+ cells in the CNPSox17 WM. This indicates that b-catenin functions as an inhibitor of the O4+ cells in the WT, and that Sox17 abolished its inhibitory effect on the lineage. We analyzed whether CCT inhibition of ABC in WT WM would restore CC1 cells in LYSO lesions. Although CCT improved regeneration of CC1 OLs and reduced apoptosis in WT lesions, unlike lesions in CNPSox17, the numbers of CC1 cells remained significantly below levels in intact WT controls. This suggests additional signaling by Sox17. Since GLI2 was found to be selectively elevated by Sox17 in O4+ cells, we tested the possibility that Sox17 increased Hedgehog (HH) signaling to protect OLs and promote the generation of O4+ cells. Stereotaxic injection of the GLI antagonist GANT61 into corpus callosum indeed caused loss of MBP, and abolished Sox17-mediated protection in LYSO lesions. Increased density of Olig2+Sonic HH+ cells was detected in the intact CNPSox17 WM at P60, indicating autocrine signaling. Stereotaxic injection of the Smoothened antagonist cyclopamine-KAAD showed a reduction of NG2+ OPCs in the WT and O4+ cells in the CNPSox17, suggesting Sox17-mediated differentiation through Smoothened. Finally, stereotaxic injection of the Smoothened agonist SAG in WT WM restored to control levels O4 and CC1 cells in LYSO lesions and reduced ABC levels. Taken together, these studies support the notion that Sox17 promotes oligodendrocyte protection and regeneration by modulating HH and Wnt signaling in OPC development.

Disclosures: **L. Chew:** None. **X. Ming:** None. **J. Dupree:** None. **V. Gallo:** None.

Poster

203. Oligodendrocyte Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 203.13/A13

Topic: A.01. Neurogenesis and Gliogenesis

Support: New York State Stem Cell Research Board (NYSTEM)

NINDS

Adelson Medical Research Foundation

Novo Nordisk Foundation

Title: Distinctions between fetal and adult human OPCs in miRNA-regulated gene expression

Authors: *N. J. KUYPERS[1], M. OSIPOVITCH[1,2], A. CORNWELL[1], D. CHANDLER-MILITELLO[1], S. SCHANZ[1], S. WANG[1], S. A. GOLDMAN[1,2];
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Abstract: Human fetal and adult oligodendrocyte progenitor cells (OPCs) differ in their cell cyclicity, migration and maturation rates and myelinogenic competence. To identify causal pathways underlying these differences, second trimester fetal human cortices (18-21 weeks g.a.) and adult temporal lobe samples (8-54 years old) were dissociated and respectively sorted on the basis of CD140a or A2B5 immunoreactivity. Total RNA was extracted, and the mRNA (RNAseq) and miRNA (microarray) profiles of each population were compared to one another. Differential expression was confirmed by Taqman low-density arrays and conventional qPCR. Of the 8 mature miRNAs enriched in adult OPCs, 5 displayed fold-change (FC) values greater than 4, which included the oligodendroglial miRNAs miR-219-2-3p (FC=48.8) and miR-338-5p (FC=13.6). Target prediction analysis suggested that these 5 miRNAs regulate the transition of fetal to adult OPCs by repressing the translation of key WNT signaling genes. In particular, a core set of WNT pathway components were down-regulated during the transition to adult OPC phenotype; these included FZD3 (FC=-7.4), FZD7 (FC=-7.7), FZD8 (FC=-3.6), BCL9 (FC=-2.3) and TCF7L2 (FC=-2.8). Their down-regulation as a group suggests that ß-catenin-dependent transcriptional activity might be suppressed accordingly. Additionally, expression of a number of protein tyrosine phosphatase receptors (PTPRB, PTPRD, PTPRH, PTPRM, PTPRR; FC range: 1.9-116.2), which can serve as negative regulators of ß-catenin-dependent signaling, increased sharply as OPCs transitioned to the adult state. In this regard, the oligodendroglial-specific cadherin 19 (CDH19; FC=333.1), which may sequester ß-catenin and further suppress the transcription of its dependent targets, was strongly upregulated with transition to adult OPC phenotype. Similarly, mRNA encoding the GSK3ß activator protocadherin 9 (PCDH9) increased concomitant with the transition to adult OPC phenotype (FC=8.6), thereby providing an additional mechanism by which ß-catenin-dependent transcription might be further reduced. Together, these results suggest the importance of miRNA-dependent suppression of ß-catenin signal competence during maturation of the oligodendroglial lineage, and with consolidation of the adult OPC phenotype.

Disclosures: **N.J. Kuypers:** None. **M. Osipovitch:** None. **A. Cornwell:** None. **D. Chandler-Militello:** None. **S. Schanz:** None. **S. Wang:** None. **S.A. Goldman:** None.

Poster

203. Oligodendrocyte Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 203.14/A14

Topic: A.01. Neurogenesis and Gliogenesis

Support: NSFC 311007

CSTC 2011jjA10029

Title: Dnmt3a regulates expression of myelin related genes during the differentiation of OPCs

Authors: X. CHEN, Y. WANG, *L. XIAO;
Dept. of Histology and Embryology, Third Military Med. Univ., Chongqing, China

Abstract: DNA methylation has been reported to impact brain development or neurodevelopment disorders by regulating genesis of neurons or astrocytes. It is however remains unclear the role of DNA methylation playing in oligodendroglial genesis? In this study, expression of Dnmt3a, one of the DNA methyltranferases (Dnmts) was observed during the differentiation of OPCs and myelination in OPC cultures and neonatal developing rat brains. The inhibitor (5'- AZA), agonist (methionine, Met), and Dnmt3a RNAi plasmid were used to observe the effect of Dnmt3a on the differentiation of OPCs, and methylated changes of myelin related genes by MeDIP-ChIP and BSP. Our results showed that Dnmt3a highly expressed in PDGFRa+ OPCs, and gradually decreased following the differentiation of OPCs. Methylated ratio of myelin genes such as Sox10, MBP and Olig1 genes increased in and the differentiation of OPCs delayed after Met treatment, which could be rescued by 5'- AZA or Dnmt3a RNAi. These results suggest that Dnmt3a mediates dynamic methylation of myelin related genes during the differentiation of OPCs.

Disclosures: X. Chen: None. **Y. Wang:** None. **L. Xiao:** None.

Poster

203. Oligodendrocyte Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 203.15/A15

Topic: B.11. Glial Mechanisms

Support: NMSS RG3951

Title: $S1P_1$ deficiency in oligodendroglial lineage cells: effect on differentiation and myelination

Authors: D. DUKALA, S. QUAN, *B. SOLIVEN;
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Abstract: Sphingosine1-phosphate (S1P) receptors are G protein-coupled receptors expressed by many cell types, including cells of oligodendrocyte (OLG) lineage. We had previously shown that targeted deletion of *S1P1* in OLG lineage cells did not result in obvious clinical phenotype or altered number of OLGs at 3 months, but there were subtle abnormalities in myelin. In this study, we examined the role of $S1P_1$ in developmental myelination and cell survival, focusing on age 3 weeks. We found that $S1P_1$ deficiency led to delayed differentiation of OLG progenitors (OPCs) into OLGs that was independent of p38 phosphorylation. This was accompanied by decreased levels of myelin basic protein (MBP) but not of myelin-OLG glycoprotein (MOG), and slight decrease in myelin thickness in the corpus callosum of *S1P1* conditional knockout (CKO) mice. $S1P_1$-deficient OLGs exhibited slower process extension, which was associated with attenuated phosphorylation of extracellular signal regulated kinases (ERKs) and p21-activated kinases (PAKs), and with upregulation of tropomodulin1. Basal levels of pAkt were not affected, though expectedly, no response to a $S1P_1$ agonist SEW2871 was observed. $S1P_1$-deficient OLGs did not exhibit increased cell death in response to cuprizone, tumor necrosis factor-α, or deprivation of nutrients and growth factors. We conclude that $S1P_1$ signaling regulates OLG development, morphological maturation and early myelination.

Disclosures: **D. Dukala:** None. **S. Quan:** None. **B. Soliven:** None.

Poster

203. Oligodendrocyte Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 203.16/A16

Topic: B.11. Glial Mechanisms

Title: Endocannabinoid signaling in oligodendrocyte maturation and myelination

Authors: ***S. M. O'NEILL**, J. R. PIRO, K. MOU, T. A. SAMAD;
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Abstract: Monoacylglycerol lipase (MAGL) is a serine hydrolase that breaks down the endocannabinoid 2-arachidonoylglycerol (2-AG). MAGL is expressed in neurons, oligodendrocytes, astrocytes, microglia, and endothelial cells in the brain. Inhibition of MAGL has been shown to be a promising therapeutic entry point for reducing pain, neuroinflammation, and neurodegenerative diseases. More recently, emerging data has suggested that MAGL inhibition reduces pathology in mouse models of multiple sclerosis such as experimental autoimmune encephalomyelitis and acute cuprizone treatment. However, the precise molecular mechanism(s) of 2-AG regulation of oligodendrocyte maturation and function has not yet been fully elucidated. In the present study, we examine whether 2-AG may exert its effects directly on immature and mature oligodendrocytes, or indirectly by acting on different cell types which then influence oligodendrocyte lineage cells. To assess the direct effects of 2-AG on oligodendrocyte

maturation and function we use a selective inhibitor of MAGL on primary oligodendrocyte precursor cells *in vitro*. We use a MAGL-deficient mouse as gain of function model of 2-AG signaling in the cuprizone model of demyelination, to assess oligodendrocyte markers and myelin levels *in vivo*. To assess indirect modulation of oligodendrocytes, we examined the effect of 2-AG signaling on activated astrocytes and microglia and the resulting modulation of oligodendrocyte maturation and survival in culture. Taken together, our data suggest a modulatory role for endocannabinoid signaling in oligodendrocyte physiology.

Disclosures: **S.M. O'Neill:** A. Employment/Salary (full or part-time):; Pfizer. **J.R. Piro:** None. **K. Mou:** None. **T.A. Samad:** None.

Poster

203. Oligodendrocyte Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 203.17/A17

Topic: B.11. Glial Mechanisms

Support: Research Grants Council, HKSAR Gov't Project No.: GRF 660813

NIH grant (NS071022)

National Key Basic Research Program of China (2013CB530900)

Title: White matter degeneration and aberrant oligodendrocyte differentiation during the progress of Alzheimer's disease

Authors: ***K. TSE**, A. CHENG, H.-M. CHOW, K. HERRUP;
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Abstract: White matter degeneration and demyelination are recognized, but little studied features of the Alzheimer's disease (AD) brain. Yet increasing evidence suggests that there is a significant association between the progress of AD pathology and the appearance of aberrant differentiation of the oligodendrocyte lineages that are the cellular antecedents of myelin formation. To explore this relationship, MRI data from healthy control, mild cognitive impairment (MCI) and AD subjects were obtained from the National Alzheimer's Coordinating Center (n = 507, mean age = 76.8 yr, female = 58.4%). The calculated MRI data of white matter and gray matter volume were analyzed using Spearman's correlation test. We augmented this human data with observations in R1.40 transgenic mice in which the entire human APP gene with the Swedish mutation is carried on a YAC insert that includes long stretches of both 3' and 5' regulatory sequences. Gene expression changes were investigated using real-time PCR, immunohistochemistry and western blotting. In the human MRI data, we find that the gray matter volume of the subjects is strongly reduced with the progress of dementia and correlates

with lower MMSE scores (R = 0.26). The reduction of the gray matter is correlated in turn with a reduction of the cerebral white matter volume (R = 0.58), especially in the temporal lobe. Moreover, the level of white matter hyperintensity, indicative of myelin lesions, increased with age in all groups, with the strongest correlation found in MCI (R = 0.48). In the R1.40 mice, we examined neocortex at 3 - 18 months of age and found high hAPP mRNA expression throughout the lifespan. As reported earlier, however, amyloid protein aggregates were only observed after 13.5 months. In the transgenic brains, levels of NG2 and PDGRFα mRNA (oligodendrocytes progenitors) as well as MBP and PLP1 (mature oligodendrocytes) were consistently higher than wild type littermates for the first 12 months. By 18 months, however, all of these oligodendrocyte markers declined to values lower than wild type. Consistently, a significant reduction of MBP protein was detected in the cortex of R1.40 at 12 months, despite the presence of abundant oligodendrocyte progenitors. White matter degeneration is a common feature of the aging population and it is highly associated with neurodegeneration in human AD. Such white matter lesions may be caused by the chronic toxicity of amyloid aggregates or inflammatory processes on the differentiation of the oligodendrocyte lineage and subsequent myelin deposition.

Disclosures: **K. Tse:** None. **A. Cheng:** None. **H. Chow:** None. **K. Herrup:** None.

Poster

203. Oligodendrocyte Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 203.18/A18

Topic: B.11. Glial Mechanisms

Title: Histone deacetylase 3 activates olig2 and suppresses jak-stat signaling to establish and maintain oligodendrocyte lineage progression

Authors: ***Z. LIGUO**, X. HE, L. LIU, M. JIANG, R. LU;
Brain tumor center, Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstract: Abstract Establishment and maintenance of oligodendrocyte identity are a prerequisite for ensuring proper myelination and myelin repair after injury in the CNS, however, the molecular underpinnings of oligodendrocyte lineage commitment remain to be fully elucidated. Here, we show that the chromatin modifying enzyme Hdac3 activates the oligodendrocyte specification factor Olig2 to establish oligodendrocyte identity and functions as a molecular switch for oligodendrocyte and astrocyte lineage development. Hdac3 ablation in the progenitors of the oligodendrocyte lineage leads to severe myelination defects and striking ectopic generation of astroglia. Genome-wide targeting analysis reveals that Hdac3 coordinates with p300 acetyltransferase to control glial subtype-specific transcriptional programs. Furthermore, Hdac3 blocks the activity of Jak-Stat signaling by modulating Stat3 acetylation and

phosphorylation states to suppress astrogliogenesis. Thus, Hdac3 is a key priming factor of oligodendrocyte development by modulating both transcriptionally-linked chromatin remodeling events and Jak-Stat signaling activity to establish and maintain the oligodendrocyte lineage. These findings suggest that modulation of Hdac3 activity may represent a potential approach to suppress astrogliosis and promote remyelination after injury.

Disclosures: **Z. Liguo:** None. **X. He:** None. **L. Liu:** None. **M. Jiang:** None. **R. Lu:** None.

Poster

203. Oligodendrocyte Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 203.19/A19

Topic: B.11. Glial Mechanisms

Support: CIHR

Title: The RNA binding protein QKI stabilizes SIRT2 transcripts and promotes SIRT2 expression during oligodendrogenesis

Authors: ***M. P. THANGARAJ**[1], J. R. DOUCETTE[2,3], S. JI[1,4], A. J. NAZARALI[1,3];
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Abstract: Oligodendrocytes (OLs) are the myelin forming glial cells, involved in the repair of the central nervous system (CNS) lesions in multiple sclerosis (MS). The cellular and molecular mechanisms that control remyelinating ability of OLs are still unclear. Sirtuin2 (SIRT2) is a class III NAD^+ dependent deacetylase that is predominantly expressed in OLs promoting process outgrowth and differentiation. However, the mechanism involved in regulating SIRT2 expression during oligodendrogial development is largely unknown. The RNA binding protein quaking (QKI) is known to regulate the expression of several myelin transcripts for normal OL development and myelination. Deletion of QKI in OLs results in severe hypomyelination in *quaking viable* (qk^v) mutant mice. Interestingly, SIRT2 protein is not expressed in qk^v mutant mice. However, whether QKI interacts directly with *Sirt2* mRNA during OL development and myelination is unknown. We have investigated the molecular mechanism by which QKI regulates SIRT2 expression during OL development. Prediction of QKI binding sites in the 3UTR of *Sirt2* mRNA revealed the presence of two quaking response elements (QREs). RNA co-immunoprecipitation experiments confirmed that QKI binds to all the three variants of *Sirt2* mRNA, implying that RNA binding of QKI plays role in stabilization of *Sirt2* transcripts. Consistent with this, mRNA stability assay showed that QKI binding stabilizes and protects *Sirt2* transcripts from degradation. QKI significantly increased the luciferase reporter activity of the

Sirt2 3UTR, which was dependent on the binding of QKI to the QRE at 1853bp in the 3UTR of *Sirt2* mRNA. In addition, overexpression of QKI promotes the expression of *Sirt2* mRNA and protein in the OLs both in growth and differentiation condition. In conclusion, our findings indicate that QKI directly binds to the *Sirt2* mRNA at 3UTR to promote the expression of SIRT2 during OL development. (Funded by CIHR)

Disclosures: **M.P. Thangaraj:** None. **J.R. Doucette:** None. **S. Ji:** None. **A.J. Nazarali:** None.

Poster

203. Oligodendrocyte Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 203.20/A20

Topic: C.09. Demyelinating Disorders

Support: NIH NIGMS training grant GM08042

AHA Predoctoral Fellowship 15PRE22580000

NIH R01NS071481

American Heart Association UCLA ASA-Bugher Stroke Center

Title: Identifying genes implicated in oligodendrocyte progenitor cell differentiation after white matter stroke using complementary *in vitro* screening systems

Authors: *D. J. DITULLIO[1,2,3], S. T. CARMICHAEL[2,3];
[1]UCLA, Los Angeles, CA; [2]Interdepartmental Program for Neurosci., [3]Dept. of Neurol., David Geffen Sch. of Medicine, Univ. of California, Los Angeles, Los Angeles, CA

Abstract: White matter stroke comprises 25% of all ischemic stroke, leading to significant cognitive and motor deficits. Death of oligodendrocytes leads to demyelination, but oligodendrocyte progenitor cells (OPCs) are limited in their ability to replenish dead cells and remyelinate axons. It is of central importance to the treatment of this widespread disease to identify genes that promote OPC differentiation and enhance recovery after white matter stroke. An OPC transcriptome has been generated at key time points after white matter stroke in a mouse model of disease. To examine the most differentially expressed genes in this set, two complementary *in vitro* culture systems are used. The CG-4 cell line was derived from rat OPCs in 1992 and has served as a tool to screen for gene effects prior to studies in other systems. On the other hand, primary OPC cultures from neonatal rats demonstrate the most genetic and functional similarity to OPCs *in vivo*. The OPC transcriptome was analyzed using relative and absolute intensity measures to identify putative regulators of OPC differentiation. Genes of interest are examined in CG-4 cells and primary OPCs. Because CG-4 cells can be generated in large numbers at low cost, these are used as a screen for genes that affect OPC differentiation,

while primary OPCs are used to confirm results in a system more genetically similar to OPCs *in vivo*. Morphology analysis and qPCR are used to assess cell maturation. Results from CG-4 cells and primary OPCs are compared to assess the viability of each system to identify genes that play a role in initiating the OPC differentiation pathway. Both qPCR and Sholl analysis of morphology suggest that CG-4 cells exhibit limited capacity for differentiation. In primary OPC cultures grown in the presence of thyroid hormone, expression of OPC markers (Pdgfra and Cspg4) decrease significantly as the cells are exposed to differentiating conditions, while oligodendrocyte markers (Cnp and Mbp) increase significantly. Sholl analysis reveals extensive elaboration of branching networks. In comparison, CG-4 cells do not demonstrate significant increases in branching complexity. Pdgfra expression does not change after induction to differentiate, and oligodendrocyte markers such as Cnp and Mbp demonstrate no change in expression and even unexpected decreases with differentiation. These results indicate that CG-4 cells, based on their limited capacity for differentiation *in vitro*, are not a reliable system in which to analyze regulators of OPC differentiation and myelination, while primary OPCs represent a valuable tool to identify genes that play a role in the physiological response to white matter stroke.

Disclosures: D.J. DiTullio: None. **S.T. Carmichael:** None.

Poster

203. Oligodendrocyte Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 203.21/A21

Topic: C.09. Demyelinating Disorders

Title: Endosulfatase regulation of fate and differentiation of human oligodendrocyte progenitors

Authors: *S. U. POL, H. SHAYYA, R. WELLIVER, J. POLANCO-GARCIA, D. BRATTON, A. MILLIRON, M. O'BARA, F. SIM;
Dept. of Pharm and Tox, Univ. At Buffalo, Buffalo, NY

Abstract: In demyelinating diseases such as multiple sclerosis, remyelination is limited by impaired human oligodendrocyte progenitor cell (hOPC) differentiation. Identification of extracellular signals that regulate human OPC differentiation may provide novel means to induce remyelination. Weighted Gene Co-expression Network Analysis (WGCNA) was applied to define hOPC differentiation specific gene networks. Using lentiviral over-expression in hOPCs, we tested the functional importance of individual genes within cross-species conserved networks. Over-expression of SULF2, a hOPC-expressed extracellular sulfatase, significantly reduced the fraction of differentiating O4+ oligodendrocytes from 17 ± 5% in controls to 11 ± 3% (p-value < 0.5, n=4). hOPCs over-expressing SULF2 or control mCherry were transplanted into neonatal hypomyelinating shiverer/rag2 mice. At 8 weeks post-implantation, SULF2 over-expression

reduced the proportion of differentiating CC1+ oligodendrocytes (9.8 ± 0.6% vs control 15.9 ± 2%, t-test $p < 0.05$, n=4) and increased the proportion of GFAP+ astrocytes (13.6 ± 2.2% vs control 7.5 ± 0.2%, t-test $p < 0.05$, n=3). To determine whether sulfatase expression was regulated during remyelination, we analyzed murine spinal cord following lysolecithin-induced demyelination. SULF2 mRNA and protein were found in demyelinating lesions. Preliminary data from OPC-specific SULF1/2 conditional knockout mice suggest that sulfatase expression regulates OPC fate following demyelination. In cultured OPCs, we found that sulfatases may act to facilitate both BMP and WNT signaling in OPCs suggesting a mechanism by which they regulate OPC fate *in situ*. As such, sulfatase inhibitors may be ideally positioned to interfere with inhibitory factors blocking OPC differentiation in chronically demylinated lesions and thereby promote remyelination.

Disclosures: S.U. Pol: None. **H. Shayya:** None. **R. Welliver:** None. **J. Polanco-Garcia:** None. **D. Bratton:** None. **A. Milliron:** None. **M. O'Bara:** None. **F. Sim:** None.

Poster

203. Oligodendrocyte Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 203.22/A22

Topic: B.11. Glial Mechanisms

Support: ERC

Swedish research council

Title: Oligodendrocyte generation dynamics in multiple sclerosis

Authors: *M. YEUNG[1], S. ZDUNEK[1], S. BERNARD[2], M. SALEHPOUR[3], K. ALKASS[4], G. POSSNERT[3], H. DRUID[4], L. BRUNDIN[5], J. FRISÉN[1];
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Abstract: Oligodendrocytes wrap layers of specialized cell membrane around nerve fibers forming myelin, which facilitate fast propagation of nerve impulses and trophic support of axons. Myelination can in theory be modified by mature oligodendrocytes generating new myelin and/or by exchanging oligodendrocytes and their myelin sheaths. In a previous study we assessed the dynamics of oligodendrocyte generation and myelination in the human brain. By analyzing the concentration of ^{14}C, derived from nuclear bomb testing during the Cold War, in genomic DNA of oligodendrocytes, we found that human white matter oligodendrocytes are remarkably stable with limited turnover, whereas myelin is exchanged at a high rate. This indicates that

myelin modulation in humans may be carried out by mature oligodendrocytes. There are different kinetics of oligodendrocyte generation and turnover in gray and white matter, with a longer period of oligodendrocyte generation and higher turnover rate throughout life in gray matter, suggesting the possibility of de novo myelination in the sparsely myelinated cortex. However, how these generation kinetics of the oligodendrocyte population may change under pathological conditions such as in the demyelination disease multiple sclerosis (MS) has been unknown. A hallmark of MS is the loss of oligodendrocytes and myelin, as well as axons, leading to conduction deficits and a variety of neurological symptoms. In early stages of MS, regeneration of myelin (remyelination) has been observed to occur. Studies in experimental animal models of MS support that new oligodendrocyte generation contributes to the remyelination process. However, it is not fully known if existing mature oligodendrocytes may contribute to remyelination. The question is to what degree oligodendrocyte generation is affected in MS, and whether remyelination occurs by generation of new oligodendrocytes or by generation of new myelin by pre-existing oligodendrocytes. To address this we are using the strategy of analyzing the content of ^{14}C in genomic DNA of oligodendrocytes from MS brain tissue. We hope to reveal how the generation pattern of the oligodendrocyte population may change during MS and further our understanding of the disease process and potentially point to targets for new therapeutic interventions aiming at affecting the remyelination process.

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Poster

204. Molecular Mechanisms of Synapse Formation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 204.01/A23

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: NIH grant 1U54HD082008

Title: Developmental deficits in auditory cortex of a fragile x syndrome mouse model

Authors: ***I. M. ETHELL**[1,2], T. WEN[2], S. REINHARD[3], H. SIDHU[2], K. TAPIA[2], D. BINDER[2], K. RAZAK[3];
[1]Univ. California Riverside, Riverside, CA; [2]Biomed. Sci. and Neurosci., [3]Psychology, Univ. of California Riverside, Riverside, CA

Abstract: Fragile X Syndrome (FXS) is a leading, genetic cause of autism and mental retardation. FXS occurs in 1 in 4000 males and 1 in 8000 males. Symptoms include language impairments, social deficits, as well as auditory processing deficits, including hyperacusis. The Fmr1 knockout (KO) mouse displays similar auditory impairments, including impaired critical

period plasticity, hypersensitivity to tones, increased response variability, reduced habituation of auditory ERPs and broader receptive fields. Determining whether these phenotypes arise during development and the mechanisms through which they occur will provide the basis for creating lasting treatments for FXS. In this study, we characterized the development of inhibitory and excitatory processes, which may underlie auditory hypersensitivity observed in the KO mouse model. We analyzed dendritic spines in excitatory neurons and perineuronal net (PNN) formation around parvalbumin (PV)-positive cells in KO mouse auditory cortex. Our previous studies suggest an important role for Matrix metalloproteinase-9 (Mmp-9), a protein that regulates the extracellular matrix and influences spine plasticity necessary for learning and memory in FXS-associated structural, functional and behavioral deficits. Mmp-9 is a target of Fmrp transcriptional regulation and has been shown to play a role in regulating spine morphology and enzymatic cleavage of the extracellular matrix, in particular PNNs formed around PV-positive interneurons. We found that Mmp-9 levels are elevated in FXS and Fmr1 ko mice, whereas genetic deletion of Mmm-9 demonstrated a reversal of some FXS phenotypes in Fmr1/Mmp-9 double KO mice. In the normal auditory cortex Mmp-9 levels peak during the first postnatal week followed by a down-regulation during second and third postnatal weeks. This mirrors an increase in PNN structures correlated with the closer of the critical period, and maturation of dendritic spines. Our data show that Mmp-9 levels remain high in the auditory cortex of Fmr1 ko mice during third postnatal week, suggesting that elevated MMP-9 levels may be responsible for the abnormal development of PV/PNN, spine maturation in the auditory cortex of Fmr1 KO mice leading to the development of auditory processing deficits. Targeting drug treatments during developmental ages may therefore be crucial for FXS treatment.

Disclosures: I.M. Ethell: None. **T. Wen:** None. **S. Reinhard:** None. **H. Sidhu:** None. **K. Tapia:** None. **D. Binder:** None. **K. Razak:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 204.02/A24

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant 1U54HD082008-01

Title: Auditory fear extinction learning in fmr1 and mmp-9 knockout mice

Authors: *S. AFROZ[1], S. REINHARD[2], I. ETHELL[1], K. RAZAK[2];
[1]Biomed. Sci., [2]Psychology, Univ. of California Riverside, Riverside, CA

Abstract: Fragile X Syndrome (FXS) is the leading heritable cause of intellectual disability and the only known genetic cause of autism. Symptoms include cognitive, behavioral and social

deficits, problems with sensory processing and speech production, as well as abnormal dendritic spine morphology. The Fragile X mental retardation-1 knockout mouse (*Fmr1* KO) is a well-established model for FXS which exhibits multiple characteristic phenotypes found in FXS, including altered dendritic spine morphology, hyper-responsiveness to sensory stimuli and reduced habituation, altered vocalizations, hyperactivity and abnormal socialization. Though cognitive impairments are a hallmark of FXS in human subjects, behavioral tests in *Fmr1* KO mice have shown mixed results perhaps due to limitations in relevant tests. Emerging evidence in FXS shows an important role for Matrix metalloproteinase -9 (MMP-9), a protein that regulates the extracellular matrix and influences spine plasticity necessary for learning and memory, which is upregulated in FXS and can exacerbate multiple phenotypes in *Fmr1* KO mice. We tested the performance of *Fmr1* KO mice on a fear conditioning and extinction paradigm and compared their performance to three models in which MMP-9 levels are manipulated: homozygous MMP-9 KOs, heterozygous MMP-9 KOs crossed with *Fmr1* KO mice for a reduction but not total deletion of MMP-9, and MMP-9/*Fmr1* double KO mice. Potential deficits in fear learning and extinction were then tested in *Fmr1* KO mice treated with Minocycline, a broad spectrum tetracycline antibiotic which reduces MMP-9 levels and which has been used in trial treatment of FXS subjects. *Fmr1* KO mice appear to take more trials to reach the same level of freezing in response to foot shock paired with a tone as WT mice and show altered trajectories during extinction learning. Similar to *Fmr1* KO mice, MMP-9 and MMP-9/*Fmr1* double KO mice show consistently lower levels of freezing across training and extinction trials, possibly due to hyperactivity of these mice. These findings may suggest the use of this fear conditioning/extinction paradigm as a potential behavioral assay for the detection of behavioral abnormalities in Fragile X Syndrome studies. *This work is supported by funding from the NIH Grant 1U54HD082008-01*

Disclosures: **S. Afroz:** None. **S. Reinhard:** None. **I. Ethell:** None. **K. Razak:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 204.03/A25

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: Eugene V. Cota-Robles Fellowship

Neurobehavioral Genetics Training Grant, NIH T32 5T32MH073526-08

Title: Linking sensory experience to abnormal synaptic development in the Fmr1 knockout mouse model of Fragile X Syndrome

Authors: *E. ARROYO, D. FIOLE, C. HUANG, C. PORTERA-CAILLIAU;
UCLA, Los Angeles, CA

Abstract: Fragile X Syndrome (FXS) is the leading cause of inherited intellectual disability and autism affecting roughly 1 in ~2,500 males. It is caused by a CGG triplet repeat expansion of the 5' UTR of the *Fmr1* gene on the X chromosome. The gene product, FMRP, is a translational repressor that has been shown to interact with mRNAs of genes involved in synaptic function at post-synaptic dendritic spines. Previous research suggests that dendritic spines in individuals with FXS exhibit an abnormally immature morphology resembling filopodia. Furthermore, several independent *in vivo* imaging studies, including our own, have shown that spines of cortical pyramidal neurons are abnormally unstable in the best-studied animal model of the disease, *Fmr1* knockout (KO) mice. Given that changes in sensory experience modulate spine dynamics in sensory cortices, it is conceivable that *Fmr1* KO mice might experience impaired experience-dependent synaptic plasticity. Interestingly, a hallmark of FXS is sensory hypersensitivity and hyper-reactivity across sensory modalities, and *Fmr1*KO mutant mice exhibit similar defects. Thus, we tested the hypothesis that hypersensitivity to external stimuli in FXS is due to impaired modulation of spine dynamics and synaptic connectivity in somatosensory cortex during a critical period of postnatal development. We performed *in vivo* time-lapse two-photon imaging of apical dendritic spines of L2/3 neurons in barrel cortex in 2 week-old wild type and Fmr1 KO mice. Here, we will report on the effects of environmental enrichment on spine density, turnover, and head volume, as well as the effects of single whisker stimulation on spine dynamics and calcium signals in both WT and *Fmr1* KO mice.

Disclosures: **E. Arroyo:** None. **D. Fiole:** None. **C. Huang:** None. **C. Portera-Cailliau:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 204.04/A26

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Title: Autism-associated mutation inhibits protein kinase C mediated neuroligin-4X enhancement of excitatory synapses

Authors: *M. A. BEMBEN[1], Q. NGUYEN[3], T. WANG[2], Y. LI[2], R. A. NICOLL[3], K. W. ROCHE[2];
[1]NINDS, [2]NIH, Bethesda, MD; [3]Univ. of California, San Francisco, San Francisco, CA

Abstract: Autism spectrum disorders (ASDs) comprise a highly heritable, diverse group of neurodevelopmental disorders, which are characterized by repetitive behaviors and impairments in social interactions. A variety of point mutations have been identified in X-linked Neuroligin-3

and 4X genes in patients with ASDs. The neuroligin (NLGN) gene family consists of five members (Nlgn1, 2, 3, 4X, and 4Y) within the human genome that encode transsynaptic cell adhesion molecules that are critical for synapse assembly, maintenance, and plasticity. Interestingly, all of the autism-associated mutations reported thus far reside in the NLGN extracellular domains except for a single point mutation in the cytoplasmic domain of NLGN4X in which an arginine is mutated to a cysteine (R704C). Here we show that NLGN4X is robustly phosphorylated by protein kinase C (PKC) at T707. The autism mutation, R704C, completely eliminates T707 phosphorylation. We observe a dramatic increase in endogenous T707 phosphorylation in NLGN4X upon PKC stimulation in human neurons. Furthermore, a phospho-mimetic mutation at T707 has a profound effect on NLGN4X-mediated synaptogenesis and excitatory potentiation. Our results now establish an important interplay between a genetic mutation, a key posttranslational modification, and robust synaptic changes, which can provide insights into the synaptic dysfunction of ASDs.

Disclosures: **M.A. Bemben:** None. **Q. Nguyen:** None. **T. Wang:** None. **Y. Li:** None. **R.A. Nicoll:** None. **K.W. Roche:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 204.06/A27

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Title: Transcriptional regulation of the rim1 promoter by neurod1

Authors: T. LEE[1,2], I. CHO[1], S. LEE[1,2], J. CHOI[1], Y. LEE[1,2], S. KIM[1,2], *H. K. SUH-KIM[1,2]; [1]Dept. of Anat., Ajou Univ, Sch. Med., Suwon, Korea, Republic of; [2]Dept. of Biomed. Sci., Suwon, Korea, Republic of

Abstract: NeuroD1 is a transcription factor with a basic-helix-loop motif, which is known to regulate differentiation and survival of neuronal cells, enteroendocrine cells, and pancreatic beta cells. Previous reports showed that targeted depletion of NeuroD1 caused decreases in Rab3 interactive molecule-1 (RIM1), RIM2, and Munc18-1. Since these proteins are known to play critical roles in exocytosis of secretory vesicles in neuronal and endocrine cells, NeuroD1 may be a master transcription factor that regulates expression of members of secretory machinery. Here, we report a potential role of NeuroD1 in the regulation of RIM1 transcription in neuronal cells. Since NeuroD1 is known to be highly phosphorylated by diverse protein kinases, we also investigated the effects diverse phosphorylation sites of NeuroD1. We will also discuss the diverse phosphorylation mutations with respect to the functions of NeuroD1.

Disclosures: **T. Lee:** None. **I. Cho:** None. **S. Lee:** None. **J. Choi:** None. **Y. Lee:** None. **S. Kim:** None. **H.K. Suh-Kim:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: NSERC discovery grant

Title: Taurine increases neurite outgrowth and promotes synapse development in the central nervous system

Authors: ***F. XU**[1,2], K. MA[1], M. QAZZAZ[1], N. I. SYED[1];
[1]Univ. of Calgary, Calgary, AB, Canada; [2]St. Louis Univ., St. Louis, MO

Abstract: Taurine (2-aminoethanesulfonic acid), is an abundant sulfur-containing amino acid that is widely present in the human brain, heart, retina, and muscle tissues. Humans obtain it from either diet or from biochemical synthesis. Taurine deficiency is associated with cardiomyopathy, renal dysfunction, abnormalities of the developing nervous system, and epilepsy. However, both its role during brain development and cellular mechanisms of action are largely unknown. Here, we investigate whether taurine affects neurite outgrowth, synapse formation, and synaptic transmission using rat cortical cell cultures and invertebrate snail (Lymnaea stagnalis) soma-soma synapses. Our study demonstrates that taurine applied at physiological concentrations enhances neurite branching and up-regulates expression of cytoskeleton proteins. Taurine also significantly increases both the density and size of synaptic puncta revealed by immunostaining of pre-synaptic synaptophysin and post-synaptic PSD-95, indicating the role of taurine in synapse development. To further deduce the functional consequence of taurine-induced anatomical changes in synaptic structures, we next examined the role of taurine in synapse formation and synaptic transmission using Lymnaea brains. Lymnaea neurons in culture reconstitute synapses between defined single pre- and post-synaptic neurons, providing an opportunity to study synapses at a resolution that cannot be achieved using mammalian cell cultures due to their intrinsic complexity. We found that taurine significantly increases both the synaptic efficacy (reflected by the percent of cells that form synapses) and synaptic strength (reflected by the amplitude of post-synaptic potentials). This study provides the first direct anatomical and functional evidence that taurine plays an important role in neurite outgrowth and synapse development in the early stage of brain development.

Disclosures: **F. Xu:** None. **K. Ma:** None. **M. Qazzaz:** None. **N.I. Syed:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

Location: Hall A

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Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: Intramural Program of NICHD, NIH (ZIA HD001607)

Title: The adaptor protein complex AP-1 modulates synaptogenesis in hippocampal neurons through sorting of neuroligin 1

Authors: *L. E. CUITINO, S. JAIN, G. FARIAS, J. S. BONIFACINO;
NICHD, NIH, Bethesda, MD

Abstract: Neurons are highly polarized cells displaying specialized somatodendritic and axonal domains. The distribution of transmembrane proteins between these two domains and synapse formation are tightly regulated processes that ensure proper neuronal connections. Abnormalities in these processes are considered to be responsible for neurodegenerative and psychiatric disorders. Several proteins, including the clathrin adaptor protein complex AP-1 and members of the neuroligin (NL) family have been proposed to be important for these processes. AP-1 is a heterotetrameric complex composed of γ, β1, μ1, and σ1 subunits, some of which occur as two or three isoforms. Four NL (NL 1-4) proteins have been identified, with NL1 and NL2 predominantly found at excitatory and inhibitory synapses, respectively. We studied the effects of overexpressing a dominant negative mutant of the obiquitous specific AP-1 μ1A subunit (μ1A-W408S), which cannot recognize specific transmembrane cargos, on synaptogenesis and NL1 sorting in rat hippocampal neurons. Upon overexpression of μ1A-W408S we detected morphological changes in dendritic spines and a decreased number of excitatory synapses in neurons at day *in vitro* 18 (DIV18). Confocal microscopy also showed a decreased number of PSD95-positive protrusions and changes in the synaptic location of NMDA glutamate receptors. In contrast, we did not observe changes in the number, morphology or composition of the inhibitory synapsis when analyzing the expression and localization of either the γ2 GABAA-receptor subunit, gephyrin or the vesicular GABA transporter (vGAT). Importantly, NL1 was restricted to the somatodendritic domain in neurons expressing wild-type μ1A, it exhibited a non-polarized distribution in neurons expressing μ1A-W408S. Further analyses showed that residues in the cytosolic tail of NL1 are important for the polarized sorting and the synaptic localization of this protein, as well as for its interaction with AP-1. These results are consistent with an important role of AP-1 in synaptogenesis, through the regulation of NL1 sorting. This work was funded by the Intramural Program of NICHD, NIH (ZIA HD001607)

Disclosures: **L.E. Cuitino:** None. **S. Jain:** None. **G. Farias:** None. **J.S. Bonifacino:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

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Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: the Research Grants Council of Hong Kong SAR (HKUST660810)

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Hong Kong Research Grants Council Theme-based Research Scheme (T13-607/12R)

the SH Ho Foundation

Title: S-nitrosylation-dependent proteasomal degradation restricts Cdk5 activity to regulate hippocampal synaptic strength

Authors: *A.-Y. FU[1,2,3], P. ZHANG[1,2,3], W.-Y. FU[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: The precise regulation of hippocampal synaptic strength requires coordinated activity and functions of synaptic proteins, which are controlled by specific post-translational modifications. While cyclin-dependent kinase 5 (Cdk5) has been suggested to regulate synaptic strength and functions through phosphorylation of its substrates at synapses, the molecular control of its activity remains unclear. Here, we show that S-nitrosylation of p35, the activator of Cdk5, by nitric oxide (NO) is important for the regulation of excitatory synaptic strength. Blockade of NO signaling reduced mature dendritic spine density and surface expression of glutamate receptor subunits, indicating structural and functional synaptic deficits. Meanwhile, reduced NO production aberrantly upregulated the phosphorylation of numerous synaptic substrates of Cdk5 and its activity. Furthermore, the NO stimulation caused the reduction of Cdk5 activity through p35 S-nitrosylation, resulting in the ubiquitination and degradation of p35. Silencing p35 protein in hippocampal neurons partially rescued the NO blockade-induced synaptic deficits. These findings collectively demonstrate that p35 S-nitrosylation by NO signaling is critical for regulating hippocampal synaptic strength.

Disclosures: **A. Fu:** None. **P. Zhang:** None. **W. Fu:** None. **N.Y. Ip:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

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Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant T32-HD007348

NIH Grant DP2-EY023190

Title: Identification of the genetic factors underlying cell-type specific synaptic organization of developing visual cortex

Authors: *J. TOMORSKY, C. DOE, C. NIELL;
Univ. of Oregon, Eugene, OR

Abstract: In visual cortex, precise synaptic connections are known to form circuits with a wide variety of functions, including the detection of orientation, contrast, and spatial frequency. However, the mechanisms that enable neurons to identify different targets during synaptogenesis, resulting in functional visual circuits, remain unknown. To identify genes important in synapse formation within the visual cortex, we utilized the transcriptional profiling technique, TU-tagging, to identify cortical layer-specific differences in gene expression that appear at eye-opening, when many cortical synapses are forming. TU-tagging enables the detection of differential gene expression between distinct cell-types within complex tissues without cell dissociation, which is important to preserve pre- and post-synaptic RNAs [1]. This technique uses the cre-lox system to express the enzyme UPRT in specific cell-types. UPRT converts injected 4-thiouracil to 4-thiouridine, which is then incorporated into newly transcribed RNA, which can be purified and analyzed by RNAseq. In this study, NR5a-cre and NP39-cre lines were used, with targeted expression in layers 4 and 2/3 of cortex, respectively. In addition, visual cortex samples were collected at two different time-points: P12 (just before eye-opening) and P16 (just after eye-opening). Layer-specific thio-tagged RNA was purified and subjected to high-throughput Illumina sequencing. Differential gene expression between time-points and cell-types was analyzed using the DESeq package. Enrichment of a particular gene in a particular layer was confirmed by examining Allen Brain Atlas *in situ*s performed at P14. Differential gene expression analysis yielded multiple genes that were enriched in layer 2/3 of visual cortex that also showed distinct temporal patterns of expression around eye-opening. A gene ontology search revealed that many of the layer 2/3 enriched genes have known functions in cell-adhesion and neuron-projection development. While the TU-tagging technique has been previously demonstrated in endothelial cells [1], this is the first time it has been shown to be effective in mouse neurons. This study has identified several genes that may be important in developing the circuits responsible for aspects of visual function such as orientation and spatial frequency selectivity. Future functional studies will determine the role of these genes in the development

and/or function of layer 2/3 visual cortical neurons. **REFERENCES: [1]** Gay, L. et al., Genes & Development, 2013. 27: 98-115.

Disclosures: J. Tomorsky: None. **C. Doe:** None. **C. Niell:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

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Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: NINDS/NIH intramural budget

Title: Regulation of Neuroligin-2 Phosphorylation

Authors: *N. F. SHANKS[1], M. A. BEMBEN[1,2], Y. LI[1], K. W. ROCHE[1];
[1]NINDS, NIH, Bethesda, MD; [2]Dept. of Biol., Johns Hopkins Univ., Baltimore, MD

Abstract: Neuroligins (NLs) are postsynaptic cell adhesion molecules involved in synapse development and function. They have a strong genetic link to autism spectrum disorders, and have been implicated in the regulation of the balance between neuronal excitation and inhibition. The NL isoform, NL-2 is located and specifically functions at inhibitory synapses, whereas other NL isoforms function only at excitatory synapses or can act at both types. Because the NL sequences are highly conserved, it is unclear how and why this synapse-specificity occurs. We are interested in investigating potential mechanisms. Here, using *in vitro* kinase methods, we demonstrate that c-AMP dependent kinase (PKA) robustly phosphorylates the cytosolic tail of NL-2. Using mass spectrometry analysis and scanning phospho-null mutants we have identified a PKA site. We have generated a phospho-specific antibody against this site, and characterized it's specificity. We observe that overexpressed NL-2 is phosphorylated in heterologous cells, and endogenous NL-2 is phosphorylated in cultured cortical rat neurons and in rodent brain. Using this antibody we are characterizing the regulation of NL-2 phosphorylation over developmental time points, in different brain regions, and in different brain fractions. We have also determined that NL-2 phosphorylation is regulated by synaptic activity, and are investigating the mechanisms by which this occurs.

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Poster

204. Molecular Mechanisms of Synapse Formation

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National Key Basic Research Program of China 2013CB530900

Hong Kong Research Grants Council Theme-based Research Scheme T13-607/12R

SH Ho Foundation

Title: Activity-dependent regulation of liprinα1 phosphorylation during synapse development

Authors: *H. HUANG[1,2], K.-O. LAI[1,2], A. FU[1,2], N. IP[1,2];
[1]Div. of Life Science,HKUST, Hong Kong, China; [2]Mol. Neurosci. Center, State Key Lab. of Mol. Neuroscience, HKUST, Hong Kong, China

Abstract: Neuronal activity plays an essential role in guiding synapse development, which underlies experience-dependent shaping of brain circuitry. Synapse development requires concerted regulation of different types of synaptic components to drive specific functions, but the manner in which neuronal activity coordinates the process is not clear. Liprinα1, a scaffold protein that mediates protein assembly of synaptic components, has been suggested to play indispensable roles in dendrite development and excitatory synapse maintenance. Here we report that liprinα1 was highly expressed at the postsynaptic regions, while knockdown of endogenous liprinα1 severely impaired dendrite and dendritic spine development. Interestingly, liprinα1 is identified as a novel substrate of the serine/threonine kinase 5 (Cdk5). The Cdk5-dependent phosphorylation of liprinα1 was reduced in the mouse visual cortex during eye opening, while dark-rearing reversed the effect, suggesting that neuronal activity regulates liprinα1 phosphorylation during development. Importantly, phosphorylation of liprinα1 inhibited spine maturation and reduced spine density as well as surface expression level of AMPA receptors, indicating the inhibitory roles of liprinα1 phosphorylation in synaptic functioning. Collectively, these findings provide molecular basis for the instructive roles of neuronal activity during synapse development.

Disclosures: **H. Huang:** None. **K. Lai:** None. **A. Fu:** None. **N. Ip:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

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National Key Basic Research Program of China (2013CB530900)

SH Ho Foundation

Title: Cdk5-dependent phosphorylation of p70 ribosomal S6 Kinase (S6K) is required for dendritic spine morphogenesis

Authors: *Z. LIANG, K.-O. LAI, E. FEI, H. HUANG, N. Y. IP;
Hong Kong Univ. of Sci. and Technol., Hong Kong, China

Abstract: The maturation and maintenance of dendritic spines depend on neuronal activity and protein synthesis. One potential mechanism involves mammalian target of rapamycin (mTOR), which promotes protein synthesis via the phosphorylation of 4E-BP and p70S6 kinase 1 (S6K). Nonetheless, the regulatory control of S6K and its cellular roles during the process remains unexplored. The present study demonstrated that S6K in neurons is phosphorylated at Ser-411 within the auto-inhibitory domain by cyclin-dependent kinase 5 (Cdk5). Ser-411 phosphorylation was regulated by neuronal activity and brain-derived neurotrophic factor (BDNF). RNAi-induced S6K knockdown in hippocampal neurons led to a loss of dendritic spines, which mimics neuronal activity blockade by tetrodotoxin. Notably, co-expression of wild-type S6K but not the phospho-deficient S411A mutant rescued the spine defects. These findings demonstrate the importance of Cdk5-mediated phosphorylation of S6K at Ser-411 in spine morphogenesis driven by BDNF and neuronal activity.

Disclosures: **Z. Liang:** None. **K. Lai:** None. **E. Fei:** None. **H. Huang:** None. **N.Y. Ip:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

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Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: NIH R01.DA037924

NSF HRD.1137725

NIH-RCMI G12 RR03051

Title: Generation and analysis of cnr1 and cnr2 zebrafish mutants

Authors: *A. ACEVEDO-CANABAL[1], L. COLON[1], M. BEHRA[1], G. YUDOWSKI[2]; [1]Univ. of Puerto Rico - RCM, San Juan, PR; [2]Univ. of Puerto Rico - Inst. of Neurobio., San Juan, PR

Abstract: Marijuana is the most commonly used drug among teenagers. The main psychoactive ingredient, Δ9-tetrahydrocannabinol (Δ9-THC), activates two cannabinoid receptors, CB1 and CB2. These receptors are encoded by the cnr1 and cnr2 genes, respectively. Both genes are expressed early on during vertebrate development and the manipulation of the cannabinoid system in chick and zebrafish embryos results in axonal fasciculation errors . It remains unclear if these neurodevelopmental changes are mediated by CB1 or CB2 receptors. Furthermore, the long-term effects of the absence of one or both of the receptors of the endocannabinoid system on the behavior of larvae and mature zebrafish have not been explored. We used CRISPR-Cas9 genome-editing tool to generate cnr1 and cnr2 mutant zebrafish lines. Those mutant lines will allow us to identify the roles of CB1 and CB2 receptors during the early stages of brain development. We have designed two targets per gene and co-injected them with Cas9 mRNA. We have identified 4 alleles for cnr1 and 2 alleles for cnr2 which have been generated in different transgenic background, namely brn3c:GFP, expressed in a subsets of neurons and mechanosensory hair cells (HC) and atoh:TOM, expressing tomato red in nascent neurons in the midbrain-hindbrain boundary in the early embryo and in maturing HC. The confirmed founders have been outcrossed to establish stable mutant transgenic lines. This will allow us to follow brain development in mutants as compared to wild type animals through a comprehensive structural/anatomical analysis. To monitor neuronal wiring and axonal fasciculation formation we did immunohistochemistry with a numbers of axonal markers. We will also use the mutants to test specific roles of the cannabinoid system during swimming behavioral tasks. To assess swimming patterns, we analyzed wild type animals for freezing, cruising, and bursting patterns after treating with different cannabinoid agonists: Δ9-THC and CP55,940 . Our studies will be crucial in identifying the roles of CB1 and CB2 during brain development and the effect of early exposure to cannabinoids.

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Poster

204. Molecular Mechanisms of Synapse Formation

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Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: NIH R01 DA007304

NIH R21 DA035663

Title: GluN2B-containing NMDA receptor antagonist ifenprodil produces long-lasting increases in dendritic spine density: Within-subject longitudinal multiphoton imaging

Authors: *J. D. RAYBUCK[1], S. A. THAYER[2];
[1]Dept. of Pharmacol., UMN, Minneapolis, MN; [2]Pharmacol., Univ. of Minnesota, Minneapolis, MN

Abstract: Serious brain disorders such as depression, cognitive dysfunction, and addiction are strongly linked to regulation of dendritic spine density in cortical and sub-cortical regions. Much work has characterized the mechanisms underlying spine regulation in *in vitro* neuronal cultures, and many of these mechanisms have been demonstrated with *ex vivo* techniques, such as Golgi staining. However, to date, little work has examined the mechanisms of dendritic spine regulation *in vivo*. Because neuronal cultures lack the complex milieu of the living brain, characterization of these mechanisms with *in vivo* models will be critical to understanding normal brain function and the treatment of brain disorders. The regulation of dendritic spines critically depends on NDMA receptor signaling. Notably, many studies have characterized a unique role for GluN2b-containing NMDA receptors in the suppression of dendritic spine formation. To begin to understand how GluN2b signaling regulates spine formation in live animals, we used *in vivo* cranial window multiphoton microscopy to image spines on apical dendrites of layer 5 pyramidal neurons. Specifically, we administered the GluN2b selective antagonist ifenprodil to Thy1-eGFP(h) transgenic mice. Repeated ifenprodil administration (4, daily doses, ip, post imaging) produced a dose-dependent increase in spine density within 24 hours of the first dose. Further, longitudinal imaging (up to 8 weeks after ifenprodil treatment) revealed that increases in spine density remained for several weeks following drug wash out, and that the duration of enhancement was dose dependent, with higher doses producing longer lasting effects. This novel finding suggests that NMDA receptors may act as a short-term regulator of spine density *in vivo*. These results may shed light on the differences between SSRIs and NMDA antagonists in the treatment of depression, as well as how spine regulation relates to this disorder. Additionally, this work has implications for synaptic changes that accompany neurodegenerative diseases, such as HIV-associated neurocognitive disorder (HAND). Further examination of the role of GluN2b receptors in spine regulation may lead to the development of more effective treatments for depression, dementia, cognitive dysfunction, and substance abuse.

Disclosures: J.D. Raybuck: None. **S.A. Thayer:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

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Title: Matrix metalloproteinase 9 inhibition may counteract neurodevelopmental abnormalities in a Xenopus tadpole model for VPA-induced neurodevelopmental disorders

Authors: *E. J. JAMES, C. AIZENMAN;
Brown Univ., Providence, RI

Abstract: Autism is a prevalent neurodevelopmental disorder that affects the brain's normal development and impairs social and communication skills. Prenatal exposure to valproic acid (VPA), a commonly prescribed antiepileptic medication, is known to greatly increase risk for autism and other neurodevelopmental disorders (NDDs). Previous research using tadpole and rodent models, as well as human studies, suggests that neurodevelopmental disorders are marked by significant increases in neural network connectivity and excitability. These abnormal networks are postulated to arise from misexpression of synaptic proteins that function to stabilize, maintain, and refine synapses. Matrix metalloproteinase 9 (MMP9), an endopeptidase involved with synaptic refinement, has been associated with various NDDs, and microarray and qPCR data from our lab revealed that chronic exposure to VPA caused significantly altered expression MMP9 mRNA. Thus, we hypothesize that inhibiting the function of MMP9, in our Xenopus laevis tadpole model for VPA-induced NDDs, would rescue the increased neural network connectivity and excitability that result from early life exposure to VPA. To test this hypothesis, we exposed developing VPA-treated tadpoles to 3uM SB-3CT, a MMP9 inhibitor, during a critical period of synaptic stabilization and refinement in the optic tectum. We then used whole cell patch clamp recording to assay for changes in intrinsic excitability and synaptic transmission. Results from these experiments revealed that chronic exposure to SB-3CT may counteract the effects of chronic exposure to VPA and produced a statistically significant increase in intrinsic cell excitability. These results suggest that manipulation of MMP9 has the potential to rescue the effects of early life exposure to valproic acid.

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Poster

204. Molecular Mechanisms of Synapse Formation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 204.17/A38

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant MH103374

Title: Opposing roles of actin signaling during the developmental stage of spine formation and maturation

Authors: *E. SPENCE, S. SODERLING;
Cell Biol., Duke Univ., Durham, NC

Abstract: Excitatory spinogenesis (dendritic spine formation) is a key neurodevelopmental process that begins with filopodia formation, leads to axonal contact, spine maturation, recruitment of AMPA type glutamate receptors, and functional synapse formation. Later, in adulthood, actin-rich spine synapses undergo both rapid changes in morphology as well as slower alterations in spine turnover that are associated with experience and are thought to encode long-term modifications in neural network connectivity. However, the underlying mechanisms and regulation of these stages of spine development are poorly understood. Results from a range of genetic and state-of the art *in vivo* proteomics will be shown that suggest a critical actin regulatory pathway that controls fundamental features of spinogenesis.

Disclosures: E. Spence: None. **S. Soderling:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 204.18/A39

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: NIH Grant MH104632

NIH Grant GM083889

Administrative Supplement GM083889-17S1

Title: Pointed-end capping of actin filaments by tropomodulin regulates dendritic spine development

Authors: *O. OMOTADE[1], V. FOWLER[2], J. Q. ZHENG[1];
[1]Dept. of Cell Biol., Emory Univ. Sch. of Med., Atlanta, GA; [2]Dept. of Cell and Mol. Biol., The Scripps Res. Inst., La Jolla, CA

Abstract: Dendritic spines are tiny, actin-rich protrusions on the surface of dendrites that serve as the platform for postsynaptic specializations of most excitatory synapses in the vertebrate central nervous system. Dynamic remodeling of the actin cytoskeleton drives the structural changes associated with the formation and modification of dendritic spines during development and synaptic plasticity, but the detailed mechanisms remain poorly understood. Actin filaments are polarized with a barbed and a pointed end: the former favors assembly whereas the latter favors disassembly. Tropomodulins (Tmods) are a conserved family of proteins that cap the pointed end of actin filaments, thereby blocking monomer exchange and inhibiting filament depolymerization. Tmod-mediated capping of pointed ends is known to regulate the stability, length, and architecture of actin networks in non-neuronal cell types. Knockout of Tmod2 in mice leads to defects in learning and memory, but the underlying cellular mechanisms are unclear. In this study, we investigated the role of Tmods in dendritic spine development and synapse formation. We find that Tmods 1 and 2, but not Tmod 3, are highly enriched in dendritic spines in cultured hippocampal neurons. Interestingly, both Tmod1 and 2 are found to concentrate in the spine neck and a small region in the center of the spine head, suggesting that Tmods may be localized to specific sub-spine regions to regulate the local actin structures. Knockdown of Tmods in cultured hippocampal neurons caused a marked loss in the number of mushroom-shaped dendritic spines with an increase in filopodia-like protrusions. These results provide the first evidence that pointed end capping of actin filaments by Tmod plays a role in the development of dendritic spines. Capping of the pointed end of actin filaments is likely important for the construction and maintenance of the actin architecture underlying postsynaptic function. This work is supported by NIH grants to JQZ (MH104632 and GM083889) and an administrative supplement to OO (GM083889-17S1).

Disclosures: O. Omotade: None. **V. Fowler:** None. **J.Q. Zheng:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 204.19/A40

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Title: nArgBP2 regulates dendritic spine formation and spine-synapse formation at glutamatergic synapses

Authors: *S.-E. LEE[1,1], Y. KIM[1], J. HAN[1], G. CESTRA[2], S. CHANG[1];
[1]Dept. of Physiol. and Biomed. Sci., Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of;
[2]Dept. of Biol. and Biotech. Charles Darwin, IBPM, Consiglio Nazionale delle Ricerche c/o Univ. of Rome La Sapienza, Rome, Italy

Abstract: nArgBP2 was originally identified as a protein that directly interacts SAPAP3, a postsynaptic scaffolding protein critical for the assembly of glutamatergic synapses. Although recent genetic deletion of nArgBP2 in mice leads to mania/bipolar-like behaviors, resembling many aspects of symptoms in bipolar disorder patients but the function of nArgBP2 at the synapse is completely unknown. Here we found that knockdown (KD) of endogenous nArgBP2 by specific shRNAs in cultured rat hippocampal neurons resulted in a dramatic reduction in dendritic spine formation. Reintroducing shRNA-resistant nArgBP2 reversed these defects. In addition, nArgBP2 KD significantly increased the levels of phosphorylated WAVE1 and Rac1, which results in the marked increase of dynamics of actin cytoskeletons in spines. We showed that nArgBP2 KD selectively impairs spine-synapse formation at glutamatergic synapse where excitatory synapses terminate mostly at dendritic shafts instead of spine heads while inhibitory synapse formation was not affected by KD. Thus, our results suggest that nArgBP2 functions to regulate spine formation and subsequent spine-synapse formation at glutamatergic synapse. Our data also raise the possibility that the reduction in expression of nArgBP2 may be related to the synaptic dysfunction observed in bipolar disorder.

Disclosures: **S. Lee:** None. **Y. Kim:** None. **J. Han:** None. **G. Cestra:** None. **S. Chang:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 204.20/A41

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Title: HIP1r required for the formation of functional excitatory synapses in cultured hippocampal neurons of rats

Authors: *L. PENG, X. XU, Y. DU, L. ZHU, J. LUO;
Sch. of Med., Zhejiang Univ., Zhejiang, China

Abstract: Huntingtin-interacting protein 1-related (HIP1r), a homologue of HIP1 and Sla2p, is an actin-binding protein, which binds to clathrin and inositol lipids via its putative central coiled-coil domain and epsin N-terminal homology (ENTH) domain, respectively. Similar to Sla2p, HIP1r also binds to F-actin with its COOH-terminal talin-like domain. It is known that HIP1r may play important roles in actin organization and endocytosis. Previous studies identified CCDC62/HIP1R as a candidate gene region for PD. However, it is little known that how HIP1r functions in the nervous system even though it is highly expressed in the brains. Here, we found that knockdown of HIP1r with shRNA in cultured hippocampal neurons induced alternation of dendritic development and synaptogenesis. Dendritic branches and complexity were reduced. PSD95 density and amplitude of mEPSC also decreased, suggesting that the number of excitatory synapses is decreased. Consistent with these resultd the total and surface expression

levels of excitatory receptors were decreased. Interestingly, inhibitory receptors remain unchanged. Take together, these data indicates that HIP1r is required for dendritic development and excitatory synaptogenesis in cultured neurons.

Disclosures: **L. Peng:** None. **X. Xu:** None. **Y. Du:** None. **L. Zhu:** None. **J. Luo:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 204.21/A42

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Title: Structural and functional insights into the latrophilin3 - FLRT3 complex that mediates glutamatergic synapse development

Authors: *F. RANAIVOSON, F. MARTINI, F. BERGAMI, S. VON DAAKE, D. COMOLETTI;
Child Hlth. Inst. of New Jersey, Rwjms/Rutger, New Brunswick, NJ

Abstract: Fibronectin leucine-rich repeat transmembrane 3 (FLRT3) was recently identified as a high affinity ligand of latrophilin-3 (LPHN3) and it was described that these proteins play an important role in glutamatergic synapse development *in vitro* and *in vivo*. LPHNs are a small family of neuronal adhesion-GPCRs originally discovered as receptors for the black widow spider toxin -latrotoxin. Mutations in LPHN3 have recently been identified as risk factors for attention deficit hyperactivity disorder (ADHD) in humans, but little is known about the physiological function of LPHNs. FLRT3 is a leucine rich repeat protein member of the FLRT protein family which contains three isoforms encoded by three distinct genes, Flrt1-3. They are predominantly expressed in central nervous system and have been implicated in specifying neuronal connectivity, functioning in axon guidance, synaptic target selection and synapse formation. Here, using a series of biochemical and biophysical techniques, including single particle electron microscopy, mass spectrometry, isothermal titration calorimetry, and protein crystallography we 1) mapped the domains of LPHN3 involved in binding to FLRT3, 2) we determined the crystal structure of the olfactomedin domain of LPHN3 in two crystal forms, and 3) we solved the crystal structure of the complex between the associating domains of LPHN3 and FLRT3. These results helped delineate the mode of association of the high affinity complex between these two synaptic adhesion proteins.

Disclosures: **F. Ranaivoson:** None. **F. Martini:** None. **F. Bergami:** None. **S. von Daake:** None. **D. Comoletti:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 204.22/A43

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Title: Structural and functional studies of adhesion G-protein coupled receptors

Authors: *Y. C. LU[1], O. NAZARKO[1], R. SANDO[2], G. SALZMAN[1], T. C. SÜDHOF[2], D. ARAÇ[1];
[1]Biochem., Univ. of Chicago, Chicago, IL; [2]Mol. and Cell. Physiol., Stanford Univ., Stanford, CA

Abstract: Adhesion GPCRs have large extracellular regions decorated by numerous adhesion domains and a conserved GPCR Autoproteolysis Inducing (GAIN) domain that mediates self-cleavage of the receptor. Discovery of the novel GAIN domain and determination of its high-resolution crystal structures revealed the mechanism of self-cleavage and provided the great hints as to why these receptors are regulated by tethered agonists hindered within the GAIN domain. The presentation will focus on our recent unpublished data on further structural/functional understanding of the extracellular domains of select adhesion GPCRs that are essential for different functions in the brain such as cortex development and synapse maturation. Our studies revealed unpredicted domains in the extracellular regions, identified the conserved surfaces of these domains, and shed light on the atomic details of how these domains interact with their extracellular ligands to regulate receptor function. In addition, we engineered and determined the structures of synthetic proteins that specifically and tightly bind to the extracellular domains of adhesion GPCRs to be used as potential lead compounds for future drug discovery. These structural studies serve as a starting point to understand and dissect the distinct roles of these domains in the functions of adhesion GPCRs.

Disclosures: Y.C. Lu: None. **O. Nazarko:** None. **R. Sando:** None. **G. Salzman:** None. **T.C. Südhof:** None. **D. Araç:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

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Program#/Poster#: 204.23/A44

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: TWU undergraduate micro grant program

TWU department of biology

Title: Small G- protein regulation of Neurexin localization in *C. elegans* neurons

Authors: *S. K. VALAPPIL[1], K. SENAGBE[1], D. HYNDS[1], T. L. GUMIENNY[2];
[2]Biol., [1]Texas Woman's Univ., Denton, TX

Abstract: Synapse formation and stabilization are key processes involved in learning and memory formation. Synaptic proteins play crucial roles in synaptogenesis, signaling across synapse and specifying synaptic function. Neurexin is a presynaptic trans-membrane protein that interacts with postsynaptic neuroligin to form a trans-synaptic cell adhesion complex. Variants of neurexins and neuroligins recruit specific proteins to the synaptic membrane to determine the type of synapse that is formed (excitatory or inhibitory). An imbalance of excitatory and inhibitory synapses results in neuropsychiatric disorders like autism spectrum and intellectual disability. Although the functional roles of neurexin are being widely explored, how neurexins are localized to the presynaptic membrane is not well elucidated. We propose that specific Rabs direct the localization of neurexin, to presynaptic terminal, and Rho GTPases regulate neurexin membrane loading. Rab and Rho proteins are molecular switches that cycle between guanosine triphosphate (GTP)-bound, active forms and guanosine diphosphate (GDP)-bound, inactive forms to transduce intracellular signals and regulate cytoskeletal dynamics. Transport of membranous proteins is regulated by the Rab family whereas Rho GTPases are best known for regulation of actin dynamics. Studies in the nematode model organism Caenorhabditis elegans have demonstrated fundamental mechanisms that direct cargo sorting, vesicle budding, and membrane localization. We employed RNA interference of specific Rab and Rho genes in *C. elegans* expressing fluorescently tagged neurexin to determine their involvement in neurexin localization in nerve cord cells. Using confocal microscopy, we demonstrate differential distribution of neurexin along the nerve cord as a result of Rab or Rho gene knock down. Results regarding small GTPase involvement in transport and localization of neurexin in primary cell cultures from *C. elegans* using RNAi and live-cell imaging will be presented. This work will contribute to a better understanding of the mechanism involved in formation of different types of synapses, potentially leading to novel strategies to treat autism and other cognitive disorders.

Disclosures: **S.K. Valappil:** None. **K. Senagbe:** None. **D. Hynds:** None. **T.L. Gumienny:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

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Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: ANR grant SynAdh

ANR grant SynSpe

Idex International PhD program

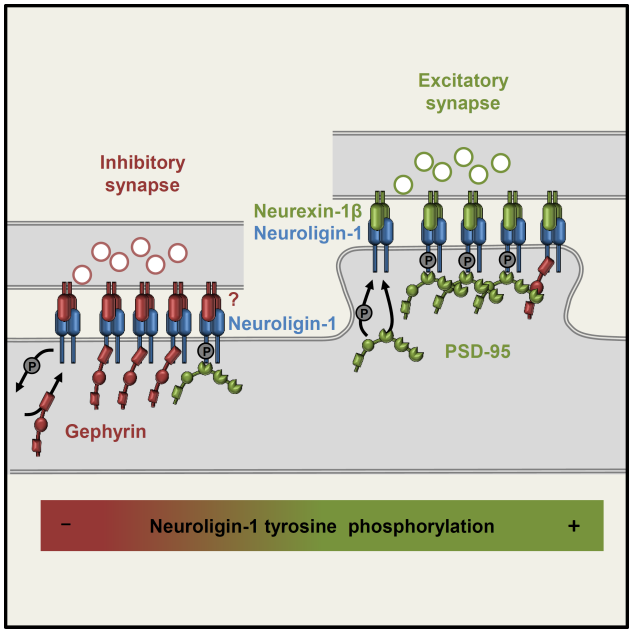
Conseil Régional Aquitaine

Fondation pour la Recherche Médicale

Title: Tyrosine phosphorylation of neuroligin-1 regulates synaptic specification

Authors: C. SAPHY[1], M. LETELLIER[1], K. CZONDOR[1], I. PAPASIDERI[1], I. CHAMMA[1], B. TESSIER[1], M. SAINLOS[1], *O. THOUMINE[2];
[1]Ctr. Natl. de la Recherche Scientifique, Interdisciplinary Inst. for Neurosci., University of Bordeaux, France; [2]UMR CNRS 5297, Interdisciplinary Inst. for Neurosci., Bordeaux Cedex, France

Abstract: The differentiation of neuronal connections into excitatory or inhibitory synapses during nervous system development is a key question in neurobiology. However, the mechanisms which govern the apposition of the proper post-synaptic neurotransmitter receptors in front of their respective glutamatergic or GABAergic pre-synaptic terminals are unclear. Neuroligins are key adhesion molecules implicated in synaptic specification which could play a role in this process. We examined here the functional impact of neuroligin-1 (Nlg1) tyrosine phosphorylation on synaptic differentiation using two previously described Nlg1 point mutants which differentially recruit the scaffolding molecules PSD-95 (Y782A) and gephyrin (Y782F) (Giannone et al., Cell Reports 2013). Expression of the Nlg1Y782A mutant in dissociated hippocampal neurons increased the density of pre-synaptic VGlut1 and post-synaptic AMPA receptor clusters, and the frequency of excitatory miniature currents, with respect to control GFP-expressing cells. In contrast, the Nlg1Y782F mutant increased the number of pre-synaptic VGAT puncta and post-synaptic GABAA receptor clusters, as well as the frequency of miniature inhibitory currents. When expressed in CA1 pyramidal cells of organotypic hippocampal slices from Nlg1 knock-out mice, Nlg1Y782A, but not Nlg1Y782F, increased the amplitude of evoked AMPA-receptor mediated currents upon stimulation of Shaffer collaterals. This effect was associated with a doubling of dendritic spine number induced by Nlg1Y782A, not observed for Nlg1Y782F. Thus, a unique tyrosine residue located in the Nlg1 intracellular domain responsible for a differential binding to scaffolding molecules, acts as a switch that determines the assembly of excitatory versus inhibitory post-synapses.



Disclosures: **C. Saphy:** None. **M. Letellier:** None. **K. Czondor:** None. **I. Papasideri:** None. **I. Chamma:** None. **B. Tessier:** None. **M. Sainlos:** None. **O. Thoumine:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 204.25/A46

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: Max Planck Florida Institute

Title: Imaging Rab GTPase activities in single dendritic spines during structural plasticity

Authors: ***J. WANG**[1,2], J. NISHIYAMA[1], R. YASUDA[1];
[1]Max Planck Florida Inst., Jupiter, FL; [2]Duke Univ., Neurobiology, NC

Abstract: Endosomal trafficking is important for synaptic delivery of AMPARs during long term potentiation (LTP). However, endosomes are heterogeneous and dynamic structures, with different distribution of Rab GTPases. For the better understanding of the molecular identity and each Rab's involvement in synaptic plasticity, we developed highly sensitive FRET sensors for four Rab GTPases, Rab4, Rab5, Rab8 and Rab10. Combining two-photon glutamate uncaging with two-photon fluorescence lifetime imaging microscopy (2pFLIM), we measured the spatiotemporal dynamics of individual Rab GTPase activity in single dendritic spines during structural plasticity. Our data show that, upon glutamate uncaging, Rab4 is transiently activated

in the stimulated spines, which decays within 15min. Rab8 is rapidly activated in the stimulated spines, which sustains for more than 30min. Surprisingly, Rab5 and Rab10 are inversely inactivated in the stimulated spines, which last for more than 30min. All these activities are highly compartmentalized in the stimulated spines. Furthermore, suppression of Rab4 only inhibits transient phase of structural plasticity while suppression of Rab8 inhibits both transient and sustained phase of structural plasticity. In contrast, suppression of Rab5 and Rab10 enhance both transient and sustained phase of structural plasticity. Our results suggest that highly compartmentalized activation of Rab4 and Rab8, as well as inactivation of Rab5 and Rab10, are important for structural plasticity in single dendritic spines.

Disclosures: J. Wang: None. **J. Nishiyama:** None. **R. Yasuda:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

Location: Hall A

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Program#/Poster#: 204.26/A47

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: Center for Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB)

Title: A novel tool for active zone protein orientation: Dually tagged full length mRFP-Bassoon-mGFP protein

Authors: T. GHELANI[1], F. GÖTTFERT[2], R. EBRECHT[3], F. WOUTERS[3], N. WITTENMAYER[1], *T. DRESBACH[1];
[1]Univ. of Goettingen Med. Sch., Goettingen, Germany; [2]Dept. of NanoBiophotonics, Max Planck Inst. for Biophysical Chem., Göttingen, Germany; [3]Dept. of Neuropathology, Univ. Med. Göttingen, Göttingen, Germany

Abstract: Bassoon is one of the largest scaffolding proteins found in the cytomatrix of the active zone (CAZ) of a neuron's presynaptic terminal. The CAZ is a specialized sub-compartment assembled in close proximity to the neurotransmitter release site, or active zone, and is comprised of interconnected active zone proteins. The CAZ and its proteins have been shown to promote short-term plasticity and long-term plasticity by enabling priming and docking of synaptic vesicles and binding to Ca2+ channels. Bassoon, one of the two large CAZ proteins, binds to other AZ proteins and provides structural stability to the protein complex. This large protein is therefore a good tool to study AZ formation. First-generation recombinant Bassoon constructs have become well-established tools for studying trafficking of active zone precursor organelles, as well as active zone assembly and maintenance. Here we introduce a dually tagged second-generation Bassoon construct. This construct carries an intramolecular mRFP tag close to its N-terminus, thus leaving the N-myristoylation consensus site of Bassoon intact. In addition, it

carries a C-terminal mEGFP tag with an optimized spacer that increases the stability of the recombinant construct. The construct possesses all of the expected properties of endogenous Bassoon and known constructs, including correct targeting and CAZ incorporation. Using specific nanobodies against the fluorescent tags and stimulated emission depletion (STED) nanoscopy, we are able to resolve and characterize the localization of the N- and C-termini of the protein at different developmental stages of maturing cultured neurons. Bassoon is one of the first proteins to be incorporated into young synapses, and tracking the changes in conformation of the protein would enhance our knowledge of AZ assembly and synapse maturation as well as serve as a ruler to compare orientation of other AZ and synaptic proteins at the synapse.

Disclosures: **T. Ghelani:** None. **F. Göttfert:** None. **R. Ebrecht:** None. **F. Wouters:** None. **N. Wittenmayer:** None. **T. Dresbach:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

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Topic: A.05. Synaptogenesis and Activity-Dependent Development

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Shenzhen Peacock Plan

Theme-based Research Scheme of the University Grants Committee (T13-607/12-R)

SH Ho Foundation

Title: Axin regulates dendritic spine development via Cdc42 signaling

Authors: *Y. CHEN[1,2,3,4], Z. LIANG[1,2,3], E. FEI[1,2,3], X. ZHOU[1,2,3], W. FANG[1,2,3], W.-Y. FU[1,2,3], A. FU[1,2,3,4], N. IP[1,2,3,4];
[1]Div. of Life Sci., [2]State Key Lab. of Mol. Neurosci., [3]Mol. Neurosci. Ctr., The Hong Kong Univ. of Sci. and Technol., Hong Kong, China; [4]Guangdong Key Lab. of Brain Science, Dis. and Drug Develop., HKUST Shenzhen Res. Inst., Shenzhen, China

Abstract: Whereas dendritic spine morphogenesis is critical for the development of glutamatergic synapses and excitatory neurotransmission, scaffold proteins are critical for orchestrating protein complexes to regulate dendritic spine development. The multi-domain scaffold protein Axin (“axis inhibitor”) regulates various developmental processes including the proliferation/differentiation of neural progenitor cells and axon formation. Our recent findings

revealed that Axin is concentrated in postsynaptic fractions and colocalizes with the postsynaptic marker PSD-95. Knockdown of Axin significantly reduced dendritic spine density in cultured neurons and in the mouse hippocampus. The stabilization of Axin by small molecule XAV939 reduced the elimination rate of dendritic spines, resulting in increased dendritic spine density and synaptic transmission. We found that Axin interacts with Ca2+/calmodulin-dependent protein kinase II (CaMKII) in synaptosomal fractions. Moreover, the dendritic spine deficits in Axin-knockdown neurons could be rescued by the small Rho-GTPase Cdc42, whose activity is regulated by CaMKII. These findings collectively suggest that Axin regulates dendritic spine morphogenesis via Cdc42-dependent signaling.

Disclosures: Y. Chen: None. **Z. Liang:** None. **E. Fei:** None. **X. Zhou:** None. **W. Fang:** None. **W. Fu:** None. **A. Fu:** None. **N. Ip:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

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Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: NSC99-2321-B-010-002

NSC102-2911-I-010-506

MOST103-2321-B-010-009

Title: Foxp2 regulates dendritic spine formation in medium-sized spiny neurons during postnatal maturation of the mouse striatum

Authors: Y.-C. CHEN[1], H.-Y. KUO[1], K.-M. LU[1], S.-Y. CHEN[1], W. ENARD[2], S. PÄÄBO[3], *F.-C. LIU[1];
[1]Natl. Yang-Ming Univ., Taipei, Taiwan; [2]Ludwig Maximilians Univ. Munich, Munich, Germany; [3]Max Planck Inst. for Evolutionary Anthrop., Leipzig, Germany

Abstract: Human-specific two amino acids substitutions in mouse Foxp2 gene results in increases of dendrite length of medium-sized spiny neurons (MSNs) and enhancement of long-term depression in humanized Foxp2H/H striatum (Enard et al., 2009). It implicates that humanized version of Foxp2H/H protein functions more efficiently than mouse Foxp2 protein in regulating neuronal morphology and function of MSNs. The dendritic spines of MSNs are the main loci in which corticostriatal and thalamostriatal synapses are localized. We examined dendritic spines of MSNs in postnatal day (P) 14 brains of Foxp2H/H mice. We analyzed dendritic spines of MSNs in the dorsolateral (DL) sensorimotor and dorsomedial (DM) associative striatum. We found that the dendritic spine density was increased in MSNs of both

DL and DM striatum of Foxp2H/H mice. To further analyze the morphology of dendritic spines in details, we classified the morphology of dendrites according to Harris et al. (1992) with slight modification. The dendrites were categorized into six types: stubby, thin/filopodia, mushroom, branched, multiple-branched and atypical. In DL striatum, the densities of thin/filopodia, mushroom, branched and multiple-branched spines were significantly increased. It was of particular interest that multiple-branched dendritic spines were found in a few Foxp2H/H MSNs. Such multiple-branched spines were barley found in wild type MSN. In DM striatum, only thin/filopodia spines were increased in Foxp2H/H MSNs. Therefore, in general, the spine morphology of Foxp2H/H MSNs was more complex than wild type MSNs. Note that the spine density was higher in DL than DM striatum either in wild type or Foxp2H/H mice at P14. Most interestingly, the multiple-branched spines mainly appeared in MSNs of DL striatum of Foxp2H/H brains, suggesting that the humanized Foxp2 has a stronger effect than mouse Foxp2 in promoting spine formation in DL MSNs. Taken together with the decrease of dendritic spines in MSNs of Foxp2-/- knockout striatum (Chen et al., 2014), our study suggests a positive regulation of corticostriatal excitatory synapses by Foxp2 in MSNs during postnatal maturation of the mouse striatum.

Disclosures: **Y. Chen:** None. **H. Kuo:** None. **K. Lu:** None. **S. Chen:** None. **W. Enard:** None. **S. Pääbo:** None. **F. Liu:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

Location: Hall A

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Program#/Poster#: 204.29/A50

Topic: A.05. Synaptogenesis and Activity-Dependent Development

Support: UFABC

FAPESP

CNPq

Title: Nitric oxide in the retina and its role in synaptogenesis processes

Authors: ***L. T. WALTER**[1], C. SCHMELTZER[2], G. S. V. HIGA[1,3], E. R. KINJO[1], G. CERCHIARO[1], A. H. KIHARA[1];
[1]Ctr. de Matemática, Computação e Cognição, Univ. Federal Do ABC, Sao Bernardo Do Campo, Brazil; [2]Inst. of Physics, Humboldt Univ., Berlin, Brazil; [3]Fisiologia de Biofísica, Univ. de São Paulo, São Paulo, Brazil

Abstract: In the nervous system (NS), nitric oxide (NO) is associated with various physiological and pathological processes. The endogenous NO production depends on nitric oxide synthases

(NOS), nNOS, eNOS and iNOS; whose names are related to the majority production in neurons, endothelium and inflammatory processes, respectively. Although structurally similar, these enzymes have different expression and regulation properties. The aim of this project was to evaluate gene expression (by real-time PCR) and protein distribution (by immunofluorescence) of the three NOS isoforms using rat retina as a model. We also measured NO production levels (by the EPR technique) during retinal development (P0, P5, P10 and P60) and under effect of pharmacological intervention (blocker L-NAME and 7-NI). Moreover, the effects caused by the blockade of genes involved in synaptogenesis were evaluated (by real-time PCR). The results showed that mRNA levels of the three isoforms are present at all studied ages. Moreover, eNOS and nNOS seems to be distributed only in vascular plexus and specific layers of retina, respectively. nNOS presence was observed in amacrine cells, double-labeling experiments and morphological observations revealed two distinct populations of nNOS positive amacrine cells, named as round and balloon cells. The data obtained from the nonspecific NOS blockers (L-NAME) and specific nNOS blocker (with 7-NI) showed a reduction of synapsin, synaptophysin and connexin 45 transcripts. Taking together, our data suggests that NOS are present in initial stages of postnatal development and nNOS pla ys an important role in electrical and chemical synapses during development of NS.

Disclosures: L.T. Walter: None. **C. Schmeltzer:** None. **G.S.V. Higa:** None. **E.R. Kinjo:** None. **G. Cerchiaro:** None. **A.H. Kihara:** None.

Poster

204. Molecular Mechanisms of Synapse Formation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 204.30/A51

Topic: B.08. Synaptic Plasticity

Title: The X-linked intellectual disability gene, DHHC9, regulates neurite outgrowth and synapse formation

Authors: *J. J. SHIMELL[1], D. B. JOVELLAR[1], G. S. BRIGIDI[1], I. TATARNIKOV[2], D. BECCANO-KELLY[2], A. J. MILNERWOOD[2], S. X. BAMJI[1];
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Abstract: Palmitoylation is a reversible post-translational modification involving the attachment of palmitate to cysteine residues on substrate proteins. This process is catalyzed by a family of palmitoyl acyltransferase (PAT) enzymes that contain a conserved DHHC motif. Palmitoylation enhances the recruitment of proteins to the membrane and the palmitoylation of a number of synaptic proteins is believed to regulate synapse organization, function and plasticity. Although this is a relatively new field of research, disruption of DHHC function has been implicated in a number of neurodegenerative and neurodevelopmental disorders, underscoring their importance

for proper brain development and function. DHHC9 loss of function mutations have been identified in patients with intellectual disability, however its role in the development and function of neural circuits is still unknown. Here we demonstrate that DHHC9 is localized to both excitatory and inhibitory neurons where it plays an important role in promoting dendritic outgrowth and arborisation and constraining exuberant synapse formation. Preliminary data suggest that this is mediated through the palmitoylation of the DHHC9 substrate, Ras. Taken together, this work suggests that palmitoylation-dependent regulation of Ras by DHHC9 modifies neural complexity by regulating neuronal growth and synaptic density.

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Poster

205. Invertebrate Transmitter Signaling

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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NIH Grant MD007600

NIH Grant GM087200

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Title: Effect of parasitism on the distribution of serotonin-like immunoreactivity in the central nervous system of Biomphalaria alexandrina

Authors: *S. ROLÓN-MARTÍNEZ[1,2], M. R. HABIB[3], L. O. VAASJO[1], R. P. CROLL[4], M. W. MILLER[1,2];
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Abstract: Schistosomiasis, or bilharzia, is estimated to affect about 200 million people worldwide. The digenetic trematode worm, *Schistosoma mansoni* that causes intestinal schistosomiasis, employs the freshwater snail genus *Biomphalaria* as its primary intermediate hosts. It has been proposed that the transition from the free-living *S. mansoni* miracidium to parasitic mother sporocyst depends upon uptake of biogenic amines, e.g. serotonin (5HT), from the snail host. However, little is known about potential sources of serotonin in *Biomphalaria alexandrina* tissues and its potential changes during the course of infection. The purpose of this investigation was to localize serotonin-like immunoreactivity (5HTli) in the central nervous

system (CNS) of *B. alexandrina* and examine 5HTli at critical points of the host-parasite interaction. 5HTli fibers were observed innervating the cephalopedal integument, the major site of *S. mansoni* miracidium penetration and transformation. However, no peripheral 5HTli neurons were detected. Clusters of 5HTli neurons were observed in the cerebral, pedal, left parietal, left pleural and visceral ganglia, suggesting that the peripheral serotonergic fibers originate from the CNS (see also Delgado et al. 2012). Specimens infected with *S. mansoni* were examined at 10 days post infection (10 dpi) and during their shedding stage. The total number of central 5HTli neurons decreased from 162.2 ± 40.0 (n = 5) under control conditions to 118.8 ± 11.9 10 dpi and 130.4 ± 6.7 at the shedding stage (one-way ANOVA, $p < 0.05$). Reductions of 5HTli were most evident in the pedal ganglion (control: 33.6 ± 8.8; 18.0 ± 3.7, 10 dpi; 25.6 ± 5, shedding; $p < 0.05$) and the left pleural ganglion (3.2 ± 2.8, control; 0 ± 0, 10 dpi; 0 ± 0, shedding; $p < 0.05$). The changes in 5HTli observed following infection by *S. mansoni* indicate that reductions in serotonin levels can occur in specific central neurons in parasitized snails and that these changes might contribute to the modifications in several behaviors that are observed during the course of infection.

Disclosures: S. Rolón-Martínez: None. **M.R. Habib:** None. **L.O. Vaasjo:** None. **R.P. Croll:** None. **M.W. Miller:** None.

Poster

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Topic: B.01. Neurotransmitters and Signaling Molecules

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Title: Direct measurement of histamine release evoked by light in the eye of the fly Calliphora

Authors: *J. BORYCZ[1], J. A. BORYCZ[1], S. R. SHAW[1], I. A. MEINERTZHAGEN[1,2]; [1]Dept. Psychology & Neurosci., [2]Biol., Dalhousie Univ., Halifax, NS, Canada

Abstract: Histamine (HA) is a neurotransmitter released at arthropod photoreceptor (PR) terminals. After release, most HA is recycled via conjugation to β-alanine by the action of Ebony, to form carcinine; carcinine is then transported back to PRs where Tan hydrolyzes it back to HA and β-alanine. So far the only means to assay light-evoked transmitter release has been indirect, from ERG recordings. Here we apply a direct method for HA measurement using *in vivo* microdialysis of the eye of the blowfly *Calliphora erythrocephala*, both wild-type and the white-eye mutant *chalky*. In *Drosophila* the total head HA is unchanged by light, does not change in wild-type flies kept in constant light, but does decrease after 3 days in constant light in the mutant *white*. White-eyed fly mutants show potentiated ERG responses to light, supposedly

due to enhanced photon capture in eyes lacking screening pigment. To measure HA release we inserted a 300 µm microdialysis probe into the left compound eye's retina in wild-type *Calliphora*, perfusing it with Ringer solution at 1 µl/min, and sampling at 30-min intervals for HA determination. If deep, the probe tip penetrated the lamina to measure HA release from PR terminals, but otherwise lay within the retina and detected recycled HA liberated by Tan. The eye was illuminated by 2 blue-white LEDs (Luxeon Star/O, LXHK- WE8). Illumination increased HA release by 82%, which plateaued after 2 h. After light-off it also took 2 h for HA release to subside to the constant dark phase level. In the white-eye mutant *chalky* HA release increased by 251% relative to wild-type after light-on and again took 2 h to plateau after light-on or -off. Our results are the first to show direct release of HA in the fly's retina, this possibly representing the Tan hydrolysis product. They also accord with our earlier studies showing reduced head HA after prolonged illumination in *Drosophila white*. We showed earlier that *Drosophila white* has abnormal head levels of other biogenic amines (Borycz et al. 2008), indicating that the effect of White might be related not only to the failure to screen out stray light, but may result also from the lack from the *white* eye of this important transporter of multiple substrates.

Disclosures: J. Borycz: None. **J.A. Borycz:** None. **S.R. Shaw:** None. **I.A. Meinertzhagen:** None.

Poster

205. Invertebrate Transmitter Signaling

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Topic: B.01. Neurotransmitters and Signaling Molecules

Title: Effect of a phytochemical on failure by oxidative damage of GABAergic neurotransmission visualized through motor behavior in the nematode Caenorhabditis elegans

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Abstract: Background: Oxidative stress (OS) is an imbalance between reactive oxygen species (ROS) and antioxidant defense system in favor of ROS. Indeed, high concentrations, ROS cause injuries in cell components (lipids, proteins and DNA). Therefore, OS has been associated with Ageing and Chronic-Degenerative diseases, including neurodegenerative and psychological diseases. From the latter, mental disorders as depression and anxiety, linked to GABA levels, could be associated with OS. Several studies in relation to oxidative stress have been executed in

animal models, including the nematode Caenorhabditis elegans (*C. elegans*). *C. elegans* is one of the simplest organisms with a nervous system, which is constituted by 302 neurons, 26 of these neurons establish GABAergic system. In this work, using *C. elegans*, we evaluated the effect of oxidative damage induced by hydrogen peroxide on GABAergic system through the "nose touch response" or Not assay, and we investigated if this noxious effect could be counteracted by antioxidative properties of the phytochemical diferuloylmethane. Methods: Age-synchronized Wild type N2 worms, grown on NGM-agar plates at 20°C seeded with E. coli OP50 were used in this study. 20 worms/well were placed in 12-well plates and exposed to H2O2 (0.1, 0.35 μM and 0.5mm in M9) at different times (15, 30, 45 and 60 min). Subsequently, worms were relocated to NGM-agar plates lacking food. Mechanosensory reflex (nose-touch avoidance response mediated by GABA neurotransmission) was evaluated. This protocol was repeated in a group of worms treated with GABA as a receptor GABAA agonist, and in a group of worms treated with Diferuloylmethane (DFM) as antioxidant agent by adding. Results and Conclusions: Worms after exposure to H2O2 (H2O2 concentrations from 0.35 μM and up) demonstrate a decreased nose touch avoidance response (around 39%) in relation to control. It follows that the oxidation induces a deterioration of the GABAergic system. The co-exposition of H2O2 plus GABA Partially reversed this effect. On the other hand, the deterioration of nose touch response was improved by co-exposition DFM with H202, although this effect was not statically significant.

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Poster

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Title: Tyrosine hydroxylase in the central nervous system of a disease-vector insect

Authors: *B. P. SETTEMBRINI[1,2], M. DE BENEDICTIS[2], S. NOWICKI[3], L. E. CANAVOSO[4];
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Abstract: Triatoma infestans, a blood feeding triatomine insect, is the main vector of Chagas'disease in Argentina and neighboring countries. Morphological, biochemical and molecular biology approaches have been employed to study the central nervous system (CNS) of this insect species. Neuropeptides have been mapped and characterized in the brain and CNS ganglia. However, within the recognized neurotransmitters only the distribution of serotonin has been reported. Cathecholamines play important roles in the brain of animal species. In insects, these neurotransmitters are involved in locomotion, learning and cuticle sclerotization among other functions. Tyrosine hydroxylase (TH) catalyzes the rate limiting step of cathecolamine biosynthesis but at present, there are few studies focused on insect TH. Two isoforms have been reported in insects; the shorter one which lacks a highly acidic region is present in neural cells. The distribution of TH in adult specimens of T. infestans was studied with our current immunocytochemistry protocol employing an antiserum developed by O' Gorman and co-workers (2007) in Manduca sexta. Our experiments have revealed the presence of numerous TH-immunoreactive (IR) cell bodies and a profuse network of neurites in all the CNS compartments. Thus, in the protocerebrum, TH-IR somata were observed in the anterior, lateral and posterior soma rinds forming clusters with variable numbers of cell bodies. Most of the immunopositive cell bodies displayed a uniform size of about 10 μm. A group of 12 somata was detected above the mushroom body calices and another one was seen at the anterolateral edge. The pars intercerebralis housed a cluster of 3 somata, their neurites were observed along the median bundle. Other protocerebral regions with TH-IR were the posteromedial soma layer and the proximal optic lobe. The antennal lobe contained TH-IR somata in both the lateral and medial soma layers; neurites from those of the lateral layer projected to the glomeruli. A small cluster of immunolabeled cell body was found in the dorsal lobe. In the prothoracic ganglion bilateral TH-IR somata were seen at the level of the exit prothoracic nerve II. Regarding the meso-metathoracic ganglionic mass, bilateral somata were found in the meso- and metathoracic neuromeres while a cell body cluster was observed in the abdominal neuromeres. A profuse network of TH-IR processes formed commissures and connectives. In homogenates of the brain and CNS ganglia, the anti-TH antibody recognized an immunoreactive band of ~ 60kDa, compatible with the values of beetles and moths. These studies revealed a widespread presence of TH in the brain and CNS ganglia of these triatomine insects.

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Poster

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Title: Fulicin neuropeptides in the central nervous system of Biomphalaria glabrata, the intermediate host for intestinal schistosomiasis

Authors: M. B. RODRIGUEZ, S. ROLÓN-MARTÍNEZ, L. O. VAASJO, *M. W. MILLER; Inst. Neurobio., San Juan, PR

Abstract: Approximately 200 million people worldwide live at risk of contracting the parasitic disease schistosomiasis. The digenetic trematode worm species *Schistosoma mansoni* that causes the most widespread form of intestinal schistosomiasis employs the freshwater snail *Biomphalaria glabrata* as its primary intermediate host. As infection of *B. glabrata* by *S. mansoni* is known to cause profound behavioral changes in the snail host, including parasitic castration and parasitic gigantism, a neural transcriptomics approach was undertaken to identify precursor prohormones that could encode neuropeptides involved in *Biomphalaria* reproductive and feeding behaviors. A transcript (1616 nucleotides) was found to encode a putative precursor polypeptide (316 amino acids) that could give rise to the neuropeptide fulicin (Phe–D-Asn-Glu-Phe-Val-NH_2; Ohta et al. 1991; Yasuma-Kamatani et al. 1995) and five additional related peptides. For this investigation, affinity purified polyclonal antibodies (rabbit) were generated against the predicted fulicin neuropeptide. Fulicin-like immunoreactivity was observed throughout the central nervous system (CNS) with distinct neurons and clusters on the ventral and dorsal surfaces, as well as in peripheral tissues. Large and small diameter fulicin-li cells were present on the dorsal and ventral surfaces of the buccal ganglion. In the cerebral and pedal ganglia, dispersed clusters of small diameter cells were observed. While no fulicin-li neurons were present in the right pleural ganglion, it was rich in immunoreactive fibers. Within the left parietal and visceral ganglia, clusters of large prominent cells appeared to give rise to axons projecting to the anal and intestinal nerves. These results suggest that fulicin and other peptides derived from the fulicin precursor could regulate behaviors related to food intake, reproduction, and growth that are altered during the course of infection in this host-parasite system.

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Poster

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CONACYT 178526

Title: Histamine immunoreactivity in nervous system of crayfish Cambarellus montezumae of F0 and F1 generations

Authors: M. RODRÍGUEZ-MUÑOZ, E. OÑA-CÓRDOVA, *E. G. ESCAMILLA-CHIMAL; Univ. Nacional Autonoma De Mexico, Mexico City, Mexico

Abstract: Cambarellus montezumae (de Saussure, 1857) is endemic crayfish inhabiting at Mexico. Today is threatened by anthropocentric alteration of their habitat, for the loss of volume of water in their environmental, by chronic pollution, overfishing and the introduction of aggressive exotic species such as carp and tilapia. Although crayfish C. montezumae has a commercial, economic, historical, biological and scientific importance, this species have not been explored potential neurotransmitters that may be playing a key role in the response to stress. The increase of histamine (HA) is an indicator that there is stress in the organism, affecting locomotor activity and HA is very important for the adaptation of animals to environmental changes. The aim of this study was to determine if there immunopositivity to HA and in what areas of eyestalk and brain of C. montezumae it is presented in adults crayfish F0 generation (collected in Michoacán, place with high levels of contaminants that are considered stressful for crayfish), and if this positivity decreased in the F1 generation (adults crayfish cultured under optimal conditions of laboratory). For that, immediately after to be collected 12 animals of F0 generation were maintained in a light dark (LD) 12:12 for 15 days, 3 animals of each were sacrificed at 07:00, 11:00, 19:00 and 23:00 hours to obtain the eyestalk and brain. 12 crayfish of F1 generation are processed in the same way as F0. Tissues were fixed and subsequently cut in 10 μm slices, several preparations were randomly chosen to perform immunohistochemistry using polyclonal antibody anti-histamine, Warp Red Chromegen Kit (Biocare Medical) and subsequently the preparations were analyzed in a microscope. The crayfish F0 generation showed immunopositivity to HA mainly in the lamina ganglionaris of eyestalk, in brain was not positivity. In organisms F1 generation not immunopositivity observed in brain, although immunoreactivity was itself presented at the lamina ganglionaris but this was lower than the F0 generation, so we can conclude that water pollution, where organisms live, it might be being a stressor in the animals, these crayfish answering with an increase in HA, primarily in the eyestalk that is the tissue that is more exposed to water pollutants, because they are located in a front position outside the body, unlike the F1 animals found with good nutrition and unpolluted water where he found little positivity to HA.

Disclosures: M. Rodríguez-Muñoz: A. Employment/Salary (full or part-time):; Depto. Ecología y Recursos Naturales, Facultad de Ciencias. 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.; PAPIIT IN210913 , CONACYT 178526. **E. Oña-Córdova:** A. Employment/Salary (full or part-time):; Depto. Ecología y Recursos Naturales, Facultad de

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Poster

205. Invertebrate Transmitter Signaling

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McKnight Brain Research Foundation

Title: Cephalopod neural transcriptomes reveal unique strategies for memory centers

Authors: *G. C. WINTERS[1], A. B. KOHN[2], N. STERN[3], B. HOCHNER[3], E. T. WALTERS[4], R. J. CROOK[4], L. L. MOROZ[1];
[1]Neurosci., Univ. of Florida- Whitney Lab. for Marine Biosci., Saint Augustine, FL; [2]Whitney Lab. for Marine Biosci., Univ. of Florida, Saint Augustine, FL; [3]Hebrew Univ. of Jerusalem, Jerusalem, Israel; [4]Integrative Biol. and Pharmacol., Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX

Abstract: Cephalopod molluscs (*Octopus, Sepia, Loligo,* and *Nautilus*) exhibit flexible behavior and are capable of advanced learning and memory. One of the most distinct cephalopod innovations is the Vertical Lobe (VL), an analog to the mammalian hippocampus containing circuitry essential for memory formation. Little is known about the molecular organization of the VL and related memory centers. We constructed, sequenced and analyzed neural transcriptomes from *Octopus* VL, CNS, SFL (Superior Frontal lobe) and arm cords, and compared these transcriptomes to neural transcriptomes of gastropod molluscs including *Aplysia californica*. We

identified 17,841 transcripts in the VL and found12,379 (69.4%) appear to be cephalopod-specific (no known annotation). Only 15 of the 50 highest expressed transcripts in the VL had homologs in other bilaterians, many of which are involved in cellular energetics and homeostasis. Only 12.4% of the transcripts detected in the *Octopus* VL share identity with transcripts from *Aplysia*. Remarkably, indicators for many memory related signal molecules and transmitters like NO (NOS), GABA (GAD), and acetylcholine (ChAT) were not identified in the VL transcriptome, suggesting a distinct cephalopod-specific complement of molecules independently recruited in the organization of learning and memory-forming circuits. Second, we characterized the complement of neuropeptide precursors expressed in the *Octopus* VL and SFL (containing presynaptic cells to the VL circuit). Two of the most abundant transcripts in the VL encode novel neuropeptides unique to *Octopus*. We used the well characterized neuropeptide precursor complement of *Aplysia* and found 22 homologs in *Octopus* VL and 23 in the SFL. Overall, we identified 50 neuropeptide precursors in cephalopods and compared them to 88 known prohormones encoded in the *Aplysia* genome. Only 31 prohormones detected in *Nautilus, Loligo, Sepia* and *Octopus* were homologous to *Aplysia* neuropeptides with 19 being cephalopod-specific. One of the unique cephalopod neuropeptides, PMEFLamide is highly expressed in *Octopus* brain, but absent in the ancestrally branching *Nautilus* CNS (which lacks a VL). This expansion of novel signaling molecules in the VL circuit is likely a key feature of the unique memory systems of cephalopods. Finally, using *in situ* hybridization we characterized expression of 17 neuropeptide mRNAs in *Octopus*, and 13 in *Loligo pealei*, and each exhibits distinct and reproducible localization patterns. Only FLRIamide, Bradykinin, Conopressin, and Buccalin have been localized to the VL, further implying extensive parallel evolution of cephalopod brains and memory circuits in particular.

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Poster

205. Invertebrate Transmitter Signaling

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McKnight Brain Research Foundation

Title: Single cell genome-wide analysis of ion channel expression in simpler neural circuits

Authors: *A. B. KOHN[1], E. C. DABE[1,2], C. BOSTWICK[1,2], L. L. MOROZ[1,2];
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Abstract: Ion channels are intimately involved in virtually every physiological process in any neural circuit but little is known about their cell-specific expression at the genomic scale. Here, we combined single-cell RNA-seq and epigenomic profiling to characterize the entire ion channel complements from identified neurons of the feeding and defensive networks of *Aplysia* californica. The developed protocols also allowed both quantification of expression respective mRNAs and analysis of their methylation status in cells of interests. The *Aplysia* genome encodes for 221 ion channels compared to the genomes of Homo, *C. elegans* and *Drosophila* with 235, 227 and 132 genes coding for ion channels, respectively. However even though the numbers of ion channels are similar for humans and *Aplysia*, their composition is vastly different. *Aplysia* as well as other molluscs, Lottia and Crassostrea have a large expansion of the ligand-gated ion channels (LGICs) however, less voltage gated ion channels compared to all other metazoans. First, we quantify the expression of ion channels in known identified sensory, motor and interneurons within a functionally characterized circuit controlling stereotyped and learned behaviors. For example, we identified and quantified on average 56 ion channels in a single presynaptic mechanosensory neuron (VC) compared to 94 in a single postsynaptic motoneuron, L7 and a single serotonergic MCC neuron expresses an average of 67 ion channels. Interestingly, the mechanosensory VC neurons and MCC express a similar number of ionotropic glutamate receptors (iGluRs) at 7 whereas L7 expresses on average 11 iGluRs. The most abundant transcript expressed in mechanosensory neuron is a voltage-gated sodium channel yet in L7 a voltage-sensitive calcium-activated chloride channel, anoctamin is most highly expressed. Second,we developed a direct and efficient method to perform unbiased capture and sequencing of newly synthesized RNA (nRNA-seq) following long-term plasticity on the scale of the entire genome and tested differential expression of ion channels following application of 5HT (2hrs) in sensory neurons. Overall, the expression levels of all the ion channel mRNAs increased 25% in the 5HT treated compared to control but the number of iGluR transcripts increased 50% under the same conditions. The obtained data about the dynamic nature of ion channel complement together with comparative genomic analysis across metazoans allows us to begin to reconstruct the molecular basis of neuronal identity and plasticity.

Disclosures: **A.B. Kohn:** None. **E.C. Dabe:** None. **C. Bostwick:** None. **L.L. Moroz:** None.

Poster

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Title: Capture of nascent RNAs during classical conditioning in *Aplysia californica*

Authors: *C. BOSTWICK[1,2], Q. YANG[3], A. B. KOHN[1], R. D. HAWKINS[3,4], L. L. MOROZ[1,2];
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Abstract: A requirement for the formation of long term memories is the synthesis of new RNA and proteins as well as epigenetic changes in the genome underlying orchestrated gene regulation, including a requirement for DNA methylation (c.f. Hawkins et al, 2015). We developed a direct and efficient method to unbiasedly capture and sequence newly synthesized RNA (nRNA-Seq) (Kohn et al., 2015), and have now applied it to behavioral learning. We used a reduced preparation of the *Aplysia* siphon withdrawal reflex (Antonov et al., 2007) in which it is possible to record the activity and synaptic connections of identified neurons during several simple forms of learning including classical conditioning, and to examine the dynamics of newly synthesized RNAs at any selected time during the learning. The reflex is mediated by neurons in the abdominal ganglion, which was removed after the end of either paired training (conditioning) or unpaired training (control) and prepared for nRNA-Seq. We sequenced three biological replicates, each comparing ganglia from paired-trained and unpaired-trained preparations. We found 55 transcripts at >2 fold change and $p< 0.05$ to be differentially expressed between preparations that had received paired compared to unpaired training. There were 23 transcripts upregulated in paired-trained ganglia. Some of these transcripts include: a DNA transposase, a multidrug resistance-associated transporter protein and apolipoprotein D (a neuronal lipoprotein thought to function in lipoprotein metabolism). We also found 32 transcripts to be downregulated, including two neuropeptides, a T-box type transcription factor, and sidekick-2, a cell adhesion protein that functions to guide synaptic growth in neurons. Also of interest are the set of differentially expressed "uncharacterized" transcripts, including non-protein-coding RNAs. These transcripts may serve a variety of roles, including regulating gene expression or post-transcriptional modifications. They may also be neuronal-specific noncoding RNAs that mediate other unknown regulatory roles within the nervous system. This study is the first to unbiasedly characterize the set of nascent RNA molecules transcribed as a result of associative learning, and

has the potential to provide insights into the transcriptional changes and cellular signaling pathways activated by classical conditioning.

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Poster

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Title: High-level mRNA expression of orthologous secreted proteins is a feature of the sea slug brain

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Abstract: Secreted proteins play key roles in animal nervous systems, where they can function as peptide neurotransmitters, hormones, growth factors, and in maintaining extracellular matrix structure. We sequenced and *de novo* assembled the CNS transcriptomes of six sea slug species, *Tritonia diomedea*, *Melibe leonina*, *Dendronotus iris*, *Hermissenda crassicornis*, *Flabellina iodinea*, and *Pleurobranchaea californica* (Gastropoda, Nudipleura), and used a novel ortholog-guided approach to predict between 718 and 841 secreted proteins for each species. Comparative gene expression profiling revealed that orthologous secretory genes are consistently enriched in the sea slug brain relative to all other genes, and that a small subset of these exhibit dramatically high expression levels. Among these are homologs for molluscan cerebral peptide 1, small cardioactive peptide, and neuroactive polyprotein R15, as well as several uncharacterized proteins. While the majority of orthologous secretome genes exhibit similar expression levels across the six species, some show species-specific enrichment, perhaps reflecting unique adaptations. The predicted secreted proteins and their corresponding gene expression profiles provide a means for probing the physiology of secretion in the sea slug brain, and for evaluating evolutionary changes in peptidergic signaling.

Disclosures: **A. Senatore:** None. **J.W. Boykin:** None. **P. Ganupuru:** None. **P.S. Katz:** None.

Poster

205. Invertebrate Transmitter Signaling

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 205.11/A62

Topic: B.01. Neurotransmitters and Signaling Molecules

Support: Natural Sciences and Engineering Research Council of Canada Grant RGPIN-2014-05565

Canadian Institutes of Health Research Grant MOP-106602

Dalhousie Medical Research Foundation Studentship

Title: Spider, Cupiennius salei, mechanosensory neurons have multiple biogenic amine receptor types, including constitutively active receptors

Authors: *P. H. TORKKELI[1], V. SUKUMAR[1], S. MEISNER[1], I. PANEK[2], A. S. FRENCH[1]; [1]Dept Physiol & Biophysics, [2]Dept. of Med. Neurosci., Dalhousie Univ., Halifax, NS, Canada

Abstract: Peripheral mechanosensory neurons of spider, Cupiennius salei, are innervated by efferent neurons that contain γ-aminobutyric acid (GABA), glutamate, acetylcholine and octopamine (OA). Strain detecting VS-3 neurons in the spider leg patella respond to these transmitters with changes in sensitivity to mechanical or electrical stimuli. OA has an excitatory effect on VS-3 neurons. A search of the spider leg peripheral tissue transcriptome revealed eight complete and three partial putative G-protein coupled receptor sequences that are homologous to invertebrate α- and β-adrenergic-like OA and tyramine (TA) receptors. However, it is not known which cells contain these receptors, so we asked if TA also modulates VS-3 neurons, and whether it acts via the same or different receptors than OA. Immunohistochemistry with an antibody against tyramine found no labeling in the efferent fibers surrounding the VS-3 neurons. However, some of the VS-3 neurons were immunoreactive to this antibody and there was strong labeling in small cells adjacent to the main leg nerve and the VS-3 neurons, suggesting that there is a local source of TA in the periphery. VS-3 neurons were stimulated electrically with pseudorandom noise before and during TA application. TA depolarized the neurons by about 5 mV and caused a dose-dependent rise in action potential firing rate with an EC50 of 15.1 μM that saturated at about 50 μM. TA concentrations above 200 μM caused a smaller change in firing rate. Frequency response analysis showed that TA increased the VS-3 neuron sensitivity significantly at all frequencies, but more in the high frequency range. These effects were similar to OA effects on VS-3 neurons, but OA effects saturated at 20 μM. Mianserin (10 μM), an antagonist of β-adrenergic-like OA receptors, blocked both OA and TA effects on VS-3 neurons. The same concentration of yohimbine, an antagonist of α-adrenergic-like TA and OA receptors, did not significantly change the TA or OA effects on firing rate, but reduced the firing under control conditions. Removal of yohimbine from superfusion caused a rebound effect with a significant increase in VS 3 neuron sensitivity. These results indicate that there are at least two different types of adrenergic-like receptors in the VS-3 neurons. Both OA and TA activate

mianserin sensitive β-adrenergic-like receptors. The yohimbine effect suggests that VS 3 neurons also have constitutively active α-adrenergic-like biogenic amine receptors. These types of receptors could provide precise control of neuronal sensitivity by using different ligands to modulate basal activity of the neuron.

Disclosures: P.H. Torkkeli: None. **V. Sukumar:** None. **S. Meisner:** None. **I. Panek:** None. **A.S. French:** None.

Poster

206. NMDA Receptors I

Location: Hall A

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Program#/Poster#: 206.01/A63

Topic: B.02. Ligand-Gated Ion Channels

Support: The Edna Ittner Pediatric Research Fund

Title: A key role for GluN2D NMDA receptor subunits in ketamine's stimulation of brain activity; implications for schizophrenia and depression

Authors: *K. SAPKOTA[1], Z. MAO[1], V. GAUTAM[2], D. MONAGHAN[1];
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Abstract: The hypothesis that N-methyl-D-aspartate receptor (NMDAR)-hypofunction underlies the symptoms of schizophrenia (SZ) is supported by many observations, including the finding that acute ketamine administration mimics many of the positive, negative, and cognitive symptoms of schizophrenia (SZ). Under *in vivo* conditions, ketamine has highest affinity for NMDARs containing GluN2C and GluN2D subunits, thus these subunits may have a dominant role in ketamine's psychotomimetic actions. Given that GluN2D subunits are expressed in the parvalbumin-containing GABAergic interneurons that are critical to SZ-related behavior and the generation of gamma neuronal oscillations, we examined the potential role of GluN2D subunits in ketamine's actions. We determined the ability of ketamine to alter regional brain activity as measured by [14C]-2-deoxy-glucose (2-DG) uptake in wildtype (WT) C57BL/6 mice and GluN2D-knockout (GluN2D-KO) mice. In the WT mouse, ketamine (30 mg/kg, i.p.) significantly increased relative regional brain activity 15 minutes post-injection in the medial prefrontal cortex (mPFC), retrosplenial cortex (RSPL), entorhinal cortex, caudate putamen, hippocampus, and presubiculum and decreased 2DG uptake in sensory cortex and inferior colliculus. In the GluN2D-KO mouse, however, there was no significant ketamine-induced increase in 2DG in any brain region. Ketamine-induced reductions in 2-DG uptake were, however, still observed in the sensory cortex and inferior colliculus of GluN2D-KO mice. Likewise, ketamine-induced increases in open field locomotor activity were present in WT mice

and absent in GluN2D-KO mice. These findings suggest a potential role of GluN2D in SZ symptoms. Further evidence for a GluN2D role is suggested by the observation that GluN2D KO mice were found to have a reduced number of PV-immunopositive cells in the mPFC, a finding that parallels that seen in SZ. Thus, GluN2D subunit hypofunction may underlie important components of SZ. Since acute ketamine administration can also reduce symptoms of depression after ketamine is eliminated, the present results also suggest a potential role for GluN2D subunits in the treatment of depression. Acknowledgement: The authors thank Dr. Masayoshi Mishina for generously providing the GluN2D-KO mouse.

Disclosures: K. Sapkota: A. Employment/Salary (full or part-time):; Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE, USA. **Z. Mao:** A. Employment/Salary (full or part-time):; Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE, USA. **V. Gautam:** A. Employment/Salary (full or part-time):; Harvard Medical School. **D. Monaghan:** A. Employment/Salary (full or part-time):; Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE, USA.

Poster

206. NMDA Receptors I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 206.02/A64

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant F31 MH105043-02

NIH Grant R01 NS089662-01

Title: The tyrosine kinase Abl2/Arg regulates GluN2B-containing NMDA receptors

Authors: *A. D. LEVY[1], X. XIAO[2], M. JEDRYCHOWSKI[4], S. GYGI[4], A. J. KOLESKE[2,3,1]; [1]Interdepartmental Neurosci. Program, [2]Mol. Biophysics and Biochem., [3]Neurobio., Yale Univ., New Haven, CT; [4]Cell Biol., Harvard Med. Sch., Boston, MA

Abstract: NMDA receptors (NMDARs) are important for learning, memory, and plasticity and regulate dendritic spine structure. Accordingly, tight orchestration of their activity, surface expression and synaptic localization by regulatory proteins is critical for proper neuronal signaling. Loss of NMDAR regulators leads to misregulation of NMDAR currents, which can cause dendritic spine loss or even cellular excitotoxicity. We have shown previously that the integrin-regulated Abl2/Arg (Arg) tyrosine kinase is required for dendritic spine stability, as *arg–/–* mice have normal spine density at postnatal day (P) 21 but lose spines and synapses by P31. In primary hippocampal neurons, late *arg* knockdown also causes spine loss and can be rescued by inhibiting NMDARs with APV or the GluN2B-specific antagonist ifenprodil. These

results suggest that Arg normally inhibits GluN2B-containing NMDAR currents to stabilize spines. In agreement with this hypothesis, we have recently shown that *arg–/–* mice have increased synaptic NMDAR currents through channels containing the GluN2B subunit as early as P21, suggesting that increased GluN2B-mediated currents may precipitate spine loss in *arg–/–* mice. However, the molecular link between Arg and GluN2B that regulates NMDAR currents and spine stability is unknown. Arg can interact with another tyrosine kinase, Src, and Src family kinases phosphorylate Y1472 on the GluN2B intracellular tail. This phosphorylation event prevents synaptic GluN2B internalization, increasing surface receptor content and NMDAR currents, suggesting a possible mechanism by which Arg can regulate GluN2B. However, we found that Y1472 phosphorylation at the synapse was not increased in *arg–/–* mice, demonstrating that Arg does not regulate GluN2B Y1472 phosphorylation to control NMDAR currents. To further pursue this mechanism, we performed a mass spectrometry screen for proteins with altered relative phosphotyrosine levels in *arg–/–* post-synaptic densities compared to wild type to identify in an unbiased manner new synaptic substrates or signaling partners of Arg that might regulate NMDAR currents. This screen identified several regulators of plasma membrane protein trafficking with significantly reduced tyrosine phosphorylation at P21 and P31 in *arg–/–*, including a known Arg substrate. These preliminary experiments suggest that Arg acts on one of these targets to regulate GluN2B trafficking.

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Poster

206. NMDA Receptors I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 206.03/A65

Topic: B.02. Ligand-Gated Ion Channels

Support: R01N5052669

Title: Differential splicing of GluN1 C-terminus controls NMDA receptor unitary conductance and pore architecture

Authors: *A. NIGAM[1], G. IACOBUCCI[1], B. GEORGE[2], G. POPESCU[1];
[1]Dept. of Biochem., [2]Dept. of Biol., Univ. of Buffalo, Buffalo, NY

Abstract: NMDA receptors (NMDARs) are glutamate-gated excitatory channels with fundamental roles in synaptic transmission and plasticity. Despite their involvement with multiple neurological disorders, a complete understanding of their functional regulation is lacking. NMDARs assemble as obligatory heterotetramers from three gene products, GluN1 through GluN3, of which the GluN1 subunit is common to all receptor isoforms. The GluN1

transcript is alternatively spliced to form four functional variants that differ in their intracellular C-termini. To investigate how this regulation affects channel properties we examined single-channel and whole cell currents from four types of recombinant GluN1-a/GluN2A receptors expressed in HEK 293 cells. We found that the GluN1 C-terminus controlled channel unitary conductance (, pS): GluN1-1, 66 ± 2 (n, 5), GluN1-2, 67 ± 2 (n, 6; $p>0.05$); GluN1-3, 74 ± 2 (n, 5; $p<0.05$), GluN1-4, 54 ± 2 (n, 5; $p<0.05$). Further, we found that these conductance changes correlated with changes in permeability to organic cations of known size. Specifically, the permeability to di-methyl ammonium, as estimated by measuring the reversal potential (mV), decreased from -40 ± 1 (n, 5) to -20 ± 1 (n, 4; $p<0.05$) for receptors containing GluN1-1 or GluN1-3 subunits, respectively. Based on these result, we propose that regulated splicing of the GluN1 C-terminus controls the unitary current amplitude of NMDARs by controlling pore size. This novel finding has important implications for understanding the structural and mechanistic bases of ionic flux through NMDARs and therefore, how the synaptic signal is controlled at central synapses.

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Poster

206. NMDA Receptors I

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH R03-NS056321

Title: Characterization of the Grin2adeltaPKC Mouse

Authors: ***D. BALU**[1], J. R. LARSON[2], J. V. SCHMIDT[3], J. P. LEONARD[3];
[1]Dept. of Biol. Sciences, Lab. of Integrative Neurosci., [2]Dept. of Psychiatry, Univ. of Illinois at Chicago, Chicago, IL; [3]Biol. Sci., Univ. of Illinois - Chicago, Chicago, IL

Abstract: The NMDA receptor subunit GluN2A, has a number of sites directly phosphorylated by kinases such as CaMKII, PKA, and PKC. Previous studies from our lab have identified Ser 1291 and Ser 1312 as two important sites for direct PKC phosphorylation; while Tyr 1292 and Tyr 1312 are two of the sites indirectly phosphorylated by PKC via Src kinase. In the oocyte expression system, mutation of those Serine sites to Alanine (that cannot be phosphorylated) in the GluN2A subunit, resulted in a decreased PKC stimulated current enhancement through the receptors compared to control (WT) NMDA receptors. To investigate the behavioral and physiological significance of those sites *in vivo*, the *Grin2adeltaPKC* mouse expressing GluN2A with those four mutated amino acids (S1291A, S1312A, Y1292F and Y1387F) was generated using homologous recombination. The mutant mice were like their WT-littermate controls in

their body weight, appearance, and general activity. Mutant and WT mice also have similar expression levels of *Grin2a* mRNA, and GluN2A protein. In this study, we provide evidence suggesting that at least one of those sites that is phosphorylated in the C-terminal domain of the GluN2A subunit contributes to anxiety-related behavior. The mice expressing the mutant GluN2A subunit show anxiolytic-like behavior in open field test, light dark emergence test, and elevated plus maze. The mutants also show mild spatial working memory deficits such as reduced spontaneous alternation in a Y maze and a decreased alternation in a T maze. Interestingly, immunostaining for c-fos (a neuronal activation marker) in the hippocampus after exposure of the animals to novel environments shows region specific differences between the mutants and WT mice. There was no increase in c-fos levels in mutants after exposure to novel environments in CA1 and CA3 compared to home-cage (basal) c-fos levels, while the c-fos increased in the WT mice in CA1, CA3 and dentate gyrus (DG). However, both groups had similar basal c-fos levels in the CA1, CA3, and DG. To determine if the mutants had impaired synaptic plasticity in the hippocampus, the Schaffer collateral-CA1 synapses were stimulated using a theta-burst protocol. In hippocampal slices obtained from mutant mice, there was no impairment in LTP. Also, the mutant mice showed no significant differences in input-output curves and paired-pulse facilitation. Taken together, these results suggest that PKC-mediated phosphorylation of at least one of those four sites regulates NMDAR-mediated signaling that modulates anxiety, and spatial working memory. *Grin2adeltaPKC* mice generation was supported by a National Institute of Health grant (R03-NS056321).

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Poster

206. NMDA Receptors I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 206.05/A67

Topic: B.02. Ligand-Gated Ion Channels

Support: CIHR

AIHS

HBI

Title: Non-canonical signaling by NMDA receptors couples Src-family kinases to pannexin-1 during excitotoxicity

Authors: *N. L. WEILINGER[1], B. D. RAKAI[1], A. W. LOHMAN[1], E. M. M. MA[1], J. BIALECKI[1], V. MASLIEIEVA[1], T. RILEA[1], M. V. BANDET[2], N. T. IKUTA[2], L. SCOTT[1], M. A. COLICOS[1], G. C. TESKEY[1], I. R. WINSHIP[2], R. J. THOMPSON[1];

[1]Univ. of Calgary, Hotchkiss Brain Inst., Calgary, AB, Canada; [2]Neurochemical Res. Unit, Dept. of Psychiatry, Univ. of Alberta, Edmonton, AB, Canada

Abstract: Over-activation of neuronal N-methyl-D-aspartate receptors (NMDAR)s causes excitotoxicity and is critical for neuronal death. In the classical view, these ligand-gated Ca2+ permeable ionotropic receptors require co-agonists and membrane depolarization for activation. We report that NMDARs signal during ligand binding that can occur independent of activation of their ion conduction pore. Pharmacological pore block with MK-801 or physiological pore block with Mg2+ prevented NMDAR currents, but failed to block secondary excitotoxic membrane currents and dendritic blebbing induced by NMDA-overstimulation. Recruitment of pannexin-1 (Panx1) channels was critical in this latent excitotoxic response. Indeed, Panx1 opening mediated the bulk of excitotoxic Ca2+ influx during NMDA receptor excitotoxicity. In contrast to MK-801, competitive antagonists that prevent ligand binding to the NMDAR prevented downstream Panx1 opening, membrane currents and blebbing. NMDARs, Src family kinases and Panx1 were found in a signaling complex and activation of Panx1 involved phosphorylation at Y308. Block of this NMDAR-Src-Panx1 signalsome was neuroprotective *in vitro* and *in situ* - dramatically reducing Panx1 currents, Ca2+-dysregulation, membrane blebbing, and cell lysis. Further, NMDAR/Src-dependent Ca2+-influx through Panx1 was critical in activating the mitochondrial permeability transition pore (mPTP) and subsequent dysfunction. Lastly, disrupting NMDAR-Src-Panx1 *in vivo* was tested by administration of TAT-Panx308 either before or 2 hours after transient middle cerebral artery occlusion (tMCAO) reduced stroke lesion volume and preserved neurological output in a skilled reaching task. Our observations provide key insights into a novel signaling modality of NMDARs that has broad reaching implications for brain physiology and pathology.

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Poster

206. NMDA Receptors I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 206.06/A68

Topic: B.02. Ligand-Gated Ion Channels

Support: T32NS007413

K12NS049453

R21NS81439

Title: Impact of human anti-GluN1 antibodies on synaptic and extrasynaptic NMDA receptors

Authors: *J. A. PANZER[1], A. RATTELLE[2], D. LYNCH[2];
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Abstract: Neuronal N-Methyl-d-aspartate receptors (NMDARs) are located at both synaptic and extrasynaptic locations; signaling from receptors at synaptic sites can promote synaptic plasticity and cell survival whereas extrasynaptic signaling has been linked to neurodegeneration. In the autoimmune disease anti-NMDAR encephalitis, antibodies bind the GluN1 subunit of the NMDAR, resulting in psychosis, altered consciousness, seizures, dyskinesias, and autonomic dysfunction. Antibodies bind to the GluN1 extracellular amino-terminal domain (ATD), resulting in transient stabilization of the receptor's open conformation, which is then followed by displacement of synaptic NMDARs and NMDAR hypofunction due to receptor cross-linking and internalization. To explore the role of receptor conformational state on antibody recognition, we have identified a GluN1 mutant that enhances antibody binding and studied its functional properties. Furthermore, we have investigated determinants of NMDAR internalization, demonstrating that enhancement of receptor crosslinking results in increased internalization, and explored approaches to block and evaluate this internalization at synaptic and extrasynaptic sites. Finally, we have investigated antibody impact on NMDAR-mediated downstream signaling at synaptic and extrasynaptic sites, demonstrating specific effects on molecular mediators of synaptic NMDAR signaling. These results may explain the unique clinical symptoms of the disease, which do not precisely replicate global NMDAR knockdown, and may point towards potential therapeutic targets in anti-NMDAR encephalitis. The unique properties of these antibodies also raise the possibility of their use as tools to explore NMDAR function.

Disclosures: J.A. Panzer: None. **A. Rattelle:** None. **D. Lynch:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent for cell-based assay testing for anti-NMDA receptor encephalitis with royalties paid to Eurimmune.

Poster

206. NMDA Receptors I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 206.07/A69

Topic: B.02. Ligand-Gated Ion Channels

Support: The Edna Ittner Pediatric Research Fund

Title: Evidence for a GluN2D NMDA receptor subunit contribution to ketamine and MK-801 stimulation of gamma oscillations; a potential role for GluN2D in schizophrenia

Authors: *Z. MAO[1], P. SYNOWICKI[2], D. LIEBER[1], K. SAPKOTA[1], V. GAUTAM[3], D. MONAGHAN[1];
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Abstract: The hypothesis that N-methyl-D-aspartate receptor (NMDAR)-hypofunction underlies the symptoms of schizophrenia (SZ) is supported by many observations, including the finding that acute ketamine and MK-801 administration mimics many of the positive, negative, and cognitive symptoms of schizophrenia (SZ). Studies have now demonstrated an impairment of gamma oscillatory activity during cognitive tasks in schizophrenic patients. Since NMDAR blockade augments basal neuronal oscillations in the gamma and other frequency bands, the acute cognitive disruptions of NMDAR blockade could be due to their ability to alter neuronal oscillations. We hypothesized that GluN2D subunits may have a preferential role in regulating gamma oscillations for two reasons: 1) Ketamine has highest affinity for NMDARs containing GluN2C and GluN2D subunits *in vivo* and 2) GluN2D subunits are expressed in the parvalbumin-containing GABAergic interneurons that are critical to the generation of gamma oscillations. Thus, we examined the potential role of GluN2D subunits in ketamine stimulation of neuronal oscillations in wildtype (WT) and GluN2D-knockout (GluN2D-KO) mice. Neuronal oscillations were measured by electrocorticography (ECoG) before and after ketamine (30 mg/kg, i.p.) in WT and GluN2D-KO mice. Frequency spectrum analysis indicated that ketamine induced a 120% increase in the upper gamma band's (45-80 Hz) oscillatory power in retrosplenial (RSPL) cortex of WT mice, but only a 20% increase in GluN2D-KO mice. Ketamine-enhanced power was also significantly reduced in the 110-140 Hz frequency band in GluN2D-KO compared to WT mice. Blockade of NMDA receptors by MK-801 caused a similar enhancement in neuronal oscillations in WT mice that were reduced in GluN2D-KO mice. These findings suggest a potential role of GluN2D in neuronal oscillations and SZ pathology. Thus, GluN2D subunit hypofunction may underlie important components of SZ by altering neuronal oscillations in specific brain regions. Acknowledgement: The authors thank Dr. Masayoshi Mishina for generously providing the GluN2D-KO mouse.

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Poster

206. NMDA Receptors I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 206.08/A70

Topic: B.02. Ligand-Gated Ion Channels

Title: Interactions of ketamine administration and mtor signaling on parvalbumin positive neurons

Authors: *M. M. BOLTON, C. F. HEANEY, A. S. MURTISHAW, M. A. LANGHARDT, J. W. KINNEY;
Psychology, Univ. of Nevada, Las Vegas, Las Vegas, NV

Abstract: Ketamine is a high affinity non-competitive antagonist of the ionotropic N-methyl-D-aspartate (NMDA) glutamate receptor. Several previous investigations in our laboratory using chronic (15 days) subanaesthetic administration of ketamine have demonstrated learning and memory deficits in rodents. We have also repeatedly observed an increase in the number and altered position of parvalbumin positive (PV+) neurons in the CA3 field of the hippocampus in ketamine treated animals. Numerous recent clinical studies have demonstrated a rapid-acting antidepressant effect of subanaesthetic ketamine, however the mechanisms responsible are not fully understood. To understand the mechanism through which ketamine induces a change in PV+ neurons, we performed an experiment to inhibit the mTOR signaling pathway using rapamycin. The same chronic, subanaesthetic dose and administration of ketamine was performed for 15 days along with bilateral hippocampal infusion of rapamycin (inhibitor of mTOR signaling). In this investigation, we demonstrated that rapamycin reduced the ketamine-induced increase in struggle in the forced swim test. In the present study, we evaluated PV+ neuronal number and position in hippocampus and cortex to determine if rapamycin ameliorated the ketamine induced changes. Our data indicate that rapamycin attenuated ketamine induced changes in number of PV+ cells in the CA1 region of the hippocampus and cortex. These data indicate mTOR signaling may be a mechanism involved in the altered PV+ neuronal number and may be associated with ketamine induced increase struggle time in the forced swim task. Further, PV+ number and position were not changed in the dentate gyrus and CA3 field by the ketamine administration consistent with our previous learning and memory investigations. These data may indicate the change in PV+ neurons in discrete regions may depend on training and activity. This study suggests that a mechanism linking ketamine administration and parvalbumin alteration may be mediated via mTOR signaling. The reduction in ketamine induced struggle time by rapamycin indicates the mechanism underlying ketamine may also involve mTOR signaling.

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Poster

206. NMDA Receptors I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 206.09/A71

Topic: B.02. Ligand-Gated Ion Channels

Support: NIMH/NIH

Title: Critical role for NMDA receptor subunit in layer 5 prefrontal cortex dopamine modulation

Authors: *J. IAFRATI[1], S. ROBINSON[1], K. SAKIMURA[2], V. SOHAL[1];
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Abstract: Prefrontal cortex (PFC) plays a key role in cognitive and emotional behaviors which are affected in many neuropsychiatric disorders. Dopamine is a major modulator of PFC activity and recent studies show that layer 5 (L5) pyramidal neurons, a major source of prefrontal output, can be divided in two main subpopulations. These L5 pyramidal subtypes project to different brain areas and exhibit specific modulation of intrinsic excitability after D1R or D2R activation (Gee et al, 2012; Seong and Carter, 2012). Indeed, we previously show than the D2-expressing subtype exhibits a prominent afterdepolarization following D2 receptor (D2R) activation and coincident NMDAR stimulation. The role of specific NMDAR subunits in this phenomenon remains unclear. Moreover the afterdepolarization specifically appears in adult mice. NMDAR subunit composition is known to switch during development, typically from prominent expression of GluN2B to increased expression of the GluN2A subunit. To test whether the D2R-induced afterdepolarization depends on specific NMDAR subunits, we used conditional transgenic mice to delete NMDAR subunits in prefrontal cortex. We used a combination of optogenetic techniques, pharmacology, and patch-clamp recordings from D1R and D2R expressing neurons. Our preliminary data suggest that the D2R-induced afterdepolarization is markedly enhanced by GluN2B deletion. This effect is cell type specific and can be suppressed by D2R antagonists. Further experiments will investigate the detailed mechanism and potential consequences of this effect. Funded by NIMH/NIH.

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Poster

206. NMDA Receptors I

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Topic: B.02. Ligand-Gated Ion Channels

Support: CIHR Grant

Title: Modulation of hippocampal extrasynaptic NMDA receptor function by stress

Authors: Y. C. TSE[1], J. LOPEZ[2], A. S. WONG[2], *T. WONG[2];
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Abstract: N-methyl-D-aspartate receptor (NMDAR) are located in both the synaptic and extrasynaptic regions. Findings from previous studies suggested distinct roles of NMDARs in these two regions in neuronal survival and synaptic plasticity. NMDAR functions are also highly sensitive to stress and stress hormone corticosterone (CORT). We have shown that synaptic NMDARs function can be enhanced by stress-level CORT in CA1 pyramidal neurons of adult rats. Impact of stress on extrasynaptic NMDAR function remains unknown. Here, we report that a brief treatment of stress-level CORT (100 nM) also reduced hippocampal extrasynaptic NMDAR function, which was induced by either TBOA or a brief tetanus (10 pulses at 100 Hz) after the blockade of synaptic NMDARs by MK801. To determine whether synaptic and extrasynaptic functions can also be modulated by chronic stress, we measured hippocampal synaptic and extrasynaptic NMDAR functions in mice that were stressed by chronic social defeat. Notably, we found that while extrasynaptic NMDAR function was reduced in mice that were susceptible to chronic social defeat by exhibiting social avoidance in a social interaction test after stress, decrease in extrasynaptic NMDAR function was not observed in mice that were resilient to chronic social defeat (i.e. mice displayed no social avoidance after stress). Hippocampal synaptic NMDAR function in both susceptible and resilient mice was not altered by chronic social defeat. Our findings suggest that stress-induced reduction of extrasynaptic NMDAR function is important for the development of social avoidance, a depression-related behavior in socially defeated mice.

Disclosures: **Y.C. Tse:** None. **J. Lopez:** None. **A.S. Wong:** None. **T. Wong:** None.

Poster

206. NMDA Receptors I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 206.11/A73

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant R01 MH045817

NIH Grant F31 MH105056

Title: Memantine and ketamine differentially alter desensitization kinetics of NMDA receptors

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Abstract: Memantine and ketamine act similarly as open channel blockers of NMDA receptors (NMDARs), yet the two drugs have remarkably divergent effects in humans. Memantine is a very well-tolerated drug that is approved for treatment of moderate to severe Alzheimer's disease, whereas ketamine causes psychotomimetic side effects, but also exhibits rapid

antidepressant effects in depressed patients. The basic mechanisms underlying the divergent clinical effects of memantine and ketamine are not well understood and are under debate. One proposed explanation for the differential effects of memantine and ketamine focuses on the hypothesis that the drugs inhibit distinct subpopulations of NMDARs. Subpopulations that might be differentially inhibited by memantine versus ketamine include receptors distinguished by the typical duration of their exposure to glutamate (e.g., synaptic versus extrasynaptic receptors), or distinguished by their subunit composition (that is, NMDAR subtype). We focus on GluN1/2A and GluN1/2B receptor subtypes because of their predominant expression in cortical and hippocampal pyramidal cells, neuron subtypes thought to be broadly involved in neurodegenerative and depressive disorders. We found that inhibition of heterologously expressed NMDARs by memantine and by ketamine depends on duration of glutamate application and on receptor subtype. We used kinetic modeling to investigate the mechanism by which inhibition of GluN1/2A receptors by memantine depends on duration of glutamate application. Our results suggest that memantine stabilizes a desensitized state of GluN1/2A receptors. Therefore, we measured the time course of recovery from desensitization of GluN1/2A and GluN1/2B receptors in the absence or presence of memantine and of ketamine. We found that memantine slows recovery from desensitization of GluN1/2A receptors, but not of GluN1/2B receptors. In contrast, ketamine does not affect the time course of recovery from desensitization of GluN1/2A receptors, but speeds recovery from desensitization of GluN1/2B receptors. Further, we found that memantine slows recovery from desensitization of GluN1/2A receptors in a Ca2+-dependent manner. Our results suggest that memantine and ketamine differentially alter NMDAR desensitization kinetics in a subtype-dependent manner.

Disclosures: **N.G. Glasgow:** None. **A. Azofeifa:** None. **J.W. Johnson:** None.

Poster

206. NMDA Receptors I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 206.12/A74

Topic: B.02. Ligand-Gated Ion Channels

Support: SIU Startup fund

Title: The mechanism underlying NMDA receptor blockage-induced fast-acting antidepressant effect

Authors: K. ZHANG[1], T. XU[1,2], V. NAGAI YAMAKI[1], M. HUANG[2], *X. CAI[1];
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Abstract: Major depressive disorder (MDD) is a serious public health problem with a lifetime prevalence of 17% in the United States. Despite the high incidence of MDD and its socioeconomic impact, the etiology of MDD remains largely unknown. Recent evidence from clinical trials shows that a single subanesthesia dose (0.5-20 mg/kg) of ketamine, a noncompetitive ionotropic glutamatergic NMDA receptor antagonist, produces rapid antidepressant responses in patients suffering from MDD. However, recent studies on the mechanism underlying ketamine's fast-acting antidepressant effect are controversial. Moreover, the psychotomimetic properties and abuse potential of ketamine necessitate caution in promoting this compound as a general treatment for depression. Understanding the underlying mechanism of action of ketamine linked to behavioral improvement is of significant importance for developing novel, safe and fast-acting antidepressants. In the current study, we provide evidence to support a new hypothesis on how NMDA receptor blockage induces fast antidepressant effect, which is quite different from the prevalent theories about fast antidepressant action of ketamine.

Disclosures: K. Zhang: None. **T. Xu:** None. **V. Nagai Yamaki:** None. **M. Huang:** None. **X. Cai:** A. Employment/Salary (full or part-time):; Southern Illinois University School of Medcine.

Poster

206. NMDA Receptors I

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Topic: B.02. Ligand-Gated Ion Channels

Support: Alana Foundation USA

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

Awakening Angels

Title: Assessment of memantine on long-term potentiation in the Ts65Dn, a mouse model of Down syndrome

Authors: *J. J. SCOTT-MCKEAN, A. L. ROQUE, A. C. S. COSTA;
Dept. of Pediatrics, Case Western Reserve Univ., Cleveland, OH

Abstract: Down syndrome (DS), or trisomy 21, is the most common genetically defined cause of intellectual disability, and the Ts65Dn is the most studied mouse model of DS. Recently, our research group has shown that the Ts65Dn mouse displays pharmacological responses consistent with a dysfunction in molecular pathways coupled to the gating of N-methyl-D-aspartate (NMDA) receptors. We and other groups also found that the uncompetitive NMDA receptor antagonist memantine rescues learning and memory deficits in these mice. In addition, we found that Ts65Dn mice showed a greater NMDA mediated long-term depression (LTD) when

compared to euploid controls, and that memantine-treated hippocampal slices from Ts65Dn mice displayed control levels of LTD. In the present study, we used electrophysiological techniques to probe the effects of memantine on long-term potentiation. To study these effects, hippocampal slices of Ts65Dn or euploid control mice were dissected out and moved to a holding chamber containing artificial cerebral spinal fluid (aCSF) and allowed to recover for at least 1 h at room temperature. Half of the slices were then moved to another holding chamber with aCSF containing 1 μM memantine, allowed to recover for an additional 4 h before being placed in the recording chamber. First, we studied the effects of memantine on either continuous high-frequency stimulation (HFS; 100 Hz for 1 s) induced LTP or theta burst stimulation (TBS; 4 trains of 5 pulses at 100 Hz, 200 ms inter-train interval) induced LTP in the CA1 region of the hippocampus in Ts65Dn and euploid control mice. Previous studies by our group have shown that Ts65Dn mice display deficits in TBS-induced LTP but not HFS-induced LTP. Second, we studied the effects of memantine on late-phase LTP (L-LTP) in the CA1 region of the hippocampus in Ts65Dn and euploid control mice. L-LTP was induced in the CA1 region of the hippocampus by 4 stimulus trains (HFS; with a 5 min inter-train intervals). In agreement with our previous studies, our results show no significant difference in HFS-induced LTP between Ts65Dn and euploid control mice, but we found a reduction in TBS-induced LTP. Interestingly, Ts65Dn hippocampal slices also showed a deficit in L-LTP when compared to euploid control mice. Memantine pretreatment at the concentration of 1 μM used in this study did not inhibit the induction or maintenance of any form of LTP tested in Ts65Dn or euploid control hippocampal slices. Given the role of NMDA receptors in synaptic plasticity and learning and memory, this research may bring us one step closer to understanding the mechanistic basis for previous electrophysiological and behavioral studies in this important mouse model of DS.

Disclosures: J.J. Scott-McKean: None. **A.L. Roque:** None. **A.C.S. Costa:** None.

Poster

206. NMDA Receptors I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 206.14/A76

Topic: B.02. Ligand-Gated Ion Channels

Title: Effect of dapsone on NMDA receptor subunits: GluN2A and GluN2B in pyramidal neurons from rat primary motor cortex

Authors: *A. JUÁREZ NÁJERA[1], C. RÍOS CASTAÑEDA[2], A. DÍAZ RUIZ[2], E. ARROYO RÍOS[1], A. VILLEGAS RONQUILLO[1], J. FLORES HERNÁNDEZ[1];
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Abstract: N-methyl-D-aspartate receptor (NMDAR) is a subtype of ionotropic glutamate receptor, which play a central role in learning, memory, synaptic development and is linked to excitotoxic neuronal death. NMDARs are assembled from four transmembrane subunits that bind to glycine or D-serine (GluN1 and GluN3A/B) or which are activated by glutamate (GluN2A-D). The GluN2A and GluN2B subunits are highly expressed in cortex and hippocampus and are the primary determinants of the functional properties of the NMDA receptors, including features such as the agonist affinity, kinetics of deactivation, the single channel conductance and the permeability to Ca2+. Nowadays, many natural and synthetic molecules have been identified as NMDAR modulators, they bind to the receptor and cause an increase or decrease in the maximum response or in the agonist affinity. Since the discovery of antagonists for binding sites of the NMDA receptor, the pharmaceutical industry has developed them as tools to study their properties and develop therapeutically useful molecules for glutamate excitotoxicity, however, despite his need no drug has proven be effective and reliable even when annual incidence of brain diseases such as traumatic brain injury and/or stroke are about 10 million people worldwide and are projected to become the third leading cause of global disease in 2020, surpassing complex diseases like breast cancer, HIV, heart disease and chronic degenerative diseases. In adition to, recent reports have proposed to dapsone (DDS, 4.40-diaminodiphenylsulfone), an antimicrobial, as a neuroprotective agent for its effects to reduce the glutamate excitotoxicity (through NMDARs), the myeloperoxidase activity, oxidative damage, the inflammatory response, and apoptotic death. Also, decrease morphological damage in models of vascular occlusion and induced injury by kainic acid and quinolinic acid. Although it is known that dapsone acts on the NMDA receptor the effect that it produces and its mechanism of action are not clear yet. In this study we analyzed NMDA activated currents from pyramidal neurons of the primary motor cortex of Wistar strain male rats (30 days), obtained with voltage-clamp technique, we made a dose response of dapsone on NMDA receptor and finally we examine the binding subunit of DDS using specific antagonists for GluN2A (TCN-201) and GluN2B (Ifenprodil).

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Poster

207. Non-NMDA Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 207.01/A77

Topic: B.02. Ligand-Gated Ion Channels

Title: Regulation of AMPA receptor acetylation by SIRT2: implications in synaptic plasticity and learning

Authors: *G. WANG[1], S. LI[3], J. GILBERT[1], H. GRITTON[2], X. HAN[2], D. SELKOE[3], H.-Y. MAN[1];
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Abstract: Sirtuins, the NAD+-dependent protein deacetylases, are well-known for their involvement in longevity and metabolism. Recently, sirtuins have been shown to play an important role in cognitive functions like learning and memory. The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) is an important glutamatergic receptor that mediates the majority of fast excitatory synaptic transmission and synaptic plasticity in the brain, serving as the substrate for learning and memory. AMPARs are subject to several post-translational modifications, such as phosphorylation and ubiquitination, to maintain normal receptor function and expression in the brain. We therefore investigated the role of sirtuins on AMPAR regulation. We found that AMPARs are subject to protein acetylation, which is regulated by SIRT2, but not other members of the sirtuin family. Inhibition/deletion of SIRT2 up-regulates GluA1 acetylation, leading to an accumulation of AMPARs and an alteration in AMPAR trafficking. Consistently, in SIRT2 knock-out mice (Sirt2-/-), AMPAR acetylation and protein levels are increased. Acute hippocampal slice electrophysiology revealed impaired synaptic plasticity (LTP/LTD) in Sirt2-/- mice. Also, Sirt2-/- mice showed a significant impairment in contextual and spatial memory. Taken together, these data indicate a novel regulatory mechanism in synaptic plasticity and memory formation via SIRT2-mediated AMPAR deacetylation.

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Poster

207. Non-NMDA Receptors

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: B.02. Ligand-Gated Ion Channels

Support: Grants-in-Aid for Scientific Research No.24240048 and 24220007, from MEXT, Japan The CREST from the JST Agency, Japan

Title: Behavioral analysis of GluD1 deficient mice with the pure genetic background

Authors: *C. NAKAMOTO[1], R. NATSUME[1], E. NAKATSUKASA[1], M. ABE[1], M. WATANABE[2,3], K. SAKIMURA[1,3];
[1]Niigata Univ., Niigata City, Niigata Prefecture, Japan; [2]Hokkaido Univ., Sapporo, Japan; [3]CREST,JST, Chiyoda-ku, Tokyo, Japan

Abstract: The delta family consisting of glutamate receptor GluD1 and GluD2 has been classified as an ionotropic receptor subunit, but recent studies have revealed that GluD2 contributes to synapse formation occurring between parallel fibers and Purkinje cells in cerebellum. GluD2 is abundantly expressed in cerebellum, but moderately expressed in other brain regions, while GluD1 is widely observed in mouse brain. Although the functional significance of GluD1 is inferred from human genetic studies reporting that the *GRID1* is a strong candidate gene for schizophrenia, bipolar disorder, major depressive disorder, and autism spectrum disorder, the relationship between molecular function of GluD1 and the onset mechanism of these diseases has been still unknown. To approach the issue we generated GluD1 conditional knockout mice from C57BL/6N strain RENKA ES cells with pure genetic background for behavioral analysis, because conventionally used GluD1KO mice were produced by backcrossing 129-derived ES cells to C57BL/6 mice, thus affecting its genetic background. We first generated a GluD1 floxed mouse in which 34-bp *loxP* sequence and neo cassette flanked by two *frt* sites were inserted into the upstream of exon4. Another *loxP* sequence was inserted into the downstream of exon4. After removing a neo cassette, the floxed mice were crossed with telencephalin Cre driver mice to generate GluD1 null KO mice, and 8-12-week-old male mice were used for behavioral analyses. GluD1 deficient mice showed hyperactivity and low anxiety in the open field test, but no difference in the elevated plus maze. They were less mobile in the forced swim test and demonstrated less social behavior in the three-chamber test. Although some of our findings are similar to those of previous reports, some contradict with other results, which is supposedly caused by the different genetic background. To find responsible regions for the abnormal behavior, we are crossing the GluD1 floxed mice with driver mice that express Cre in different brain regions.

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Poster

207. Non-NMDA Receptors

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Topic: B.02. Ligand-Gated Ion Channels

Support: DFG individual grant (GE2519/3-1)

Jena Center for Sepsis Control and Care (CSCC)

Interdisciplinary Center for Clinical Research (IZKF) Jena

Title: Molecular pathophysiology of human anti-glutamate receptor 2 autoantibodies on AMPA-receptor mediated synaptic transmission

Authors: *C. GEIS, H. HASELMANN, B. GRÜNEWALD, C. WERNER;
Dept. of Neurol., Jena Univ. Hosp., Jena, Germany

Abstract: Background: Autoantibodies (AB) to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) were recently described in patients with autoimmune encephalitis. In this study we purified AB from plasma exchange material of a patient with autoimmune encephalitis to determine the molecular effects of the patient IgG fraction on the synaptic glutamatergic transmission. Methods and findings: Patient IgG was highly specific to the critical glutamate receptor 2 (GluR2) subunit of AMPAR as shown by using transfected HEK293 cells, brain slices of GluR1 or GluR2 deficient mice, and mass spectrometry. Primary hippocampal neurons preincubated with anti-GluR2-specific IgG showed a time-dependent reduction of GluR2-specific fluorescent puncta. We iontophoretically applied glutamate to individual synapses of cultured hippocampal neurons that were identified by intravital FM1-43-labelling and measured single-synapse evoked EPSCs by whole-cell patch-clamp recording. We found increased AMPAR-mediated EPSC amplitudes and disturbed receptor kinetics after preincubation with anti-GluR2-specific patient IgG. Furthermore, short-term plasticity was affected by reduced recovery after desensitization. Interpretation: These neurophysiological properties are consistent with previous findings of GluR2 deficient neurons. We suggest that anti-GluR2-specific patient IgG leads to a decrease of GluR2-containing receptors in active synapses (e.g. by internalization). Synaptic scaling mechanisms may generate a compensatory overexpression of other than GluR2 subunits resulting in synaptic AMPA-receptor insertion with higher conductivity and increased calcium-permeability. The present data show a possible mechanism of how anti-GluR2 specific IgG can directly affect synaptic transmission in the hippocampus possibly resulting in cognitive deficits and behavioral abnormalities in anti-AMPAR mediated autoimmune encephalitis.

Disclosures: C. Geis: None. **H. Haselmann:** None. **B. Grünewald:** None. **C. Werner:** None.

Poster

207. Non-NMDA Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 207.04/A80

Topic: B.02. Ligand-Gated Ion Channels

Title: Dissecting the role of AMPARs in development and function of hippocampal CGE-derived interneurons

Authors: *G. AKGUL[1], L. BARKSDALE[2], K. A. PELKEY[1], C. J. MCBAIN[1];
[1]Natl. Inst. of Hlth., Bethesda, MD; [2]Federtion of American Societies for Exptl. Biol., Bethesda, MD

Abstract: Disrupting the excitatory synaptic recruitment of GABAergic inhibitory interneurons can promote excitation-inhibition imbalance within cortical circuits that can precipitate neurological diseases such as schizophrenia and epilepsy. In the hippocampus, two major lineages of inhibitory interneurons, derived from caudal ganglionic eminence (CGE) and medial ganglionic eminence (MGE), contribute distinct subgroups, which give rise to microcircuit complexity. The specific biophysical properties of excitatory input onto distinct interneurons strongly correlate with cellular lineage and dictate the circuit recruitment of discrete forms of feedforward and feedback inhibition. CGE-derived interneurons primarily express calcium impermeable AMPARs. In contrast MGE-derived interneurons typically express GluA2-lacking calcium permeable AMPARs. To investigate the role of AMPAR-mediated glutamatergic input in the development of CGE-derived interneurons and its significance for brain activity, we created two knockout (KO) mouse lines that eliminate either the GluA2 subunit (A2-KO) or GluA1&A2&A3 subunits (A1/2/3-KO) selectively in CGE-derived interneurons. KO mice have been tested for AMPAR activity in CGE-derived interneurons and compared with the activity in wild type (WT) CGE-derived interneurons. A2-KO CGE-derived interneurons display inwardly rectifying AMPAR-mediated EPSCs with fast kinetics confirming successful targeted loss of GluA2 within this interneuron cohort. In A2-KO and A1/2/3-KO CGE-derived interneurons, spontaneous excitatory input was reduced more than 50% and 95% respectively, revealing a deficit in the circuit integration CGE-derived interneurons in both mutant lines. In addition, Scholl analysis on the dendritic tree of the recorded interneurons showed that the loss of all three AMPAR subunits in CGE-derived interneurons (A1/2/3-KO) leads to decreased dendritic branching. Early in cortical and hippocampal development, dendrite targeting CGE interneurons play a role in creating a patterned activity in nascent local circuits called giant depolarizing potentials (GDPs). GDPs are an essential step in the maturation of newly formed synaptic connections and circuits. In A1/2/3-KO's, we observed a significant increase in hippocampal GDP frequency inside the CA3 subfield at age P7-8 compared to WT, while the average peak amplitudes of GDP's did not change. In contrast GDPs monitored in A2-KO's showed comparable properties to WT's across all age ranges tested (P5-10). The data highlight a role for AMPARs on CGE interneurons for maturation and correct assembly of the newly formed circuits.

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Poster

207. Non-NMDA Receptors

Location: Hall A

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Topic: B.02. Ligand-Gated Ion Channels

Support: The Academy of Finland (137569)

Magnus Ehrnrooth Foundation

Title: Structure-function analysis of stargazin-dependent ampa receptor autoinactivation

Authors: *K. P. KEINANEN, T. MÖYKKYNEN, S. K. COLEMAN;
Dept Biosciences, Univ. of Helsinki, Helsinki, Finland

Abstract: Stargazin, a transmembrane AMPA receptor regulatory protein, increases glutamate responses of AMPA receptor channels by slowing down desensitization. This allosteric regulatory effect is short-lived due to functional uncoupling (autoinactivation) in the stargazin-receptor complex. The extent of autoinactivation is strongly dependent on receptor subunit but underlying mechanisms are poorly understood. Here, we have examined the role of C-terminal tail, Q/R editing and alternatively spliced flip/flop segment in stargazin-dependent autoinactivation in homomeric AMPA receptors. Wild-type and mutated receptors were expressed in 293 cells in the presence of stargazin and their agonist responses were studied by using patch clamp electrophysiology. Our results indicate that neither Q/R site editing in the channel pore, native in GluA2 or artificially introduced in GluA4, nor the length of the carboxyl terminal tail (in GluA2 and GluA4) have any significant effect on the strength of autoinactivation. In GluA2 receptors , residues within the flip/flop segment have a strong influence of the extent of autoinactivation. These data help delineate the critical structures responsible for the stability of stargazin-AMPA receptor modulatory interaction.

Disclosures: K.P. Keinanen: None. **T. Möykkynen:** None. **S.K. Coleman:** None.

Poster

207. Non-NMDA Receptors

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Title: Subtype-specific effects of auxiliary subunit GSG1L on AMPA receptor function

Authors: *T. P. MCGEE, C. BATS, M. FARRANT, S. G. CULL-CANDY;
UCL, London, United Kingdom

Abstract: AMPA-type glutamate receptors (AMPARs) mediate fast excitatory neurotransmission in the mammalian brain and are integral to a variety of processes, including

postsynaptic plasticity and synapse development. They also play a key role in triggering neuron damage in a number of neurological conditions. The AMPAR pore-forming subunits, GluA1-4, are expressed differentially with respect to brain region and ontogenetic period, providing considerable functional diversity. This diversity is increased by a variety of auxiliary transmembrane proteins. These coassemble with AMPAR subunits to form macromolecular complexes, the composition of which can influence receptor trafficking, gating and pharmacology. Proteomic studies recently identified a number of new AMPAR-interacting proteins, among which is the tetraspanin GSG1L. Structurally similar to transmembrane AMPA receptor regulatory proteins (TARPs), GSG1L has been validated as a bona fide AMPAR auxiliary protein capable of modulating receptor gating and cell surface expression. To investigate the potential influence of GSG1L at different synapses we have explored its functional effects in different AMPAR assemblies. Specifically, we recorded currents activated by fast application of glutamate to outside-out membrane patches from cells expressing various combinations of AMPAR pore-forming subunits and auxiliary proteins. To constrain receptor stoichiometry in these assemblies we generated AMPAR-GSG1L tandem constructs, in which the N-terminus of GSG1L was directly linked to the C-terminal tail of the GluA subunit. Using these methods we found GSG1L to have novel and subtype-specific effects on AMPARs.

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Poster

207. Non-NMDA Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 207.07/A83

Topic: B.02. Ligand-Gated Ion Channels

Title: Desensitized ligluk2 receptors are immobilized at glutamatergic synapses

Authors: *A. I. POLENGHI[1], E. M. PETRINI[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 short- and long-term modulation of synaptic responses. 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 exploited light-sensitive glutamate receptors (LiGluK2) in combination with the single particle tracking technique. LiGluK2 are engineered glutamate receptors 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 manipulating the different conformational states of LiGluK2 with light, we investigated the lateral mobility of LiGluK2 in cultured hippocampal neurons. We report that desensitized LiGluK2 receptors are strongly and reversibly immobilized at excitatory synapses. In contrast the desensitization state did not alter the lateral mobility of extrasynaptic LiGluK2, suggesting a selective mechanism for synaptic receptors. First, we aimed at assessing whether the light-induced immobilization of LiGluK2 lateral diffusion might be due to changes in intracellular Ca^{2+} induced by LiGluK2 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 LiGluK2 at synapses. Next, we hypothesized that the mechanism underlying this phenomenon could be due to altered interactions between scaffold proteins and synaptic receptors in the desensitized state. To this end, we generated a mutant form of LiGluK2 lacking of the PDZ binding motif. Interestingly, we found that the deletion of the PDZ binding domain completely abolished the desensitization-induced trapping of LiGluK2 at synapses. We propose here that the conformational changes occurring during desensitization may strengthen the interaction between receptors and scaffold proteins, thus promoting the trapping of LiGluK2 at synapses.

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Poster

207. Non-NMDA Receptors

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH 2R01NS065920

Title: Extending the influence of the cerebellar climbing fiber through non-synaptic signaling

Authors: *A. K. NIETZ[1], L. CODDINGTON[2], L. OVERSTREET-WADICHE[2], J. I. WADICHE[2];
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Abstract: Sensory information arrives at the cerebellar cortex through mossy fibers (MFs) originating from many areas of the cortex, brainstem, and spinal cord. In the granule cell layer, MFs synapse onto excitatory granule cells (GCs) and inhibitory Golgi cells (GoCs). GoCs provide feed-forward and feedback inhibition to GCs to regulate the initial stage of cerebellar processing of sensory input. Excitatory climbing fibers (CFs) that originate in the inferior olive make synaptic contacts with Purkinje cells (PCs). Recently, it has been shown that CFs also signal to molecular layer interneurons through a form of glutamatergic transmission mediated

entirely by neurotransmitter spillover in the absence of anatomically defined synaptic connections [1-3]. Here, we show that CF-mediated spillover transmission also influences GoC activity supporting functional connectivity despite the lack of reported anatomical connections between CFs and GoCs [4,5]. We recorded electrically-stimulated or channelrhodopsin-driven climbing fiber (CF)-mediated currents in GoCs from acute mouse slices. CF-GoC EPSCs showed marked depression to paired-pulse stimulation ($EPSC_2/EPSC_1$ 0.28 ± 0.03, n = 18) in contrast with facilitation of MF/PF evoked synaptic responses (1.27 ± 0.10, n = 20). CF-EPSCs were slower to rise (1.31 ± 0.15 ms; n = 20) than MF/PF-EPSCs (0.61 ± 0.06 ms; n = 21) and sensitive to glutamate transporter inhibition (241.4 ± 36.3%, n = 18). CF stimulation increased the probability of spiking (2.0 ± 0.2 action potentials, n = 12) and generated a pause of spontaneous spiking that increased the next inter-spike interval (67.1 ± 22.9%, n = 7). Our ongoing experiments are aimed at understanding the mechanisms underlying these observations that reveal an extended influence of CFs over the cerebellar cortex beyond signaling to Purkinje cells and molecular layer interneurons. [1] Szapiro and Barbour *Nat Neuro* 10: 735 - 742, (2007). [2] Mathews et al. *J Neurosci* 32: 17988-17997, (2012). [3] Coddington et al. *Neuron* 78: 1050-1062, (2013). [4] Galliano et al. *Frontiers in Neural Circ* 7: 1-9, (2013). [5] Schulman and Bloom *Brain Res* 210: 350-355, (1981).

Disclosures: **A.K. Nietz:** None. **L. Coddington:** None. **L. Overstreet-Wadiche:** None. **J.I. Wadiche:** None.

Poster

207. Non-NMDA Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 207.09/A85

Topic: B.02. Ligand-Gated Ion Channels

Support: Wellcome Trust grant 086185/Z/08/Z (to SGC-C and MF)

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AHA 11GRNT7890004 (to VJ)

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Title: TARPs affect the gating of AMPARs by sub-saturating glutamate concentrations

Authors: *I. D. COOMBS[1], D. M. MACLEAN[2], V. JAYARAMAN[2], M. FARRANT[1], S. G. CULL-CANDY[1];

[1]UCL, London, United Kingdom; [2]Univ. of Texas Hlth. Sci. Ctr., Biochem. and Mol. Biol., Houston, TX

Abstract: AMPARs are tetrameric glutamate-gated ion channels that mediate fast excitatory synaptic transmission. Functional and proteomic studies have revealed native AMPARs can interact with numerous classes of ancillary proteins, including the transmembrane AMPAR regulatory proteins (TARPs). TARPs are crucial for the correct trafficking of AMPARs, and also affect their functional properties. Notably, TARPs increase glutamate potency and slow receptor deactivation and desensitization, thereby directly shaping phasic synaptic transmission (1). Both synaptic and extrasynaptic AMPARs can also be exposed to low glutamate concentrations following synaptic spillover (2,3). We set out to investigate how TARPs affect AMPAR channel activation and desensitization at low agonist concentrations by recording glutamate-evoked currents from outside-out patches taken from HEK293 cells transfected with GluA1 or GluA1 + TARP γ-2. As expected, γ-2 increased the potency of glutamate (measured from either peak- or steady-state currents) by ~3-fold. This TARP-induced increase in glutamate potency was due, at least in part, to increased efficacy at low occupancy, as directly revealed by both macroscopic and single-channel analysis. The γ-2-induced increase in efficacy was also apparent with heteromeric GluA1/2 receptors. In contrast with the enhancement of channel activation, we found that TARPs reduced by ~8-fold the potency of glutamate to desensitize GluA1. This was also apparent from decreased rates of entry into desensitized states at a range of sub-saturating glutamate concentrations. Taken together, our data suggest that under conditions of low receptor occupancy the presence of TARPs greatly alters the balance between activation and desensitization. These findings suggest that TARPs may profoundly influence the response of AMPARs to glutamate spillover. 1)Tomita et al. Nature 435, 1052-8 (2005). 2)DiGregorio et al. Neuron 32, 521-33 (2002). 3)DiGregorio et al. J.Neurosci. 27, 8344-57 (2007).

Disclosures: I.D. Coombs: None. **D.M. MacLean:** None. **V. Jayaraman:** None. **M. Farrant:** None. **S.G. Cull-Candy:** None.

Poster

207. Non-NMDA Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 207.10/A86

Topic: B.02. Ligand-Gated Ion Channels

Title: Lk6 regulates synaptic levels of the glutamate receptor subunit, GluRIIA, at the *Drosophila* neuromuscular junction

Authors: *F. L. WYGLE-LIEBL, T. DELANEY, N. HUSSEIN;
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Abstract: Synaptic communication depends on the spatially correct formation of presynaptic terminals and the localization of postsynaptic receptors. The development and assembly of glutamatergic synapses is of particular importance because the majority of excitatory transmission in the mammalian central nervous system occurs via ionotropic glutamate receptors. We found, through a forward genetic screen, that the translational regulator, Lk6, is important for the levels of GluRIIA-containing receptors at the *Drosophila* neuromuscular junction. Lk6 is the *Drosophila* homolog of MNK1/2, which is downstream of the mitogen activated protein kinase (MAPK) pathway. Our data suggests that Lk6 specifically regulates GluRIIA as mutations in lk6 do not affect the synaptic levels of other proteins including Brp and DLG and the glutamate receptor subunits GluRIIB and GluRIIC. Lk6 is known to regulate translation by influencing eIF4E activity. Knockdown of eIF4E in postsynaptic cells results in a reduction in the synaptic levels of GluRIIA similar to that of the lk6 mutant. Further, overexpression of eIF4ES251A, a nonphosphorylatable eIF4E, results in a reduction in synaptic GluRIIA. Our results indicate that Lk6 and Target of rapamycin (TOR) signaling regulate eIF4E activity in parallel thereby influencing synaptic levels of GluRIIA.

Disclosures: **F.L. Wygle-Liebl:** None. **T. Delaney:** None. **N. Hussein:** None.

Poster

207. Non-NMDA Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 207.11/A87

Topic: B.02. Ligand-Gated Ion Channels

Support: NINDS, NIH Intramural Research Program

Title: The role of GSG1L in the regulation of AMPA receptor-mediated synaptic transmission

Authors: X. GU[1], X. MAO[1], M. LUSSIER[1], M. HUTCHISON[1], L. ZHOU[1], K. HAMRA[2], K. W. ROCHE[1], *W. LU[1];
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Abstract: Trafficking and function of AMPA receptors (AMPARs) are regulated by a number of auxiliary subunits, including TARPs and CNIH2/3, which are important for AMPAR forward trafficking to synapses. Recently, a number of new membrane proteins have been found in neuronal AMPAR complexes. However, their function in the regulation of synaptic transmission remains largely unclear. We have investigated the role of the membrane protein GSG1L and found that it is important for AMPAR targeting to the neuronal surface and synapses. In addition, we have determined the GSG1L domains important for its function. Interestingly, GSG1L also uniquely modulates AMPAR deactivation and desensitization kinetics in hippocampal neurons. Finally, we also examined the role of GSG1L in the regulation of AMPAR synaptic transmission

in GSG1L knockouts. These data suggest that GSG1L represents a new class of auxiliary subunit with distinct modulatory properties for AMPARs.

Disclosures: **X. Gu:** None. **X. Mao:** None. **M. Lussier:** None. **M. Hutchison:** None. **L. Zhou:** None. **K. Hamra:** None. **K.W. Roche:** None. **W. Lu:** None.

Poster

207. Non-NMDA Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 207.12/A88

Topic: B.02. Ligand-Gated Ion Channels

Title: Molecular determinants underlying heterogeneity in AMPAR content across CA1 Schaffer collateral synapses

Authors: ***M. YAMASAKI**[1], M. FUKAYA[2], M. YAMAZAKI[3], M. ABE[3], K. SAKIMURA[3], M. WATANABE[1];
[1]Hokkaido Univ., Sapporo, Japan; [2]Kitasato Univ., Sagamihara, Japan; [3]Niigata Univ., Niigata, Japan

Abstract: Retaining AMPA-type glutamate receptors (AMPARs) at synapses requires specific interaction with postsynaptic density (PSD)-95 via transmembrane AMPAR regulatory proteins (TARPs). AMPAR content varies depending on synapse-type; however, the underlying molecular mechanisms remain unclear. By quantitative immunogold analysis, we compared three Schaffer collateral synapses in hippocampal CA1: non-perforated and perforated synapses on pyramidal cell spines, and synapses on parvalbumin-positive dendrites (PV-synapses). Although PSD-95 labeling was similar across three types of synapses, AMPAR labeling was increased in PV- and perforated synapses. While TARP γ8 labeling was homogeneous across three types of synapses, γ2 labeling was limited to PV- and perforated synapses. In γ2 knockout mice, AMPAR labeling in PV- and perforated synapses was markedly decreased, effectively eliminating the heterogeneity in AMPAR content. This raises the possibility that γ2 is more potent in accommodating synaptic AMPARs than γ8. Taken together, our results indicate that differential composition of TARPs may underlie synapse-type specific regulation of AMPAR content among CA1 Schaffer collateral synapses.

Disclosures: **M. Yamasaki:** A. Employment/Salary (full or part-time):; full. **M. Fukaya:** None. **M. Yamazaki:** None. **M. Abe:** None. **K. Sakimura:** None. **M. Watanabe:** None.

Poster

207. Non-NMDA Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 207.13/A89

Topic: B.02. Ligand-Gated Ion Channels

Support: American Heart Association Founders Affiliate Fellowship

Title: Modulation of AMPA receptor tetramerization by auxiliary subunits

Authors: *Q. GAN, L. P. WOLLMUTH;
Neurobio. and Behavior, Stony Brook Univ., Stony Brook, NY

Abstract: Ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels that play critical roles in excitatory neurotransmission. Functional iGluRs are tetramers composed of four identical or similar core subunits and are usually associated with transmembrane proteins known as auxiliary subunits. Transmembrane AMPA receptor regulatory proteins (TARPs) and cornichons (CNIHs) are the major auxiliary subunits for AMPA-type iGluRs. They regulate the trafficking, localization as well as the functional properties of AMPA receptors, thereby modulating the strength and kinetics of AMPAR-mediated synaptic transmission. However, the roles played by auxiliary subunits in the earlier stages of the AMPAR biosynthetic pathway are yet unclear. The present study focuses on the effects of CNIH-2/3 and TARP γ-2 on the tetrameric assembly of GluA1 and GluA2 homo-tetramers. We used blue-native PAGE (BN-PAGE) to assess the oligomeric state of AMPARs either expressed alone or co-expressed with auxiliary subunits. Previously we identified a face in the third transmembrane segment (M4) of AMPARs that is critical to the tetramerization process. Here we find that co-expression with CNIH-2 completely rescues the deficit in tetramerization caused by moderate mutations within this face of M4. Co-expression with CNIH-3 has a similar albeit slightly weaker effect. However, cornichons are unable to counteract the abolishment of tetramerization caused by more radical mutations. Compared to cornichons, TARP γ-2 does not significantly enhance the mutants' tetramerization, regardless of the types of mutations introduced. Furthermore, we found that deletion of the intracellular carboxy-terminal domain (CTD) disrupts tetramerization, an effect that is also rescued by cornichons. Our results suggest that cornichons, but not TARP γ-2, act as chaperones that enhance the ability of AMPARs to form tetramers. This is consistent with previous findings that cornichons associate with AMPARs in ER/cis-Golgi, where tetrameric assembly takes place. Understanding how cornichons modulate AMPAR tetramerization may help us gain better insight into the biogenesis of AMPARs and its regulation by other neuronal proteins.

Disclosures: Q. Gan: None. **L.P. Wollmuth:** None.

Poster

207. Non-NMDA Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 207.14/A90

Topic: B.02. Ligand-Gated Ion Channels

Title: Anticonvulsant and improved side-effect potential of ly3130481: the first γ−8-selective transmembrane ampa receptor regulatory protein (tarp) antagonist

Authors: *J. M. WITKIN[1], A. KATO[2], S. GLEASON[2], D. GERNERT[2], P. ORNSTEIN[2], W. PORTER[2], J. REEL[2], K. GARDINIER[2];
[1]Lilly Corp Ctr., Indianapolis, IN; [2]Lilly Res. Labs, Indianapolis, IN

Abstract: AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors are a primary protein involved in epileptogenic phenomenon and the AMPA receptor antagonist, perampanel (®Fycompa), was recently approved in Europe and the United States for the treatment of partial seizures. Transmembrane receptor regulatory protein (TARP) γ-8 is an auxiliary protein associated with some AMPA receptors and has high brain localization in the hippocampus. We hypothesized that we could maintain the antiepileptic functionality while eliminating the motoric side effects inherent in globally-acting (non-TARP-dependent) AMPA receptor antagonists (i.e., perampanel) with a small molecule selectively targeting forebrain AMPA receptors. Specifically, AMPA receptors associated with TARP γ-8 (high localization in hippocampus associated with seizures) while simultaneously avoiding pharmacological interaction with TARP γ-2 (high localization in cerebellum associated with dizziness and motor coordination). We discovered LY3130481 (6-[(1S)-1-[1-[5-(2-hydroxyethoxy)-2-pyridyl]pyrazol-3-yl]ethyl]-3H-1,3-benzothiazol-2-one)), an orally-bioavailable compound and the first molecule rationally designed to target selective regions of the central nervous system while providing a relative sparing of interactions with non-desired brain regions. LY3130481 produced broad-ranging anticonvulsant efficacy in rodents including in models utilizing both acute and subchronic convulsant stimulation and either chemical or electrical stimuli. As AMPA receptors are involved in sensitization within the central nervous system, the blockade of seizure sensitization (kindling) was also demonstrated, thereby revealing a potential for disease modification of epilepsy. Moreover, the relative sparing of cerebellar Perkinje neurons from blockade of AMPA currents translated to a marked reduction in deleterious effects on motor function. Given these findings, the door has opened to the utilization of the TARP scaffolding proteins as a means of designing brain region-specific interactions along with the large therapeutic potential it offers.

Disclosures: **J.M. Witkin:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly and Company. **A. Kato:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **S. Gleason:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. **D. Gernert:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **P. Ornstein:** None. **W. Porter:** A. Employment/Salary (full or part-

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Poster

207. Non-NMDA Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 207.15/A91

Topic: B.02. Ligand-Gated Ion Channels

Title: An assay system for the detection of transmembrane AMPA receptor regulatory protein (TARP) γ-8-selective antagonists: *In vitro* characterization of LY3130481

Authors: C. DING, B. HEINZ, A. S. KATO, H. YU, K. M. GARDINIER, D. L. GERNERT, D. S. BREDT, J. M. WITKIN, *K. D. BURRIS;
Lilly Res. Labs., Indianapolis, IN

Abstract: Alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptors, a subtype of ionotropic glutamate receptors, mediate fast excitatory amino acid transmission in the mammalian central nervous system. AMPA receptors are hetero- or homomeric tetramers constructed from iGluA1-4 subunits, each of which can be alternatively spliced to yield two isoforms (flip and flop). Transmembrane AMPA receptor regulatory proteins (TARPs) are auxiliary subunits that associate with AMPA receptors and modulate both trafficking and channel activity. TARPs consist of six isoforms (stargazin/ γ-2, γ-3, γ-4, γ-5, γ-7 and γ-8) which show distinct expression patterns in the brain. These properties make specific AMPA-TARP receptors promising targets for treating CNS diseases. The goal of these studies was to develop high-throughput assays to selectively screen and profile TARP subtype-selective AMPA receptor antagonists. The ability of compounds to block glutamate-stimulated increases in intracellular Ca^{++} was examined in CHO-S cells expressing iGluA1flip alone (TARP-dependence) or iGluA1flop plus different TARP subtypes (TARP selectivity) using fluorescence imaging plate reader (FLIPR)-based assays. GYKI-53784, a nonselective AMPA receptor antagonist, blocked glutamate-stimulated responses in cells expressing iGluA1flip or iGluA1flop co-expressed with either TARPγ-2 or TARPγ-8. In contrast, LY3130481 blocked responses to glutamate in cells co-expressing iGluA1flop and TARPγ-8, but had no effect in cells expressing iGluA1flop with TARPγ-2 or in cells expressing iGluA1flip alone. LY3130481 represents the first molecule targeting specific populations of AMPA receptors in the brain. The development of assay systems to screen for TARP subtype-selective AMPA receptor modulators and the identification of LY3130481 as a TARPγ-8-selective AMPA receptor antagonist provide a new opportunity for selectively regulating AMPA receptor activity for therapeutic benefit. Given the relative densities of TARPγ-8 hippocampus vs. cerebellum (γ-2 dominant), it was predicted that

LY3130481 would have efficacy in hippocampal neural circuits (e.g., antiepileptic) without major effect in cerebellum (e.g., dizziness, ataxia).

Disclosures: C. Ding: A. Employment/Salary (full or part-time):; Eli Lilly and Co. **B. Heinz:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **A.S. Kato:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **H. Yu:** Other; Former Employment at Eli Lilly and Co. **K.M. Gardinier:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **D.L. Gernert:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **D.S. Bredt:** Other; Former employment at Eli Lilly and Co. **J.M. Witkin:** A. Employment/Salary (full or part-time):; Eli Lilly and Co. **K.D. Burris:** A. Employment/Salary (full or part-time):; Eli Lilly and Co..

Poster

207. Non-NMDA Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 207.16/A92

Topic: B.02. Ligand-Gated Ion Channels

Title: Discovery of a selective TARP-g8 dependent AMPA receptor antagonist (TDAA)

Authors: *D. GERNERT, K. M. GARDINIER, J. WITKIN, W. PORTER, J. REEL, K. BURRIS, C. DING, V. BARTH, F. STEVENS, P. HAHN, P. SPINAZZE, S. HOLLINSHEAD, D. MAYHUGH, J. SCHKERYANTZ, A. KHILEVICH, D. BREDT, H. YU, P. ORNSTEIN; Discovery Chem., Eli Lilly, Indianapolis, IN

Abstract: Modulation of AMPA receptors has long been proposed as a method to treat various neurological disease states, however antagonists of AMPA receptors has, to date, suffered from motor impairment at efficacious doses. The discovery that Transmembrane AMPA Receptor Regulatory Proteins (TARPs), which are auxiliary subunits of AMPA receptors that modulate pharmacology and trafficking of AMPA receptors, show differential expression levels in the central compartment led us to hypothesize that by targeting specific AMPA-TARP complexes that we could selectively target forebrain and not hindbrain excitatory synaptic transmission. We present here our discovery of the first known antagonist molecules which are both selective for, and dependent on the AMPA-TARP-γ8 complex.

Disclosures: D. Gernert: A. Employment/Salary (full or part-time):; Eli Lilly & Co. **K.M. Gardinier:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **J. Witkin:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **W. Porter:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **J. Reel:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **K. Burris:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **C. Ding:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **V. Barth:** A. Employment/Salary (full or

part-time):; Eli Lilly & Co. **F. Stevens:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **P. Hahn:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **P. Spinazze:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **S. Hollinshead:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **D. Mayhugh:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **J. Schkeryantz:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **A. Khilevich:** A. Employment/Salary (full or part-time):; Eli Lilly & Co. **D. Bredt:** A. Employment/Salary (full or part-time):; Janssen Pharmaceutical. **H. Yu:** A. Employment/Salary (full or part-time):; Janssen Pharmaceutical. **P. Ornstein:** A. Employment/Salary (full or part-time):; Roosevelt Univerity.

Poster

207. Non-NMDA Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 207.17/A93

Topic: B.02. Ligand-Gated Ion Channels

Title: Discovery of a forebrain-specific AMPA receptor antagonist: electrophysiological characterization of a TARP gamma-8-dependent AMPA receptor antagonist

Authors: *A. KATO[1], C. DING[1], K. M. GARDINIER[1], D. L. GERNERT[1], H. YU[1], R. ZWART[2], H. WANG[1], Y. QIAN[1], F. PASQUI[2], E. SHER[2], K. BURRIS[1], J. T. R. ISAAC[2], J. M. WITKIN[1], D. S. BREDT[1], E. S. NISENBAUM[1];
[1]Eli Lilly & Co., Lilly Res. Lab., Indianapolis, IN; [2]Eli Lilly & Co., Lilly Res. Lab., Windlesham, United Kingdom

Abstract: AMPA receptors are pivotal glutamate-operated cation channels for fast excitatory synaptic transmission in mammalian brain. AMPA receptors function *in vivo* in concert with various types of auxiliary subunits, including TARPs and cornichons (CNIH-2/3). TARPs associate with most of native AMPA receptors if not all, and modulate their functions dramatically in various aspects, e.g. trafficking, gating and pharmacology. TARPs are a multi-gene family protein group with differential expression in brain areas. TARP gamma-8 is expressed in forebrain with especially high enrichment in hippocampus, but not in cerebellum. The regional specificity of TARP expression and the marked changes of AMPA receptor pharmacology by TARPs inspired us to screen compounds that specifically block gamma-8-containing AMPA receptors with the hypothesis of retaining AMPA receptor blocking efficacy while minimizing cerebellar-driven ataxia. Indeed, we successfully identified a gamma-8 specific AMPA receptor antagonist, LY3130481, which selectively antagonizes gamma-8 containing AMPA receptors but not the other TARP, using FLIPR (a high-throughput intracellular-calcium detection system) and electrophysiological methods for recombinants. This compound has no selectivity in AMPA receptor principal subunits, i.e. it blocks GluA1, GluA2, GluA3 or GluA4,

if gamma-8 is co-expressed. The compound potently blocks glutamate-evoked currents through native gamma-8-containing AMPA receptors in forebrain, but not gamam-8-lacking cerebellar Purkinje neurons. Field EPSP recordings revealed that LY3130481 potently but partially blocks hippocampal synaptic transmissions. The inhibitory effects of LY3130481 for native AMPA receptors are abolished in TARP gamma-8 -null mice. LY3130481 is the first brain-region specific AMPA receptor antagonist.

Disclosures: **A. Kato:** A. Employment/Salary (full or part-time):; Eli Lilly and Company (full). 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.; Eli Lilly and Company. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly and Company. **C. Ding:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. 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.; Eli Lilly and Company. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly and Comapny. **K.M. Gardinier:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. 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.; Eli Lilly and Company. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly and Company. **D.L. Gernert:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. 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.; Eli Lilly and Company. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly and Company. **H. Yu:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. 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.; Eli Lilly and Company. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly and Company. **R. Zwart:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. 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.; Eli Lilly and Company. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly and Company. **H. Wang:** A. Employment/Salary (full or part-time):; Eli Lilly and Company. 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.; Eli Lilly and

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drug study, report that research relationship even if those funds come to an institution.; Eli Lilly and Company. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly and Company.

Poster

207. Non-NMDA Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 207.18/A94

Topic: B.02. Ligand-Gated Ion Channels

Title: Tarp gamma-8 dependent ampa receptor antagonists as a novel therapeutic approach for chronic pain

Authors: K. L. KNOPP, R. M. A. SIMMONS, W. GUO, B. L. ADAMS, J. M. WITKIN, K. M. GARDINIER, D. L. GERNERT, W. PORTER, P. ORNSTEIN, J. REEL, C. DING, H. WANG, Y. QIAN, K. BURRIS, A. NEED, V. BARTH, F. PASQUI, R. ZWART, E. SHER, K.-C. CHOONG, A. S. KATO, T. M. WALL, *E. S. NISENBAUM;
Neurosci. Discovery Res., Eli Lilly & Co., Indianapolis, IN

Abstract: Non-selective ionotropic glutamate receptor antagonists are efficacious in chronic pain conditions, but are accompanied by significant tolerability issues, likely arising in part from the ubiquitous expression of AMPA receptors in the CNS. Recently, a glutamate receptor antagonist, LY3130481, has been identified which selectively blocks TARP γ8-containing AMPA receptors. Expression studies show that γ8 is heterogeneously distributed in the CNS with highest expression in hippocampus, but significant expression also in spinal cord, anterior cingulate (ACC) and somatosensory (SS) corticies. In addition to having potential therapeutic utility in epilepsy (see Witkin et al., SfN., 2015), these data have suggested that LY3130481 may provide a novel approach to chronic pain conditions, providing the focus of the current studies. Potent and selective blockade of recombinant γ8-containing AMPA receptors (IC50=62 nM) by LY3130481 was also evident in rodent ACC (IC50=73 nM) and SS (IC50=142 nM) pyramidal neuron and human spinal neuron (IC50=480 nM) AMPA receptors, but not rodent Purkinje or human cerebellar neuron AMPA receptors (IC50>10 uM) which do not express γ8. LY3130481 (0.3-3 uM) partially suppressed, whereas the non-selective AMPA antagonist GYKI53784 (50 uM) completely blocked glutamatergic EPSPs in ACC neurons. LY3130481 (1-10 mg/kg i.v.) similarly attenuated discharge of wide-dynamic range spinal sensory neurons *in vivo* in response to trains of stimulation of peripheral nerve afferents. Consistent with an effect on neurotransmission in ascending pain pathways, LY3130481 significantly reduced nocifensive behavior in the formalin assay (ED50=3.7 mg/kg, p.o.) that was correlated with occupancy of CNS γ8-containing AMPA receptors, but was not effective in γ8-/- mice. LY3130481 also dose-dependently (1-30 mg/kg, p.o) attenuated gait disturbances in a model of established joint pain in

the absence of motor side effects. Collectively, these data demonstrate that LY3130481 can selectively reduce excitatory transmission in pain pathways expressing γ8-containing AMPA receptors and significantly reduce nocifensive behaviors in multiple preclinical models of pain in the absence of motor impairment. These results suggests that selective blockade of TARP γ8-containing AMPA receptors may provide an effective and safer approach for the treatment of chronic pain conditions.

Disclosures: **K.L. Knopp:** A. Employment/Salary (full or part-time):; Eli Lilly. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly. **R.M.A. Simmons:** A. Employment/Salary (full or part-time):; Eli Lilly. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly. **W. Guo:** A. Employment/Salary (full or part-time):; Eli Lilly. **B.L. Adams:** A. Employment/Salary (full or part-time):; Eli Lilly. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly. **J.M. Witkin:** A. Employment/Salary (full or part-time):; Eli Lilly. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly. **K.M. Gardinier:** A. Employment/Salary (full or part-time):; Eli Lilly. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly. **D.L. Gernert:** A. Employment/Salary (full or part-time):; Eli Lilly. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly. **W. Porter:** A. Employment/Salary (full or part-time):; Eli Lilly. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly. **P. Ornstein:** A. Employment/Salary (full or part-time):; Eli Lilly. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly. **J. Reel:** A. Employment/Salary (full or part-time):; Eli Lilly. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly. **C. Ding:** A. Employment/Salary (full or part-time):; Eli Lilly. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly. **H. Wang:** A. Employment/Salary (full or part-time):; Eli Lilly. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly. **Y. Qian:** A. Employment/Salary (full or part-time):; Eli Lilly. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly. **K. Burris:** A. Employment/Salary (full or part-time):; Eli Lilly. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly. **A. Need:** A. Employment/Salary (full or part-time):; Eli Lilly. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly. **V. Barth:** A. Employment/Salary (full or part-time):; Eli Lilly. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly. **F. Pasqui:** A. Employment/Salary (full or part-time):; Eli Lilly. E. Ownership Interest (stock, stock options,

royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly. **R. Zwart:** A. Employment/Salary (full or part-time):; Eli Lilly. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly. **E. Sher:** A. Employment/Salary (full or part-time):; Eli Lilly. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly. **K. Choong:** A. Employment/Salary (full or part-time):; Eli Lilly. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly. **A.S. Kato:** A. Employment/Salary (full or part-time):; Eli Lilly. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly. **T.M. Wall:** A. Employment/Salary (full or part-time):; Eli Lilly. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly. **E.S. Nisenbaum:** A. Employment/Salary (full or part-time):; Eli Lilly. 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.; Eli Lilly. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly.

Poster

207. Non-NMDA Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 207.19/A95

Topic: B.02. Ligand-Gated Ion Channels

Support: 1R15GM114807

Title: Sap97 and cask block the rxr er retention signal of grik5

Authors: *S. H. STANDLEY, X. HONG, M. RONILO, M. AVETISYAN;
Grad. Col. of Biomed. Sci., Western Univ. of Hlth. Sci., Pomona, CA

Abstract: In several prior publications, it has been demonstrated that Grik5 has an arginine-based RXR endoplasmic reticulum (ER) retention signal in its cytoplasmic C-terminus. The classical function of these trafficking signals is to impose a requirement for assembly with other cognate subunits, in this case Grik2, before exiting the ER. However, assembly has been reported to have little effect on ER exit or surface expression. Here, we show that SAP97 and CASK block the RXR endoplasmic reticulum (ER) retention signal in Grik5. Heterologous cells co-transfected with Grik2/Grik5 were largely ER-retained as has been previously described. Co-transfection of SAP97 alone had little effect on ER exit or cell surface expression of Grik2/Grik5, however when co-transfected with CASK, ER exit was dramatically enhanced.

Further experiments using truncations and fragments of SAP97 indicated that the SH3 and GuK domains of SAP97 were critical for blocking the ER retention signal of Grik5. SAP97 and CASK have previously been shown to be necessary for sorting of the NMDA receptor, GluN1-1, into a local non-conventional secretory pathway. GluN1-1 also has an RXR ER retention signal in its C-terminus that does not appear to function classically. We propose that these ER retention signals are imposing a requirement for binding SAP97/CASK and thereby sorting into a local, non-conventional secretory pathway.

Disclosures: **S.H. Standley:** None. **X. Hong:** None. **M. Ronilo:** None. **M. Avetisyan:** None.

Poster

207. Non-NMDA Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 207.20/A96

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant R01NS080598

Title: N-linked glycans modulate AMPA and kainate receptor functional properties

Authors: ***C. VERNON**, J.-Z. YAN, B. A. COPITS, Y. F. GUZMÁN, G. T. SWANSON; Dept. of Pharmacol., Northwestern Univ., Chicago, IL

Abstract: Diverse types of post-translational modification are known to affect the function of ionotropic glutamate receptors (iGluRs), for example by altering receptor affinity for interacting proteins or by controlling receptor localization and stability at the cell membrane. *N*-linked glycosylation is one such modification and iGluRs, including the AMPA and kainate receptor (KAR) subfamilies, are glycosylated at multiple sites. Glycosylation is known to be important for proper iGluR folding and exit from the secretory pathway and is more generally important for proper nervous system development and function, but its impact on the functional properties of signaling proteins like ligand- and voltage-gated ion channels is not well defined. We hypothesize that *N*-linked glycans on iGluRs contribute not only to receptor folding but also to biophysical functions of these receptors. To test this, we pharmacologically restricted glycan maturation on recombinant AMPARs and KARs by inhibiting α-mannosidases. We find that α-mannosidase inhibition alters receptor biophysical properties such as the rate of desensitization, recovery from desensitization, and apparent glutamate affinity. Effects on receptor function vary dependent upon both the processing enzyme targeted and the receptor subunit composition. When α-mannosidase-I is inhibited, GluA1/2-containing AMPAR desensitization slows from 10.4±0.6 ms (n=27) to 21.6±3.4 ms (n=22) but by contrast, GluK2-containing KAR desensitization speeds from 4.0±0.2 ms (n=27) to 2.9±0.1 ms (n=26). We also tested whether enriching for complex glycans impacted receptor biophysical properties. The charged glyco-

epitope HNK-1 altered receptor biophysical properties for a subset of receptors including GluK2-containing KARs, which we find to be a substrate for HNK-1 conjugation in the mouse CNS. We are investigating how glycan content impacts native receptor properties, as our findings suggest that regional variation in oligosaccharide processing could result in differential synaptic function of iGluRs. The work presented here supports the idea that the molecular composition of *N*-glycans contributes to diversity in AMPAR and KAR functional properties, increasing receptor complexity beyond that provided by different subunit combinations. Glycan processing is dynamically regulated throughout development and diseases altering glycosylation enzymes are known to severely impact CNS development and function. Greater understanding of iGluR modulation by *N*-glycans is essential to fully appreciate receptor function during normal development as well as in disease states. Supported by NIH R01NS080598 to G.T.S.

Disclosures: **C. Vernon:** None. **J. Yan:** None. **B.A. Copits:** None. **Y.F. Guzmán:** None. **G.T. Swanson:** None.

Poster

207. Non-NMDA Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 207.21/A97

Topic: B.02. Ligand-Gated Ion Channels

Support: NIMH F31 MH099807

NIMH R01 MH099114

Title: Kainate receptors mediate endocannabinoid mobilization in the striatum

Authors: *J. MARSHALL, A. CONTRACTOR;
Physiol., Northwestern Univ. - Dept. Of Physiol., Chicago, IL

Abstract: The striatum is the main input structure of the basal ganglia, integrating information from the thalamus and cortex to control planning and modulation of movement. The principal neurons of the striatum, spiny projection neurons (SPNs), express high levels of kainate receptors, members of the ionotropic glutamate receptor family, whose functional roles within SPNs have not been well-characterized. Kainate receptors play diverse modulatory roles at synapses in many different brain regions where they are expressed. In addition to contributing to excitatory post-synaptic currents (EPSCs) they have been shown to presynaptically regulate neurotransmitter release and in some cases are metabotropically coupled to intracellular signaling pathways—activating downstream signaling independent of ionotropic currents. Here we demonstrate that kainate receptors are present at postsynaptic sites at corticostriatal synapses onto SPNs where they are activated by endogenously released glutamate. Direct activation of kainate receptors with low concentrations of exogenously applied agonist, that produces little or

no inward current, reduces glutamate release at corticostriatal excitatory synapses onto SNPs. In a subset of recordings, the suppression was blocked by a cannabinoid type 1 (CB1R) antagonist, indicating that activating kainate receptors leads to endocannabinoid (eCB) mobilization. Furthermore, this effect of low-agonist activation of kainate receptors is sensitive to a number of postsynaptic pharmacological manipulations that suggest kainate receptors mobilize eCBs through a metabotropic signaling pathway that is independent of ion flux through the channel pore. Cannabinoid signaling itself has a well-established role in the striatum playing a crucial role in different forms of striatal plasticity. Therefore these results suggest a novel and significant role for kainate receptors in tuning striatal synapses and regulating striatal activity.

Disclosures: J. Marshall: None. **A. Contractor:** None.

Poster

207. Non-NMDA Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 207.22/A98

Topic: B.02. Ligand-Gated Ion Channels

Support: NINDS R01 NS071952

Diversity Supplement R01 NS080598-S1

Title: Identification of novel Neto2-interacting proteins in the central nervous system

Authors: *Y. F. GUZMAN[1], J. N. SAVAS[2], G. T. SWANSON[1];
[1]Northwestern Univ. Dept. of Pharmacol., Chicago, IL; [2]Dept. of Neurol., Northwestern Univ., Chicago, IL

Abstract: Kainate receptors, like other ionotropic glutamate receptors in the central nervous system, exist as part of macromolecular signaling complexes. These complexes are composed of scaffolds, enzymes, and auxiliary proteins that directly impact receptor function and localization. Neuropilin and tolloid-like protein 2 (Neto2) is thought to be an important member of the signaling complex for kainate receptors. Interaction of kainate receptors with Neto2 increases the open probability of the receptor and slows the decay kinetics of glutamate-gated currents from these receptors. In addition, Neto2 distributes the GluK1 subunit of kainate receptors to neuronal synapses. The precise mechanism by which Neto2 contributes to kainate receptor trafficking is unknown. Identification of Neto2-interacting proteins might provide some insights on how Neto2 modulates native kainate receptor function or localization of these receptors in the central nervous system. The aim of the current study is to identify Neto2-interacting proteins by use of high-scale proteomic analyses. We performed immunoaffinity purification of native Neto2 followed by liquid chromatography-tandem mass spectrometry. As expected, successful purification of Neto2 with a specific antibody showed an enrichment of the Neto2 protein.

Preliminary results validated the known interaction of Neto2 with GluK2 and GluK3 kainate receptor subunits suggesting successful co-purification of Neto2 protein and its interacting proteins. This pilot screen did not find evidence of association with KCC2 or NMDA receptors subunits, both putative Neto2-interacting proteins. The majority of proteins identified in this preliminary screen have known trafficking functions, including members of the ASAP and Arf families. Interestingly, several proteins involved in metabotropic signaling pathways were also identified, such as G(o) alpha and G(I)/G(S)/G(T) beta-1 and beta-2. Thus far, we have established a possible interaction of Neto2 with proteins involved in cellular trafficking and metabotropic signaling. Future work will focus on validating some of these interactions and identifying a functional role for these proteins in Neto2 and kainate receptor physiology. These future studies are compelling because they might address unresolved questions in the kainate receptor field. Firstly, this work might help elucidate the mechanisms by which Neto proteins aid kainate receptor trafficking. Secondly, these studies might provide insight on how these ionotropic receptors can mediate metabotropic signaling. Funding from NINDS R01 NS071952 to GTS and Diversity Supplement R01 NS080598-S1 to YGF.

Disclosures: **Y.F. Guzman:** None. **J.N. Savas:** None. **G.T. Swanson:** None.

Poster

207. Non-NMDA Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 207.23/A99

Topic: B.02. Ligand-Gated Ion Channels

Support: MRC Grant

Title: Retromer-dependent trafficking of the kainate receptor auxiliary proteins Neto1 and Neto2

Authors: ***K. WILKINSON**, D. L. ROCCA, J. M. HENLEY;
Sch. of Biochem., Univ. of Bristol, Bristol, United Kingdom

Abstract: Retromer is an endosomal sorting complex that has recently been shown to mediate the recycling of endocytosed surface proteins back to the plasma membrane. In neurons, retromer regulates the recycling of AMPA and NMDA-type glutamate receptors via binding of the C-termini of the receptor subunits GluA1 and NR1, respectively, to SNX27, a PDZ domain-containing cargo adaptor for retromer. However, a role for SNX27-retromer in the trafficking kainate-type glutamate receptors (KARs) has not been investigated. Here, we show that GluK2-containing KARs associate with SNX27/retromer. SNX27 immunoprecipitates with GluK2 from cultured neurons and brain, however, binding of SNX27/retromer to GluK2 is not direct, but instead mediated by the recently identified KAR auxiliary subunits Neto1 and Neto2, which have been primarily implicated in regulating the channel properties of KARs. Neto1 binds SNX27 via

its C-terminal PDZ ligand, while Neto2 associates with retromer in a manner independent of SNX27. Notably, GluK2 can only be immunoprecipitated with SNX27 in the presence of Neto1, indicating that the Neto proteins couple GluK2-containing KARs to retromer. Knockdown of SNX27 or the core retromer component Vps35 in cultured cortical neurons dramatically reduces the steady-state levels of both Neto1 and Neto2, suggesting SNX27/retromer may function in rescuing Neto-containing KAR complexes from lysosomal degradation. Moreover, in heterologous systems, binding to retromer regulates the surface expression and stability of the Neto proteins. We are now examining the effects of knockdown of SNX27 or retromer on synaptic KAR responses. Together, these data highlight a role for retromer in the regulation of KARs, and identify trafficking of the Neto proteins as a potential mechanism to control KAR function.

Disclosures: K. Wilkinson: None. **D.L. Rocca:** None. **J.M. Henley:** None.

Poster

207. Non-NMDA Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 207.24/A100

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant NS35812

Title: Kainate and AMPA receptors are required for error correction during taxis behaviors

Authors: *P. J. BROCKIE, P. MALDONADO, J. E. MELLEM, D. M. MADSEN, A. V. MARICQ;
Dept. of Biol., Univ. of Utah, Salt Lake Cty, UT

Abstract: In order to survive animals must locate necessary resources and environs. This can be achieved by navigating along gradients of sensory cues, moving towards those that are favorable and away from those that are harmful. A relatively simple neural circuit required for navigation along temperature and chemical gradients has been described in the nematode *C. elegans*. Central to this circuit is the pair of RIA interneurons that receive glutamatergic synaptic inputs from several different sensory neurons. We have shown that the GLR-3 and GLR-6 kainate receptor subunits are exclusively expressed in the RIA neurons, which also express the GLR-1 AMPA receptor. Heterologous expression of GLR-3 and GLR-6 demonstrated that they constitute the components of a heterologous ionotropic glutamate receptor that can be gated by both glutamate and kainate. To determine the role of GLR-3/GLR-6 kainate receptors in taxis behaviors, we generated null mutations in both the genes. *In vivo* electrophysiological analysis of these mutants showed that GLR-3 and GLR-6 mediate a component of the excitatory glutamate-gated current in RIA that is kinetically distinct from that mediated by GLR-1 AMPA receptors.

Using behavioral assays that test a worm's ability to chemotax towards an attractive odor, we have shown that both *glr-3* and *glr-1* mutants are less proficient at this behavior than wild-type animals. We propose that GLR-3/GLR-6 kainate receptors are required for subtle changes in navigation necessary to steer the worm towards an attractant, whereas GLR-1 AMPA receptors are recruited when a worm moves dramatically off course. Together, our results indicate that kainate and AMPA receptors in RIA mediate distinct components of taxis behavior that together allow animals to successfully navigate along sensory gradients thus increasing their chances of locating important resources.

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Poster

207. Non-NMDA Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 207.25/A101

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH/NINDS 5R21NS082785

Title: The role of the embryonic form of the GluK2 kainate receptor subunit in regulating neuronal excitability

Authors: *T. NOMURA, J. CATCHES, H. FERNANDES, A. CONTRACTOR;
Physiol., Northwestern Univ., Chicago, IL

Abstract: Kainate receptors (KARs) are glutamate-gated ion channel receptors that have multiple roles in modulating synapses and circuits. While the cellular and synaptic roles of KARs have been well described, we currently have an incomplete picture of how they impact circuit and network activity during development. As with subunits of the AMPA receptors (AMPARs), GluK2, one of the principal subunits of KARs is subject to RNA editing. RNA editing of KARs is strictly developmentally regulated, where the GluK2 subunit is unedited in the embryo and neonate, but >85% of GluK2 mRNA transcripts are edited by two weeks after birth. RNA editing at a site within the channel pore (Q/R site) is known to impact on KAR function by altering the Ca2+ permeability and single channel conductance of the receptor; however it still remains elusive what the role of this modification is during early embryonic and postnatal development. Here we found that the unedited embryonic / neonatal form of GluK2 plays a critical role in the regulation of intrinsic neuronal excitability in the developing hippocampus. We performed *in vitro* patch clamp recordings from hippocampal slices from mutant mice in which the editing complementary sequence of GluK2 was deleted (GluK2ΔECS). The GluK2 transcript cannot be edited and therefore all GluK2 receptors expressed in this mouse

are in the embryonic form unedited form (Vissel et al. Neuron 2001). We found that CA3 neurons in the hippocampus of neonatal GluK2ΔECS mice displayed higher spontaneous action potential firing rates than WT mice. The slow afterhyperpolarization (sAHP) recorded in these same cells was reduced compared to that in littermate controls. There were no detectable changes in basic membrane properties of CA3 neurons in these mice, including resting membrane potentials and action potential thresholds. Long term treatment of slices with the AMPARs / KARs antagonist NBQX resulted in an elevated sAHP in GluK2ΔECS mice to an amplitude equivalent to that in WT mice. However the AMPAR specific antagonist GYKI had no effect, suggesting that specific activation of unedited KARs chronically suppresses sAHP. Treatment with an inhibitor of Gi/o G proteins, pertussis toxin, also resulted in an elevated sAHP, suggesting that G protein mediated signaling was required for the effect. We conclude that the embryonic / neonatal form of the GluK2 subunit of KARs plays an important role in regulating neuronal excitability by a chronic metabotropic mediated suppression of the sAHP. Supported by NIH/NINDS 5R21NS082785

Disclosures: **T. Nomura:** None. **J. Catches:** None. **H. Fernandes:** None. **A. Contractor:** None.

Poster

207. Non-NMDA Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 207.26/A102

Topic: B.02. Ligand-Gated Ion Channels

Title: Glutamate receptor-like channels in plants: putative agonist profile and intersubunit interactions

Authors: ***T. LU**[1,2,3], D. TAPKEN[1], M. HOLLMANN[1];
[1]Biochem. I – Receptor Biochem., Ruhr-University Bochum, Bochum, Germany; [2]Grad. Sch. of Chem. and Biochem., [3]RUB Res. Sch., Ruhr Univ. Bochum, Bochum, Germany

Abstract: Glutamate receptor-like proteins in the model plant Arabidopsis thaliana (AtGLRs) are homologous to mammalian ionotropic glutamate receptors (iGluRs) in sequence and predicted membrane topology. Since their discovery in the late 1990s, their functional characterization has progressed slowly. Only in the last years, two of the 20 receptor subunits have been found to be gated by a broad spectrum of amino acids: AtGLR1.4 is activated by seven amino acids: methionine, tryptophan, phenylalanine, leucine, tyrosine, asparagine and threonine [1]; AtGLR3.4 is activated by asparagine, serine and glycine [2]. We used two-electrode voltage clamp (TEVC) in Xenopus oocytes to test the putative agonists of additional AtGLR subunits with a focus on the 20 naturally occurring amino acids. In addition, we employed a bimolecular fluorescence complementation assay (BiFC) to study potential

interactions between different AtGLR subunits that might be required to assemble functional ion channels. Initial BiFC data indicate that a long linker sequence is required when attaching half fluorophores to the C termini of AtGLR subunits of interest, in order to allow assembly and successful fluorophore reconstitution. With this method we have identified several heteromeric combinations and analyzed them electrophysiologically. So far, however, we have not found any influence of such heteromerization on the properties of functional AtGLR subunits. 1.Tapken D, Anschütz U, Liu L, Huelsken T, Seebohm G, Becker D, Hollmann M: A plant homolog of animal glutamate receptors is an ion channel gated by multiple hydrophobic amino acids. Sci Signal 2013, 6:ra47. 2.Vincill ED, Bieck AM, Spalding EP: Ca2+ conduction by an amino acid-gated ion channel related to glutamate receptors. Plant Physiol 2012, 159:40-6.

Disclosures: **T. Lu:** None. **D. Tapken:** None. **M. Hollmann:** None.

Poster

207. Non-NMDA Receptors

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 207.27/A103

Topic: B.08. Synaptic Plasticity

Support: IBS-R001-D1

DA 020087

HHMI

Title: LARGE, an intellectual disability-associated protein, regulates AMPA receptor trafficking and memory

Authors: ***M.-G. KANG**[1,2,3], T. CHO[1], D. Z. LEE[1], S. KANG[1], B. Y. LEE[1], S.-W. KIM[1], K. A. CUNNINGHAM[3], K. T. DINELEY[3], T. A. GREEN[3], J.-C. BÉÏQUE[4], R. L. HUGANIR[5], H.-S. SHIN[1];
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Abstract: Dynamic AMPA receptor (AMPA-R) trafficking at the postsynaptic membrane is a major mechanism underlying the cellular basis of normal learning and memory as well as psychiatric and neurological disorders marked by cognitive dysfunction. We identified like-acetylglucosaminyltransferase (LARGE) as a novel component of the AMPA-R complex. Patients with LARGE mutations have intellectual disabilities as well as muscular dystrophy.

Here, our functional studies demonstrated that LARGE downregulates the surface and synaptic targeting of AMPA-Rs by modulating AMPA-R trafficking from the Golgi apparatus to the plasma membrane. Knockdown of LARGE in the hippocampus resulted in occlusion of hippocampal long-term potentiation (LTP) and deficits in associative fear memory, which were attributable to an increase in the number of AMPA-Rs at the synapse. These results thus identify LARGE as a central component in the machinery that regulates synaptic targeting of AMPA-Rs, synaptic plasticity and, ultimately, memory.

Disclosures: **M. Kang:** None. **T. Cho:** None. **D.Z. Lee:** None. **S. Kang:** None. **B.Y. Lee:** None. **S. Kim:** None. **K.A. Cunningham:** None. **K.T. Dineley:** None. **T.A. Green:** None. **J. Béïque:** None. **R.L. Huganir:** A. Employment/Salary (full or part-time):; Millipore Corporation. **H. Shin:** None.

Poster

208. Potassium Channels II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 208.01/A104

Topic: B.04. Ion Channels

Support: 5R03NS079877-01

R21-NS085471-01

8P20GM103449

Title: The WNK signaling pathway as a link to altered intracellular chloride and trafficking of voltage-gated ion channels following stimulation of GABA receptors in cerebellar neurons

Authors: *E. M. CILENTO[1], B. BALLIF[2], J. FUCHS[3], J. GREEN[3], M. WILLIAMS[5], A. MORIELLI[4];
[1]Neurosci. Grad. Program, [2]Biol., [3]Psychological Sci., [4]Pharmacol., Univ. of Vermont, Burlington, VT; [5]Dept. of Physiol. and Neurobio., Dartmouth Col., Lebanon, NH

Abstract: Kv1.2 is a voltage-sensitive ion channel that influences neuronal excitability. GABA, the principle inhibitory neurotransmitter of the brain, minimizes Purkinje cell (PC) excitability in the cerebellum in part by influencing the expression of Kv1.2 at the cell surface. Activation of GABAA receptors is known to alter chloride passage inside neurons, however it is unknown if intracellular chloride ([Cl-]i) influences Kv1.2 trafficking, leaving the mechanism by which GABA influences Kv1.2 trafficking unclear. Recently, the family of chloride-sensing WNK kinases have been hypothesized to respond to GABA-induced [Cl-]i changes by signaling events to restore both chloride homeostasis and neuronal excitability (Alessi et al., 2014). In this study, we investigated WNK signaling as a potential mechanism to link GABAergic inhibition of Kv1.2

and the alterations of [Cl-]i that follow. We used mass spectrometry to develop an interactome of Kv1.2 in the rat cerebellum and provide, to our knowledge, the first report of WNK family kinases associating with a voltage-gated ion channel in the brain. Using immmunoflourescence, we further demonstrate that both WNK1 and SPAK strongly localize to PCs. With overexpression systems, we show that WNK1 and SPAK operate through duel, opposing mechanisms to balance the trafficking of Kv1.2, however disrupting the presence of WNK1, the ability for WNK kinases to bind SPAK, or even extracellular tonicity can dramatically alter this balance. As a result, we propose a model where GABAergic inhibition of PC excitability alters PC [Cl-]i. The resulting level of [Cl-]i then determines the manner and degree of WNK signaling driven trafficking of Kv1.2. Finally, we highlight our model with a demonstration that, following cerebellar learning, WNK1 has decreased detected interaction with Kv1.2.

Disclosures: **E.M. Cilento:** None. **B. Ballif:** None. **J. Fuchs:** None. **J. Green:** None. **M. Williams:** None. **A. Morielli:** None.

Poster

208. Potassium Channels II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 208.02/A105

Topic: B.04. Ion Channels

Support: NIGMS-SC1-GM088019

NIMHD-G12-MD007583

P031M105050

R25GM110513

Title: Hyperglycemia reduces astrocytic potassium channels and function

Authors: *D. E. RIVERA-APONTE[1], M. P. D. MENDEZ-GONZALEZ[1], A. RIVERA-PAGAN[1], Y. KUCHERYAVYHK[1], L. KUCHERYAVYHK[1], S. N. SKATCHKOV[2], M. J. EATON[1];
[1]Biochem., [2]Biochemistry/Physiology, Univ. Central Del Caribe, Bayamon, PR

Abstract: Diabetics are at risk for a number of serious health complications including an increased incidence of stroke and poorer recovery after ischemic stroke. Astrocytes play a critical role in protecting neurons by maintaining extracellular homeostasis and preventing neurotoxicity through glutamate uptake and potassium buffering. These functions are aided by the presence of potassium channels, such as Kir4.1 inwardly rectifying potassium channels, in the membranes of astrocytic glial cells. The purpose of the present study was to determine if hyperglycemia alters Kir4.1 potassium channel expression and homeostatic functions of astrocytes. We used q-PCR,

Western blot, patch-clamp electrophysiology and a colorimetric glutamate clearance assay to assess Kir4.1 channel levels and homeostatic functions of astrocytes grown in normal and high glucose conditions. We found that astrocytes grown in high glucose (25mM) had an approximately 50% reduction in Kir4.1 mRNA and protein expression as compared with those grown in normal glucose (5mM). These reductions occurred within 24 hours of exposure to hyperglycemia, whereas reversal occurred within 7 days after return to normal glucose. The decrease in functional Kir channels in the astrocytic membrane was confirmed using barium to block Kir channels. In the presence of 100μm barium, the currents recorded from astrocytes in response to voltage steps were reduced by 45%. Furthermore, inward currents induced by stepping extracellular $[K^+]_o$ from 3 to 10 mM (reflecting potassium uptake) were 50% reduced in astrocytes grown in high glucose. In addition, glutamate clearance by astrocytes grown in high glucose was significantly impaired. Taken together, our results suggest that down-regulation of astrocytic Kir4.1 channels by elevated glucose may contribute to the underlying pathophysiology of diabetes-induced CNS disorders and contribute to the poor prognosis after stroke.

Disclosures: D.E. Rivera-Aponte: None. **M.P.D. Mendez-Gonzalez:** None. **A. Rivera-Pagan:** None. **Y. Kucheryavyhk:** None. **L. Kucheryavyhk:** None. **S.N. Skatchkov:** None. **M.J. Eaton:** None.

Poster

208. Potassium Channels II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 208.03/A106

Topic: B.04. Ion Channels

Support: NIH Grant HD067517

Title: Dephosphorylation of N-terminal serines promotes biogenesis of the sodium-activated potassium channel Slack-B

Authors: *G. E. KIM[1], M. R. FLEMING[1], J. KRONENGOLD[1], L. K. KACZMAREK[2]; [1]Pharmacol. Dept., [2]Pharmacol. Dept. and Cell. and Mol. Physiol. Dept., Yale Univ., New Haven, CT

Abstract: The Slack sodium-activated potassium (KNa) channel is the largest known potassium channel. Encoded by KCNT1, Slack is expressed throughout the brain. Human KCNT1 mutations lead to severe learning disability that is linked to intractable seizures with early-to-mid-life onset. Slack exists as multiple isoforms, of which Slack-B is the largest, and is expressed predominantly in brainstem regions and the olfactory bulb. Using liquid-chromatography tandem mass spectrometry, we determined that two serine residues (S34 and S44) close to the N-terminus of Slack-B are phosphorylated under basal conditions. To test the

biological role of these sites we performed site-directed mutagenesis and generated 8 different combinations of S34 and S44 mutations that mimic either phosphorylation or dephosphorylation at each site. We found that levels of the "dephosphorylated" mutant channel (AA Slack) are greatly increased in cRNA-injected Xenopus oocytes and transiently transfected HEK293 cells compared to wild type Slack. This is correlated with an increase in currents measured in two-electrode voltage clamp experiments using oocytes and immunofluorescent localization of the protein in HEK cells. The increase in current was not associated with alterations in channel kinetics or single channel properties. Similarly, changes in Slack protein expression could not be explained by our choice of codons in generating the AA or EE mutant channels. Next, to determine whether the increase in Slack protein accumulation resulted from an increase in Slack translation or a decrease in its degradation, we measured the time constant for Slack protein accumulation, Slack protein half-life and the effect of preventing proteasomal degradation. Protein accumulation was biphasic with a rapid increase that tailored off into a plateau for AA Slack in both cell systems, whereas there was a much slower, linear accumulation for wild-type Slack and EE Slack ("phosphorylated" mutant). The rate of accumulation of AA Slack was significantly greater, and inhibiting proteasomal degradation with MG-132 in HEK cells for six hours could not enhance the level of wild-type expression to that of AA Slack. Finally, measurements of protein half-life in the presence of the protein synthesis inhibitor cycloheximide revealed no difference among the three groups. Taken together, the results indicate that dephosphorylation of these two N-terminal residues is a key step during biogenesis of Slack. Our findings also suggest that regulation of the phosphorylation state of S34 and S44 during biogenesis may allow neurons to alter channel abundance rapidly in response to stimulation.

Disclosures: G.E. Kim: None. **M.R. Fleming:** None. **J. Kronengold:** None. **L.K. Kaczmarek:** None.

Poster

208. Potassium Channels II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 208.04/A107

Topic: B.04. Ion Channels

Support: NIH Grant NS078184

Title: Slick (Kcnt2) sodium-activated potassium channels expressed in peptidergic CGRP neurons regulate nociception and peptide release

Authors: *D. L. TOMASELLO[1], A. BHATTACHARJEE[2];
[1]Program in Neurosci., [2]Pharmacol. & Toxicology, SUNY at Buffalo, Buffalo, NY

Abstract: Previous work has identified the Slack (Kcnt1) K_{Na} channel as a key contributor to excitability in dorsal root ganglion (DRG) neurons and pain signaling, however little is known about the functional role of the closely related Slick (Kcnt2) K_{Na} channel. We studied the phenotypic consequences of *Slick* deletion using a novel Slick-lacZ reporter knockout (KO) mouse line. Utilizing the sciatic nerve cuff model for neuropathic pain we tested thermal hyperalgesia on KO and wild type (WT) littermates. Prior to nerve injury Slick KO mice exhibited sensitized basal nociception, and following injury KO mice had exacerbated hyperalgesia compared to WT mice in both male and female groups. Staining of Slick in the DRG indicated prominent staining localized to the cytoplasm of calcitonin-gene related peptide (CGRP) small and medium sized neurons only. CGRP is a neuroinflammatory peptide. Slick staining was also identified in the spinal cord and within the paw indicating Slick is localized to cell bodies and to nerve endings. Strikingly, a pool of Slick channels localized to large dense core vesicles (LDCV) containing CGRP as determined by immunohistochemistry. Fractionation of DRGs confirmed that Slick channels migrate with LDCV positive fractions. CGRP immunolabeling within Slick KO mouse DRG neurons displayed lower intensity and dispersed localization within the cytoplasm, however no change in Substance P staining was observed. Next we tested the electrophysiological properties of peptidergic DRG neurons from KO and WT mice. We found a decrease in the total outward potassium current, a decrease in action potential rheobase, and an increase in the height of action potential from DRG neurons isolated from Slick KO mice compared to WT neurons indicating that Slick deletion produces hyperexcitability. Our data indicate that Slick channels limit the excitability of CGRP peptidergic neurons and reduce pain behavior after nerve injury. Moreover, Slick channels are likely recruited to the membrane during CGRP release functioning to constrain excessive CGRP release. Given the specificity of Slick expression to CGRP positive neurons only, Slick channels are desirable pharmacologic targets for the treatment of pain and inflammation.

Disclosures: **D.L. Tomasello:** None. **A. Bhattacharjee:** None.

Poster

208. Potassium Channels II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 208.05/A108

Topic: B.04. Ion Channels

Support: McDonnell Center for Systems Neuroscience

Title: Non-invasive control of cellular excitability using focused ultrasound: Effects on ion channels

Authors: *J. KUBANEK[1], J. SHI[2], J. CUI[2];
[1]Washington University, Sch. of Med., Saint Louis, MO; [2]Biomed. Engin., Washington Univ., St. Louis, MO

Abstract: Ultrasound (US) has recently been found to modulate cellular excitability in the brain. US propagates through the skull and can be well focused. These properties make US an attractive new method to stimulate particular brain regions with the goal to treat diseases of the nervous system non-invasively. However, to arrive to such clinical applications, it is necessary to first determine the mechanism/s by which US mediates membrane excitability. Using the Xenopus oocyte expression system, we found that focused US (10 MHz frequency, 100--500 kPa amplitude) is capable of activating ion channels of the K2P family (TREK-1, TREK-2, TRAAK) natively present in neuronal, retinal, and cardiac cells. The transmembrane current due to the US exhibits an early rapid response (within about 20 ms), and then steadily increases with the time of the sonification. The average peak effect represents about 20% modulation of the transmembrane current. The effect is not present when the channels are blocked using BaCl2. An effect of US on ion channels presents a safe mechanism of US action on cellular excitability, and thus paves the way to future non-invasive treatments of neurological or cardiac diseases.

Disclosures: J. Kubanek: None. **J. Shi:** None. **J. Cui:** None.

Poster

208. Potassium Channels II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: B.04. Ion Channels

Support: NIH Grant NS069898

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Start-up funds from The University of Iowa

Srart-up funds from Washington University School of Medicine in St. Louis

Title: Pituitary adenylate cyclase-activating peptide modulates neuronal Kv4.2 channel via convergent phosphorylation by PKA and ERK1/2

Authors: *R. GUPTE[1,2], R. MERRILL[2], A. J. SHEPHERD[3], S. STRACK[2], D. P. MOHAPATRA[3,2];
[1]Dept. of Anesthesiol., Washington Univ. In St. Louis Sch. of Medic, Saint Louis, MO; [2]Pharmacol., The Univ. of Iowa, Iowa City, IA; [3]Dept. of Anesthesiol., Washington Univ. Sch. of Med. in St. Louis, Saint Louis, MO

Abstract: The endogenous neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) exerts various neuromodulatory functions, including enhancement of synaptic activity, and chronic inflammatory and neuropathic pain conditions. However, it remains unclear whether PACAP signaling could directly influence the function of intrinsically excitable ion channels, thereby altering the excitability of neurons. Kv4.2, the major dendritic voltage-gated K^+ channel in mammalian neurons, is a key regulator of neuronal excitability and modulation of synaptic inputs, and plays a critical role in central sensitization of inflammatory pain. We determined whether PACAP signaling could dynamically influence neuronal Kv4.2 channel activity. We found that isoforms of the PACAP receptor, PAC1, are expressed in rodent brain neurons, and their activation led to the downstream activation of protein kinase A (PKA) and extracellular signal-regulated protein kinase-1/2 (ERK1/2). Using cultured rat hippocampal neurons and HEK293T-based heterologous expression system, we show that PACAP induces phosphorylation of Kv4.2 protein, and consequently decreases channel current density. Such modulation of Kv4.2 was dependent on the activity of both PKA and ERK1/2. PACAP effects on Kv4.2 channel density was abolished in phospho-disruptive mutations at three ERK1/2-phosphorylation sites, T602, T607 and S616, in Kv4.2; however, it was unaffected in phospho-disruptive mutation at PKA-phosphorylation site, S552. PACAP38 did not induce any significant change in the voltage-dependence and time course of activation of Kv4.2 channel. Although PACAP38 did not alter the voltage-dependence of channel's steady-state inactivation, it led to a decrease in the time course of fast-inactivation, which was attenuated with the inhibition of ERK1/2. Conversely, PACAP38 exposure led to an increase in the extent of non-inactivating current component of the channel, which was attenuated with the inhibition of PKA, but not ERK1/2, and was abolished in the Kv4.2-S552A channel. These results indicate a convergence of PACAP-mediated PKA and ERK1/2 activation, in order to influence the current density and kinetic properties of Kv4.2 channel. Our findings suggest that PACAP/PAC1 signaling modulates Kv4.2 channel function in a manner that could lead to increased intrinsic excitability of neurons. Since PACAP levels are elevated in multiple pathological conditions, such as stroke-reperfusion injury, chronic inflammatory and neuropathic pain, modulation of Kv4.2 channel function could lead to hyperexcitability in these pathologies.

Disclosures: **R. Gupte:** None. **R. Merrill:** None. **A.J. Shepherd:** None. **S. Strack:** None. **D.P. Mohapatra:** None.

Poster

208. Potassium Channels II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 208.07/B2

Topic: B.04. Ion Channels

Support: NSF IOS Award #1122115

Title: Functional consequences of amino acid variation in pain-pathway kv1 channels in a wild rodent (Onychomys) exhibiting reduced pain sensitivity

Authors: *K. SHERER[1], R. BARAJAS[2], A. ROWE[2];
[1]Neurosci., [2]Zoology, Michigan State Univ., East Lansing, MI

Abstract: In 1931 French physician Dr. Albert Schweitzer declared, "Pain is a more terrible lord of mankind than even death itself." A variety of pain conditions affect more people in the United States than diabetes, cancer and heart disease combined. Unfortunately, current drugs are inadequate for managing pain, mainly due to our incomplete understanding of how pain states develop. Small- and medium-diameter sensory neurons transmit pain signals to the central nervous system via action potentials (APs). While sodium (Na^+) ion channels initiate AP firing in sensory neurons, potassium (K^+) ion channels are responsible for inhibiting APs and returning the neuron to its resting potential, thus terminating pain signals. Currently, we understand that pathologic pain states are associated with excessive firing, or hyperexcitability, of nerves, creating the sensation of pain. Rodent pain models indicate that altered K^+ channel expression and function are associated with hyperexcitability of sensory neurons and pain-related behaviors. Grasshopper mice (*Onychomys*) provide a unique model for studying the K^+ ion channels that regulate pain signals. Grasshopper mice prey on scorpions (*Centruroides*) whose venom induces burning pain and hypersensitivity to touch in humans. However, the mice exhibit minimal pain-related behaviors in response to the scorpions' defensive stings. Grasshopper mice sensory neurons exhibit decreased excitability compared to house mice. Thus, this model provides the opportunity to study the role of K^+ channels in a hypoexcitable system. RNA-seq data (confirmed by Sanger sequencing) revealed multiple amino acid variants in both the coding and regulatory regions of $K_v1.1$, $K_v1.2$, and $K_v1.4$ *Shaker* channel α-subunits and the leak channel TWIK1 compared to the house mouse (*Mus musculus*). K_v1 channels produce A- and D-type currents that accelerate repolarization of neurons after AP firing. TWIK1 is a weak inward rectifier that contributes to the resting membrane potential of neurons. Thus, K_v1 and TWIK1 are critical for controlling the excitability of sensory neurons. I hypothesize that amino acid variants in grasshopper mice K^+ channels produce functional changes that inhibit sensory neuron excitability and decrease pain signaling. Current experiments are aimed at understanding the functional consequences of amino acid variants in K_v1 and TWIK1 channels. Elucidating how structural and functional changes in K^+ channels lead to alterations in AP firing in sensory neurons will contribute to our understanding of how pain states develop.

Disclosures: K. Sherer: None. **R. Barajas:** None. **A. Rowe:** None.

Poster

208. Potassium Channels II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 208.08/B3

Topic: B.04. Ion Channels

Support: NIH Grant MH090694

NIH Grant MH094839

NIH Grant MH100510

FRAXA Research Grant

Title: Cell-type specific channel phenotypes in the prefrontal cortex of the fmr1-/y mouse model of Fragile X syndrome

Authors: B. E. KALMBACH, D. JOHNSTON, *D. H. BRAGER;
Ctr. for Learning and Memory, Univ. Texas at Austin, Austin, TX

Abstract: Fragile X syndrome (FXS) is caused by a single gene mutation resulting in the loss of expression of fragile X mental retardation protein (FMRP). FXS patients display several behavioral phenotypes associated with prefrontal cortex (PFC) dysfunction. Voltage-gated ion channels, some of which are targets of FMRP-regulation, heavily influence neuron function. In the *fmr1-/y* mouse model of FXS we tested for alterations to ion channels in L5 pyramidal neurons of medial prefrontal cortex (PFC). Using somatic and dendritic patch clamp recordings we show that the functional expression of h-channels (I_h) is downregulated, whereas a rapidly inactivating A-type K^+ current is upregulated in pyramidal tract-projecting (PT) PFC neurons. These channel phenotypes are opposite to our published findings from hippocampus. Additionally, we found that a slowly inactivating K^+ current is downregulated at the soma of PT neurons, resulting in increased excitability. Importantly, these K^+ channel phenotypes are not found in neighboring L5 intratelencephalic-projecting (IT) PFC neurons. Thus, the absence of FMRP has divergent effects on the function of individual types of ion channels not only between brain regions, but also across cell types within the same brain region.

Disclosures: B.E. Kalmbach: None. **D. Johnston:** None. **D.H. Brager:** None.

Poster

208. Potassium Channels II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 208.09/B4

Topic: B.04. Ion Channels

Support: NIH Grant NS080889

Title: Kir2 leak conductance dampens the excitability of lamina I projection neurons in the neonatal rat

Authors: *N. C. FORD, M. L. BACCEI;
Anesthesiol., Univ. of Cincinnati, Cincinnati, OH

Abstract: Background leak conductances are implicated in determining the excitability and modulating the firing properties of CNS neurons. Previous studies demonstrated that classic inward-rectifying potassium (Kir2) conductance contributes to membrane excitability and shapes burst-firing in interneurons of the neonatal rat superficial dorsal horn (SDH). However, little is known about which types of leak conductance are responsible for regulating the intrinsic excitability of lamina I projection neurons (PNs), which correspond to a major output of the spinal nociceptive network. The present study investigated the contribution of Kir2-mediated leak conductance to the intrinsic membrane excitability in identified lamina I PNs during the neonatal period. Lamina I PNs were back-labeled via injection of DiI into the rat parabrachial nucleus (PB) or periaqueductal gray (PAG) at postnatal day 1 (P1), and whole-cell patch clamp recordings were obtained from these cells using an *in vitro* intact spinal cord preparation at P3-5. $BaCl_2$ (200 μM) was bath applied to block Kir2 conductance. Ba^{2+} block of Kir2 channels in spino-PB neurons significantly depolarized the resting membrane potential, decreased rheobase, and increased the membrane resistance, rate of spontaneous activity, AP duration and repetitive firing rate. AP threshold was not affected by blocking Kir2 channels in spino-PB neurons. Perfusion with the Kir2-selective antagonist ML133 (10μM) also enhanced the excitability of spino-PB neurons. Similarly, Ba^{2+} block of Kir2 channels in spino-PAG neurons significantly depolarized the resting membrane potential, increased AP duration and repetitive firing rate, while decreasing rheobase. However, AP threshold, membrane resistance and spontaneous activity were not affected by blocking Kir2 channels in spino-PAG neurons. Comparing the level of Ba^{2+} sensitive K^+ conductance between spino-PB and spino-PAG neurons revealed no significant differences. We conclude that Kir2-mediated leak conductance plays a role in dampening the excitability and shaping the firing properties of lamina I PNs in the neonatal rat. These data suggest that changes in Kir2-mediated conductance during early life could significantly alter the level of ascending nociceptive transmission to the developing brain.

Disclosures: N.C. Ford: None. **M.L. Baccei:** None.

Poster

208. Potassium Channels II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 208.10/B5

Topic: B.04. Ion Channels

Support: MRC

Action on Hearing Loss

Title: Kv3 channels in the auditory brainstem are composed of Kv3.1 and Kv3.3 subunits

Authors: D. M. LINLEY[1], S. ROBINSON[2], J. R. STEINERT[2], C. KOPP-SCHEINPFLUG[3], *I. D. FORSYTHE[1];
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Abstract: Fast high fidelity action potential (AP) firing is vital for accurately encoding sound and for extracting sound localisation cues in the ascending auditory pathway. High frequency firing is aided by the presence of high voltage activated Kv3 channels, which have rapid kinetics and promptly repolarise APs. We investigated the contribution of different Kv3 subunits (Kv3.1-Kv3.4) to AP repolarisation in three nuclei of the superior olivary complex (SOC) in the auditory brainstem. Respective tissue samples were collected from recently sacrificed CBA/Ca mice, Kv3.1 knockout (KO) and Kv3.3-KO strains (both backcrossed onto CBA/Ca). RT PCR was used to assess the distribution of Kv3 subunit mRNA across the SOC (in animals aged P13-P33), and immunohistochemistry was conducted using antibodies targeting Kv3 subunits. Whole-cell patch recording in transverse brainstem slices was performed in both voltage and current-clamp mode (P14-P19). Immunohistochemistry and RT PCR revealed that Kv3.1b and Kv3.3 subunits are co-expressed in the medial nucleus of the trapezoid body (MNTB) and the superior paraolivary nucleus (SPN) in wild-type (WT) mice. In the lateral superior olive (LSO), Kv3.3 subunits appear to predominate over Kv3.1b. Kv3.2 and Kv3.4 subunits showed negligible expression across the whole SOC. Current-clamp recordings in the WT SPN gave AP half-widths (HW) of 0.27±0.02ms (mean ± SEM) which were prolonged in the Kv3.1-KO and slowed further in the Kv3.3-KO mice (respectively, 0.50±0.09ms and 0.59±0.10ms). Voltage gated repolarising K^+ currents in the SPN were reduced in both the Kv3.1-KO and Kv3.3-KO compared to WT (respectively, 17.5±3.9nA, 23.3±5.4nA and 42.7±2.2nA at +50mV). However, in the LSO, AP HW in the Kv3.1-KO was unchanged compared to WT (respectively 0.27±0.03ms and 0.28±0.02ms), while AP HW in the Kv3.3-KO was prolonged to an even greater extent than in the SPN (0.7±0.06ms). Voltage-clamp data from the LSO showed no significant change in the Kv3.1-KO compared to WT (respectively, 46.4±3.5nA and 39.8±7.9nA at +50mV; p=0.792) but voltage gated K^+ currents in the Kv3.3-KO were greatly reduced (7.9±1.7nA). Preliminary studies in the MNTB suggest that KO of either Kv3.1 or Kv3.3 produces only a small change in AP duration and K^+ current amplitudes. This will be further investigated. In conclusion, Kv3.3 is co-expressed with Kv3.1b in the auditory brainstem. We postulate that Kv3.3 influences AP repolarisation to a greater extent in the more lateral nuclei of the SOC.

Disclosures: **D.M. Linley:** None. **S. Robinson:** None. **J.R. Steinert:** None. **C. Kopp-Scheinpflug:** None. **I.D. Forsythe:** None.

Poster

208. Potassium Channels II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 208.11/B6

Topic: B.04. Ion Channels

Support: CIHR Grant

EYES High PDF Fellowship

Title: L-type voltage gated calcium channels activate IKCa channels to generate the slow afterhyperpolarization in CA1 pyramidal cells

Authors: *G. SAHU, J. MICLAT, G. W. ZAMPONI, R. W. TURNER;
Hotchkiss Brain Inst., Univ. of Calgary, Calgary, AB, Canada

Abstract: Calcium activated potassium (KCa) channels control the frequency and pattern of spike discharge in hippocampal pyramidal neurons. The role of big conductance (BK) and small conductance (SK) channels in generating the fast (~10 ms) and medium (~100 ms) afterhyperpolarization has been long established. The molecular identity of the channel responsible for the slow afterhyperpolarization (sAHP) that lasts for seconds was recently identified as intermediate conductance KCa (IKCa) channels. Previous work suggested that L-type calcium channels provide calcium influx to drive the sAHP in CA1 pyramidal cells. This study tested if the L-type channel isoforms CaV1.2 or CaV1.3 are capable of activating IKCa channels and the sAHP in CA1 pyramidal cells. Transient transfection of CaV1 cDNA in tsA-201 cells established that the initial onset and peak current for CaV1.2 channels was evoked at -30 mV and +10 mV, and for CaV1.3 channels -40 mV and 0 mV at physiological levels of [Ca]o. We found that either CaV1.2 or CaV1.3 was sufficient to activate IKCa channels when coexpressed in tsA-201 cells. Moreover, when coexpressed IKCa channels were activated in a voltage-dependent manner that closely followed the activation voltages of the CaV1 isoforms, including a decrease in IKCa amplitude as calcium conductance decreased upon approaching ECa. Importantly, a step command for 5-150 msec to +10 mV for CaV1.2 and 0 mV for CaV1.3 to maximally activate the CaV1 isoforms produced a graded tail current for IKCa that lasted 1-5 sec, effectively recreating an sAHP current (IsAHP). By comparison, neither IKCa nor CaV1 channel isoforms expressed in isolation produced a long-lasting tail current. In concurrence with the expression results, CA1 cells produced an IsAHP in the presence of blockers against all CaV channel isoforms except L-type channels, as well as SK, BK, KV1, KV7, and Na channels. Moreover, internal perfusion of 1 μM TRAM-34, a selective blocker for IKCa channels, abolished the IsAHP/IKCa currents in either CA1 cells or tsA-201 cells. Immunocytochemistry revealed an overlapping expression pattern for IKCa and both Cav1.2 and Cav1.3 immunolabels in tsA-201 cells and CA1 cells but no obvious co-localization with either L-type channel isoforms. The CaV1-mediated activation of IKCa channels was also blocked by internal perfusion of 5 mM EGTA or BAPTA, suggesting a microdomain based interaction. The results suggest that L-type calcium channel isoforms are sufficient to activate IKCa channels in a manner consistent with the sAHP in CA1 pyramidal neurons. This work was supported by Canadian Institute of Health Research (CIHR) and a University of Calgary Eyes High PDF (GS).

Disclosures: **G. Sahu:** None. **J. Miclat:** None. **G.W. Zamponi:** None. **R.W. Turner:** None.

Poster

208. Potassium Channels II

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Program#/Poster#: 208.12/B7

Topic: B.04. Ion Channels

Support: MRC

Title: The role of neuronal Kv2.1 potassium channels under physiological conditions

Authors: ***J. L. SMALLEY**, B. PIGOTT, I. D. FORSYTHE;
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Abstract: Kv2.1 is a voltage-dependent potassium channel that is highly expressed in the cortex and hippocampus. It is postulated that this delayed rectifier is a key regulator of neuronal excitability and integral for co-ordinating intrinsic plasticity. The channel is tetrameric with each subunit consisting of a short N-terminal domain, 6 membrane-spanning domains, and a long intracellular C-terminal domain that is highly phosphorylated. Previous work *in vitro* has demonstrated that Kv2.1 is localized in clusters that are dispersed during hypoxia and plays a role in axonal injury and synaptic excitation. Clustered Kv2.1 is highly phosphorylated, while declustering is associated with distinct dephosphorylation, although the mechanism(s) of phosphorylation/dephosphorylation and changes in localisation that occur upon neuronal excitation have not been well characterised. Here we use an unbiased proteomic-based approach and bioinformatic analysis to identify the proteins and biochemical processes associated with Kv2.1 *in vivo*, and combine this with an *ex vivo* system of acute cortical slices to determine phosphorylation and neuronal excitability. We carried out LC-MS/MS on freshly isolated and fractionated cortical tissue and achieved a high degree of Kv2.1 sequence coverage. Our results suggest that Kv2.1 phosphorylation *in vivo* occurs at a few, but not all of the C-terminal sites formerly identified *in vitro*. Our data also suggests that under normal conditions, Kv2.1 is expressed within clusters with a unique proteome containing endoplasmic reticulum (ER), Golgi, and synaptic vesicle proteins. Transition through these cellular compartments would be expected for any large and complex protein and therefore it would not be surprising that ER/golgi remnants remain. However during hypoxia/excitation, the majority of clusters disperse, indicating that these Kv2.1 structures are not simply extensions of the ER or Golgi, but are a distinct vehicle for the storage and delivery of Kv2.1. Unbiased functional analyses of Kv2.1 binding proteins, using several large curated databases of protein interactions, suggest a prominent role for kinases/phosphatases, including several kinases not previously implicated. The analysis also highlighted microtubule-associated trafficking proteins, which may be involved in the movement and dispersion of Kv2.1 clusters. This work demonstrates how the use

of native tissue-based systems combined with shotgun proteomics and bioinformatics can be used to generate new insights into the function and regulation of an important neuronal signalling protein.

Disclosures: **J.L. Smalley:** None. **B. Pigott:** None. **I.D. Forsythe:** None.

Poster

208. Potassium Channels II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 208.13/B8

Topic: B.04. Ion Channels

Support: DE018661

Title: Effects of cold temperatures on trigeminal neuron excitability and KCNQ channel activity

Authors: ***H. KANDA**[1], R. IKEDA[2], J. G. GU[1];
[1]Dept. of Anesthesiol. and Perioperative Med., Univ. of Alabama at Birmingham, Birmingham, AL; [2]Dept. of Orthopaedic Surgery, Jikei Univ. Sch. of Med., Tokyo, Japan

Abstract: In the present study we studied the effect of cooling temperatures on trigeminal afferent neuron excitability and KCNQ channel activity by using the whole mount trigeminal ganglion (TG) preparation and the whole-cell patch-clamp recording technique. In 49% of small-sized TG neurons recorded, cooling temperature of 15 oC increased neuronal excitability as was evidenced by the reduction of action potential rheobase and increases of action potential firing numbers in response to membrane depolarization. In the remaining 51% of TG neurons cooling temperatures decreased or had little effect on their excitability. Since KCNQ channels play an important role in controlling neuronal excitability, we studied whether cooling temperatures affected KCNQ channel activity (M-currents) and thereby causing the increases of neuronal excitability in some TG neurons. We found that M-currents in TG neurons were significantly inhibited by cold in a cooling temperature-dependent manner. Inhibition of M-currents pharmacologically by linopirdin (10 μM) could increase TG neuron excitability in a manner similar to cold-induced increases of TG neuron excitability. Furthermore, retigabine, a KCNQ channel activator, enhanced M-currents and abolished cold-induced increases of neuronal excitability. Our results suggest that cold temperatures increase trigeminal afferent neuron excitability via KCNQ channel inhibition.

Disclosures: **H. Kanda:** None. **R. Ikeda:** None. **J.G. Gu:** None.

Poster

208. Potassium Channels II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 208.14/B9

Topic: B.04. Ion Channels

Title: Inhibition of hERG Channel by Acepromazine

Authors: Y. JOO, *K.-W. SUNG;
The Catholic Univ. of Korea, Sch. of Medicine, Dept. of Pharmacol., Seoul, Korea, Republic of

Abstract: Acepromazine is a first generation of antipsychotics as phenothiazine group, like chlorpromazine. Phenothiazine derivative antipsychotic drug has been shown its sedative effects by blocking of dopamine receptors in the central nervous system. Chlorpromazine is still used in human medicine for treatment of psychosis, but acepromazine isn't because it has a little therapeutic effect. Whereas acepromazine is used in veterinary field as a seditave medicine in a case of a captive wild animals or pre-treatment of anesthesia. Chlorpromazine that is closely related analogue, acepromazine, is known as a drug that inhibited hERG channel. Human ether-a-go-go-related gene(hERG) encodes for a protein known as Kv11.1 that is the alpha subunit of the rapid delayed rectifier potassium current(*Ikr*). The channel is highly expressed in cardiomyocytes and the most critical factor in the repolarization process. It is terminated cardiac action potentials and can affect the action potential duration. Inhibition of hERG channel by genetic defect or drug side effect cause long QT syndrome which is prolonged QT interval in the electrocardiogram and sever arrhythmias. We studied whether acepromazine affect hERG channel. This study was investigated using the whole cell patch clamp technique in human embryonic kidney (HEK293) cells expressed human ether-a-go-related gene (hERG) channels. The hERG channels were inhibited by acepromazine in a concentration-dependent manner with an IC50 value 1.2μM and Hill coefficient of 0.81. Acepromazine blocked hERG currents voltage-dependent manner between -40 and +60mV. Before and after application of the 1μM acepromazine, the half activation potential(V1/2) was -37.9mV and -44.57mV, slope value was 5.70 and 4.60. There was no frequency-dependent inhibition on hERG channel by the acepromazine. Acepromazine also blocked the hERG current during the repolarization pulse in a concentration-dependent manner. The extent of the blocking by acepromazine in the repolarizing current was more than that in the depolarizing pulse, implying that acepromazine has a high affinity for the open state of the channel, with a relatively lower affinity during the inactivated state of hERG channel. Keywords: hERG Channel, Acepromazine, Patch clamp technique

Disclosures: Y. Joo: None. **K. Sung:** None.

Poster

208. Potassium Channels II

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Topic: B.04. Ion Channels

Support: NIH grants R01 NS43394

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Title: Coupling of distinct ion channel types in neurons mediated by AKAP79/150

Authors: *J. ZHANG, M. S. SHAPIRO;
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Abstract: M-type K+ channels, comprised of KCNQ2-5 (Kv7.2-7.5) subunits, play key roles in the regulation of neuronal excitability in the peripheral and central nervous systems. In diverse neurons, L-type Ca2+ channels (LTCCs) drive transcriptional regulation via NFAT transcription factors, and in sensory neurons, TRPV1 cation channels excite neurons in response to heat, acidity or chemical ligands, driving nociception. The A-kinase-anchoring protein (AKAP)79/150 has been shown to orchestrate regulation of all three types of channels by PKC, PKA, calcineurin or NFAT transcription factors. Using stochastic optical reconstruction microscopy (STORM), which offers sub-diffraction (~20 nm) resolution, we have directly visualized individual signaling complexes containing endogenous and cloned AKAP79/150, these three ion channels and G protein-coupled receptors in neurons and tissue-culture cells. We also observe AKAP150-mediated clustering of KCNQ, LTCCs and TRPV1 channels at the single-complex level. Thus, AKAP79/150 links different channel types together, raising the possibility of their functional, as well as physical, coupling. In nodose ganglia sensory neurons, application of capsaicin induced the translocation of YFP-tagged PH probe from plasma membrane into the cytoplasm, suggesting membrane PIP2 depletion triggered by TRPV1 activation. In neurons isolated from AKAP150+/+ mice, application of very low concentration of capsaicin (100 nM) for a very short time, which is believed to trigger only local PIP2 depletion, induced ~40% suppression of M-current amplitude (IM), suggesting the close localization of TRPV1 and M-channels, which thus can be suppressed by capsaicin-induced local PIP2 depletion. However, in AKAP150-/- neurons, IM was shown to be not affected by this modest activation of TRPV1 channels, implying the critical role of AKAP79/150 in bringing together complexes involving these two ion channels. Furthermore, with the application of the LTCC blocker, nifedipine, but not the N-type Ca2+ channel blocker, ω-conotoxin GVIA, IM was significantly suppressed, and both acute desensitization and tachyphylaxis of TRPV1 current was greatly impaired, suggesting the functional coupling of LTCCs with KCNQ, and TRPV1 channels, consistent with our findings suggesting their physical coupling at the single-complex level with STORM. We will further investigate the role of AKAP79/150 in the functional coupling of these three ion channels in sensory neurons and their physiological roles in tuning the nociceptive response to painful stimuli.

Disclosures: J. Zhang: None. **M.S. Shapiro:** None.

Poster

208. Potassium Channels II

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Topic: B.04. Ion Channels

Support: Medical Research Council, UK

Title: Scaling of pyramidal neuron excitability in auditory cortex by nitric oxide-mediated inhibition of K_v2 currents during postnatal development

Authors: *B. PIGOTT, J. L. SMALLEY, M. HAMMETT, I. D. FORSYTHE;
Univ. of Leicester, Leicester, United Kingdom

Abstract: The intrinsic and synaptic properties of cortical pyramidal neurons change throughout postnatal development in a manner that enables the cortex to adapt its output to fluctuating sensory input, for example, as sense organs become active. Alterations in voltage-gated K^+ (K_v) currents have been well-documented, and could underlie adjustments in neuronal excitability, but the identity of the channels involved and the mechanisms underlying their regulation are unknown. To address these questions, standard techniques were used for whole-cell patch-clamp recording in slices of mouse auditory cortex. Data are mean values ± SEM. In good agreement with past reports, outward K_v currents recorded in layer 2/3 pyramidal neurons increased significantly from hearing onset (P11-14) to maturity (> P28; respective currents were 13 ± 0.5 nA and 24 ± 2 nA at +50 mV; unpaired *t* test, $p < 0.05$; n = 11-30 cells). This increase was explained by the addition of a current that was sensitive to the K_v2 channel inhibitor gunangxitoxin-1E (100 nM), which reduced the total current at +50 mV by 34 % in neurons from mature mice (P28-32; $p < 0.05$ compared to control; n = 6-8 cells), but had no effect in slices from animals aged 11-14 days old ($p = 0.36$; n = 6-7 cells). The absence of a K_v2 current in neurons from the younger mice could not be explained by a lack of K_v2 protein, as determined immunohistochemically (n = 3 mice). Rather, K_v2 currents were suppressed in these animals by endogenous nitric oxide (NO); the NO synthase inhibitor L-nitroarginine (100 μM) increased the total outward current from 13 ± 0.5 nA to 21 ± 1 nA ($p < 0.05$; n = 12-23 cells) and the percentage block by guangxitoxin-1E (100 nM) from 13 % to 25 % at +50 mV (n = 6-7 cells) but was without effect in slices from mature mice ($p = 0.35$; n = 5-11 cells). The NO signal appeared to be generated by the endothelial isozyme of NO synthase, which was detected in blood vessels throughout layer 2/3, and transduced via activation of the guanylyl cyclase-coupled NO receptor and protein kinase G. Under current clamp, NO synthase inhibition (100 μM L-nitroarginine) reduced the peak frequency of action potential firing from 39 ± 1 Hz to 29 ± 3 Hz in slices from the young mice ($p < 0.05$; n = 12-14 cells), indicating that NO augments neuronal excitability at this age. These data show that native K_v2 currents are subject to significant regulation during postnatal development. We postulate that the inhibition of K_v2 by NO at

hearing onset allows neurons to scale their output (in terms of action potentials) to their synaptic input, such that the firing of L2/3 pyramidal neurons will be facilitated at a time when hearing thresholds are high and hence cortical input (acoustic or synaptic) is low.

Disclosures: **B. Pigott:** None. **J.L. Smalley:** None. **M. Hammett:** None. **I.D. Forsythe:** None.

Poster

209. Synaptic Transmission: Pharmacology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 209.01/B12

Topic: B.07. Synaptic Transmission

Title: The role of glutamate release in the nucleus accumbens core during cocaine reinstatement in rats with a history of both alcohol and cocaine self-administration

Authors: *B. STENNETT, L. KNACKSTEDT;
Univ. of Florida, Gainesville, FL

Abstract: One of the difficulties in successful treatment of cocaine addiction is reducing the high risk of relapse that exists even after long periods of abstinence. Relapse can be modeled in animals using the extinction-reinstatement paradigm. This paradigm involves training animals to lever-press for cocaine reinforcement in an operant chamber. The operant response is then extinguished and reinstated either with cues previously paired with the response made to attain cocaine delivery, or an IP injection of cocaine. Previous research has established the role of nucleus accumbens glutamate transmission in the reinstatement of cocaine-seeking and has shown that the antibiotic ceftriaxone prevents relapse to cocaine seeking in rats. However, it is estimated that 60% to 90% of cocaine addicts use alcohol with cocaine. The combination of alcohol and cocaine potentially produces unique neuroadaptations that differ from those produced by either drug alone. Therefore, we developed a model of poly-drug addiction in which rats self-administered cocaine for two hours in an operant chamber and subsequently drank alcohol (20% v/v) from bottles in the home cage for 6 hours. Following two weeks of drug consumption, animals underwent extinction training for a minimum of two weeks. Animals were treated with IP ceftriaxone (200 mg/kg) or vehicle for 6 days prior to being tested for cue- and/or cocaine-primed reinstatement. During reinstatement testing, animals were probed with a microdialysis cannula in the nucleus accumbens to measure glutamate levels during relapse to drug seeking. In agreement with previous work by our group and others, an increase in glutamate was found during the reinstatement to cocaine seeking in rats that did not consume alcohol. However, in animals that self-administered both cocaine and alcohol, cocaine+cue-primed reinstatement of the operant response that previously delivered cocaine was not accompanied by an increase in glutamate in the nucleus accumbens. In addition, we found that ceftriaxone was not effective in preventing relapse in animals that consumed both alcohol and cocaine, and in

these animals we observed a decrease in glutamate relative to baseline during the reinstatement test. In conclusion, glutamate transmission in the nucleus accumbens does not mediate relapse to cocaine-seeking in animals that consumed ethanol with cocaine. These findings indicate that medications targeting glutamate may not be effective therapies for preventing relapse in humans that drink alcohol with their cocaine.

Disclosures: **B. Stennett:** None. **L. Knackstedt:** None.

Poster

209. Synaptic Transmission: Pharmacology

Location: Hall A

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Topic: B.07. Synaptic Transmission

Support: NSFC 31320103906 to TB

Title: Inhibition of eEF2K enhances hippocampal synaptic transmission by presynaptic p38 MAPKα in rat

Authors: ***W. WENG**, T. BEHNISCH;
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Abstract: The strength of synapses does not only depend upon their history of use but undergoes compensatory scaling together with other synapses to normalize neuronal activity. Whereas compensatory scaling that takes days is widely investigated the mechanisms of fast compensatory scaling are not well understood. Here we present data showing that a 60 min inhibition of NMDA-receptors by MK-801 is sufficient to induce a lasting potentiation of hippocampal synaptic transmission of Schaffer-collateral CA1 synapses. It was suggested that de-novo protein synthesis by altering the activity of the eukaryotic elongation factor 2 kinase (eEF2K; CaMKIII) could represent an underlying mechanism. Thus we studied if inhibition of eEF2K is sufficient to induce an enhancement of synaptic transmission. We found that inhibition of eEF2K by A484954 induces a potentiation of excitatory postsynaptic field potentials (fEPSP) within 10 minutes that lasted for up to 2 hours. In contrast to the initial hypothesis, this enhancement was not prevented by co-application of protein synthesis inhibitors and also not by inhibitors of Erk and Akt. However, inhibition of p38 mitogen-activated protein kinase α and β isoforms (p38 MAPKα/β) was very effective to prevent eEF2K-mediated fEPSP enhancement. Analysis of mEPSCs and paired-pulse stimulated fEPSPs before and after eEF2K inhibitor application points towards a presynaptic origin of the enhanced synaptic transmission. Thus, the data suggest that modulation of p38 MAPKα/β activity by eEF2K could contribute to synaptic scaling by altering presynaptic vesicle release probabilities.

Disclosures: **W. Weng:** None. **T. Behnisch:** None.

Poster

209. Synaptic Transmission: Pharmacology

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: B.07. Synaptic Transmission

Title: Proper statistical analyses of electrophysiological data

Authors: *E. C. CHURCH[1], S. ALFORD[2];
[1]Grad. Program in Neurosci., Univ. of Illinois At Chicago, Chicago, IL; [2]Univ. of Illinois at Chicago, Chicago, IL

Abstract: Many of the most commonly used statistical techniques (e.g. t-tests, ANOVAs) require the data analyzed be normally distributed. As others have pointed out, looking for "statistically significant" deviations from normal can prove less than ideal, particularly when experimental sample sizes are small and more is known about the underlying population from whence the data came. Here, we use simulations in R to test hypotheses regarding patch clamp data analysis and statistician-recommended approaches of data manipulations for distributions common in electrophysiology. Additionally, if we consider a problem that has interested neuroscientists for more than 60 years, probability of release can give rise to a number of different skewed distributions of synaptic response amplitudes that do not meet requirements for commonly used statistical approach. Other analysis approaches must be used to investigate ratio distributions in neuroscience (eg paired pulse ratio) that are mathematically skewed. Different statistical approaches can at times produce wildly different results. Here, we use simulations in R to test hypotheses regarding patch clamp data analysis and statistician-recommended approaches of data manipulations for distributions common in electrophysiology.

Disclosures: E.C. Church: None. **S. Alford:** None.

Poster

209. Synaptic Transmission: Pharmacology

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Topic: B.07. Synaptic Transmission

Support: HFSP Long-Term Fellowship

NIMH Silvio Conte Center 1P50MH094271

Title: Functional characterization of the genomic imprinting of the Bcl-xL gene in mouse cortex

Authors: ***M. D. CAIATI**, L. NEEDLEMAN, C. DULAC, T. K. HENSCH;
Mol. and Cell. Biol., Harvard Univ., Cambridge, MA

Abstract: Genomic imprinting, the epigenetic silencing of either the paternal or maternal allele, leads to the expression of only one working copy of the imprinted gene. Although limited to a small fraction of genes, genomic imprinting plays a crucial role in brain development as its dysfunction has been linked to neurodevelopmental disorders, such as Angelman and Rett syndromes. Here we report the functional consequences of a selective neuronal deletion of either the maternal or paternal allele of the Bcl-xL gene, an anti-apoptotic gene showing a predominant paternal expression in mouse cortex. Mice in which only the maternal allele (MAT mice) had been deleted from cortical neurons were largely comparable to controls. However, several anatomical and physiological alterations were observed in mice devoid of the paternal allele (PAT mice): lower brain size, reduced cortical thickness and reduced neuronal cell number as adults. Interestingly, only glutamatergic but not GABAergic neurons were affected, as confirmed by largely normal inhibitory transmission even in young animals. Instead, excitatory postsynaptic currents recorded from layer 2/3 pyramidal cells in primary visual cortex of P16-18 mice, occurred at higher frequency in PAT mice. Accordingly epileptiform bursts, recorded in the presence of GABAA receptor antagonist bicuculline, produced more spikes/burst in PAT mice, further supporting a dys- regulation of cortical excitation following loss of the paternal allele. Evoked AMPA and NMDA currents upon layer 4 stimulation exhibited normal amplitude and kinetics in both PAT and MAT mice. However, AMPA currents displayed an inward rectification only in PAT mice, indicating the presence of calcium permeable AMPA receptors and confirmed by lower GluR2 AMPA subunit mRNA expression. Notably, GABAB receptor expression was also reduced in PAT mice, implicating their dysfunction in the altered glutamatergic transmission. Altogether, these findings provide strong evidence in support of the functional significance of genomic imprinting in neuronal synaptic transmission, cell signaling and network activity.

Disclosures: **M.D. Caiati:** None. **L. Needleman:** None. **C. Dulac:** None. **T.K. Hensch:** None.

Poster

209. Synaptic Transmission: Pharmacology

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: B.07. Synaptic Transmission

Support: NIH R01 grant NS 040723

Title: Prolonged ketamine exposure induces increase in the expression and activity of GluN2B-containing NMDARs in the neurons of neonatal rat brain

Authors: *S. KOKANE, R. STEVENS, X. ZOU, Q. LIN;
Psychology, Univ. of Texas At Arlington, Arlington, TX

Abstract: Ketamine is a widely used pediatric anesthetic with a strong potential to cause widespread neuroapoptosis in the developing brain when administered repeatedly or for prolonged periods of time. Since ketamine is a non-competitive antagonist of N-methyl-D-aspartate receptor (NMDAR), several studies have proposed that ketamine-induced neuroapoptosis occurs via its effects on NMDAR expression and activity. At the molecular level, prolonged ketamine exposure has been associated with consequent increase in the expression of NMDARs. GluN2 (GluN2A-D) sub-units are involved in regulating the functions of the NMDARs and are differentially expressed across developmental stages - specifically, the expression pattern of GluN2A and GluN2B subunits. Being that GluN2B-containing NMDAR sub-type is predominantly expressed in the brain during the neonatal stage. We hypothesized that this NMDAR sub-type is the basis of increased neuronal vulnerability of the developing brain to ketamine-induced compensatory up-regulation of NMDARs, ultimately resulting in neuroapoptosis. Previous data from our group have shown that ketamine led to time dependent increase in NMDAR channel activity beginning 4 h after the last ketamine dose and peaked at 6 h after the last dose. In the present study, we used Ro 25-6981, a specific GluN2B antagonist, to selectively block GluN2B subunits. We then recorded the channel activity of NMDARs, using whole-cell patch clamp, from forebrain neurons of neonatal (PND 7) rats that were treated with either ketamine (20 mg/kg, six times at 2 h intervals) or saline (control). Our results demonstrated that at 6 h after the last ketamine dose, application of Ro 25-6981 significantly decreased spontaneous excitatory postsynaptic currents (EPSC) mediated by NMDARs. This indicated that NMDAR-mediated channel activity in neurons of the developing brain is majorly regulated by GluN2B sub-units. Western blot analysis revealed that there was also a time-dependent increase in expression and phosphorylation of the GluN2B sub-unit after ketamine administration. Based on these findings, we conclude that prolonged ketamine exposure induces an up-regulation of GluN2B-containing NMDARs in neurons of the developing brain. We propose that this increased expression is closely associated with the neuroapoptosis in these neurons. We thus propose a novel mechanism for ketamine-induced neuroapoptosis mediated specifically by GluN2B sub-unit of the NMDARs in the neurons of the developing brain.

Disclosures: **S. Kokane:** None. **R. Stevens:** None. **X. Zou:** None. **Q. Lin:** None.

Poster

209. Synaptic Transmission: Pharmacology

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Topic: B.07. Synaptic Transmission

Support: CIHR MOP-102617

CIHR New Investigator MOP-109357

Title: Glucose modulation of inputs and outputs in the ventral tegmental area dopamine neurons

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Abstract: Glucose is the only energy source for the human brain under normal conditions. Historically, glucose concentrations in artificial cerebrospinal fluid (aCSF) for brain slice electrophysiology are much higher than in the living brain (10-26 mM), likely to support the health of tissues undergoing hypoxia by providing increased lactate. Brain glucose concentrations are 2.5 mM under physiological conditions, but can rise to 4.5 mM after a meal. Dopamine neurons in the ventral tegmental area (VTA) play a critical role in reward learning and can serve as primary metabolic sensors. While early studies have indicated that alterations in VTA glucose can change dopamine output, little is known if changes in glucose level influences excitatory synaptic transmission onto dopamine neurons in the VTA. Therefore, we investigated differing glucose concentrations on excitatory synaptic transmission of putative dopamine neurons in midbrain slices using whole cell path clamp. When aCSF was prepared with 11 mM glucose, frequency of spontaneous or miniature excitatory postsynaptic currents (EPSCs) was significantly less when aCSF was prepared with 4.5 mM glucose. Glucose concentration did not alter sEPSC or mEPSC amplitude suggesting that glucose concentration primarily influences presynaptic glutamate release probability, but not postsynaptic number or function of AMPA receptors. We also examined glucose effect on dopamine release in the nucleus accumbens (NAc) with *in vivo* fast scan cyclic voltammetry. We observed a decreased evoked DA release in the NAc core with intra-VTA application of 11 mM glucose. Taken together, concentrations of glucose typically used in electrophysiological recordings decrease release probability of glutamate onto VTA neurons.

Disclosures: S. Liu: None. **J. Huang:** None. **S. Borgland:** None.

Poster

209. Synaptic Transmission: Pharmacology

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Title: Alterations in dopamine system regulation of inhibition in an animal model of transcriptional dysregulation and hippocampal circuit dysfunction

Authors: *L. BRADY, A. F. BARTLEY, Q. LI, L. E. DOBRUNZ;
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Abstract: Hippocampal circuit dysfunction and transcriptional dysregulation occur in many neuropsychiatric disorders including schizophrenia. Parvalbumin-positive (PV+) GABAergic interneurons, which play an essential role in hippocampal network activity, are regulated by the transcriptional coactivator PGC-1α. Genetic deletion of PGC-1α in mice results in decreased expression of PV in interneurons, as is seen in schizophrenia. Previous data from our lab has shown that the inhibitory/excitatory (I/E) ratio is increased in PGC-1α-/- mice at the Schaffer collateral - CA1 pathway, caused by enhanced inhibition. This causes a reduction in the spread of activation in hippocampal CA1 as measured with voltage-sensitive dye imaging. PGC-1α-/- mice also have impaired nesting behavior, indicating hippocampal circuit dysfunction. Dopamine (DA) has been shown to modulate GABAergic inhibitory synaptic transmission in hippocampus. Previously we found that haloperidol, a DA receptor antagonist with selectivity for D2-like receptors, has different effects on synaptic transmission and circuit function in PGC-1α-/- mice, suggesting that there are alterations in DA system regulation of the I/E ratio. It is not yet known how alterations in the DA system in PGC-1α-/- mice affect hippocampal circuit function. Here we use PGC-1α-/- mice to investigate the interaction between dysfunction of the dopaminergic and GABAergic systems caused by interneuron transcriptional dysregulation. We find that DA has a disinhibitory effect on synaptic responses in WT and PGC-1α-/- slices, although there was no significant difference in the magnitude. There was also no difference in the effects of a D2 receptor antagonist; instead we saw differences in the effects of D4 antagonists, which increased the field potential in PGC-1α-/- mice due to disinhibition. These data suggest that there is a tonic effect of D4 activation to enhance feedforward inhibition in PGC-1α-/- slices, which may contribute to the enhanced I/E ratio in these mice. DA D4 receptors are located on PV+ GABAergic interneurons in hippocampus, and have been shown to be important for regulating gamma oscillations. Consistent with this, we find enhanced power of kainate-induced gamma oscillations in hippocampal slices from PGC-1α-/- mice. These results suggest that alterations in the DA system's modulation of inhibition, through changes in D4 receptor effects, are involved in the circuit dysfunction caused by deletion of PGC-1α. These results may lead to a greater understanding of circuit dysfunction in disorders of I/E imbalance and provide information for the development of alternate pharmacotherapeutic approaches.

Disclosures: L. Brady: None. **A.F. Bartley:** None. **Q. Li:** None. **L.E. Dobrunz:** None.

Poster

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International Anesthesia Research Society

Title: Differential effects of anesthetics on network activity versus monosynaptic responses in auditory cortex

Authors: *B. M. KRAUSE[1], H. HENTSCHKE[3], A. RAZ[2], M. I. BANKS[2];
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Abstract: Introduction: Loss of consciousness under volatile anesthetics is likely mediated via direct actions on the cortico-thalamic network, but how these agents alter activity at the network level is unclear. The volatile anesthetic isoflurane (ISO) is well known to depress Up states: synchronous, propagating network discharges which share key characteristics with the aroused cortical state and occur spontaneously or are evoked by afferent input. We have previously demonstrated that ISO depresses short-latency ('early', presumably monosynaptic) corticocortical (CC) afferent responses to a higher degree than early thalamocortical (TC) responses (Raz et al., Frontiers in Systems Neuroscience 8:191-2014). Here, we hypothesized that this differential early anesthetic susceptibility translates to the susceptibility of evoked Up states. We investigated the magnitude and horizontal propagation of early responses and Up states elicited by CC or TC stimulation. Methods: Acute auditory thalamocortical brain slices were prepared from 4-13 wk mice. Local field potentials (LFPs) and multiunit activity (MUA) were recorded using silicon multielectrodes (16 shanks, 100μm spacing, 1 site/shank) oriented rostro-caudally in layer 5 of auditory cortex. TC and CC afferents were stimulated with bipolar tungsten electrodes. Early responses were quantified from MUA in a time interval of [1 10] ms post-stimulus. Up states were quantified from the same signal in an interval of [1 1000] ms. Results: Afferent stimulation of both pathways reliably evoked UP states; early responses were not discernible in all cases. Early responses to CC afferent stimulation were usually strongest close to the stimulation site, whereas those evoked by TC stimulation were more dispersed along the recording sites and were usually stronger. Up state activity propagated several mm at a rate dependent on stimulus efficacy (range 8 - 42 μm/ms). Although the lags and direction of propagation of Up states often differed for CC and TC stimuli, their amplitudes and shapes were similar. ISO (0.2 -2.4%) reversibly decreased both early responses and Up states, but the latter much more dramatically. Specifically, at 0.2% ISO, Up states evoked by CC stimuli were already depressed by about 80%, whereas those elicited by TC stimuli were depressed by about half. Early responses were depressed by about 30% (CC) and 15% (TC). Conclusions: The observation that early spiking responses were less affected by ISO than Up state activity suggests

that ISO acts preferentially on synapses within the cortical network, or that small modulatory effects of ISO accumulate across multiple synapses in a polysynaptic chain, or both.

Disclosures: **B.M. Krause:** None. **H. Hentschke:** None. **A. Raz:** None. **M.I. Banks:** None.

Poster

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DTRA/JSTO CBM.THRTOX.01.10.RC.021

DTRA/JSTO CBM.THRTOX.01.10.RC.014

Title: Discovery of novel compounds that restore synaptic communication in synapses intoxicated with botulinum neurotoxin

Authors: *P. H. BESKE, K. M. HOFFMAN, A. B. BRADFORD, P. M. MCNUTT;
US Army Med. Res. Inst. of Chem. Def., Aberdeen Proving Ground, MD

Abstract: Botulinum neurotoxins (BoNTs) are highly lethal bacterial toxins produced by *Clostridium botulinum, C. butyricum*, and *C. barati*. Following internalization into presynaptic termini, BoNTs act with exquisite selectivity to cleave SNARE proteins associated with synaptic vesicle release, thereby preventing the exocytosis of neurotransmitters. The combination of efficient neuronal targeting and presynaptic activity renders BoNTs the most potent substances known. BoNTs are epidemiologically associated with food-borne botulism, but the ease of production, extreme potency, and prolonged intracellular persistence of BoNTs also render the toxin a Tier-1 Biothreat agent. Despite the description of the intracellular target and proteolytic mechanism of BoNTs over 20 years ago, effective therapies to reverse intoxication or restore synaptic function have yet to be identified. To address this gap, we developed a novel synaptic function-based assay termed the “Restoration of Quantal Exocytosis” (ResQuE) assay, which we employed in therapeutic screening studies. Following comprehensive synaptic impairment with BoNT/A, /D, or /E, we applied whole-cell patch-clamp electrophysiology to *in vitro* CNS neuron populations to measure the ability of repurposed drugs to rescue neurotransmission. Using this approach, we have identified a novel category of drugs that restores activity in synapses that are fully paralyzed by BoNT/A. Further testing of effective drugs was also performed in *ex vivo* phrenic nerve-hemidiaphragm preparations. The identification of clinically viable compounds that effectively restore synaptic communication following BoNT/A poisoning represents a

promising therapeutic approach by which BoNT/A-induced paralysis can be reversed.
Disclaimer: The views expressed in this abstract are those of the authors and do not reflect the official policy of the Department of the Army, Department of Defense, or the U.S. Government.

Disclosures: P.H. Beske: None. **K.M. Hoffman:** None. **A.B. Bradford:** None. **P.M. McNutt:** None.

Poster

209. Synaptic Transmission: Pharmacology

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Topic: B.07. Synaptic Transmission

Title: Development of a moderate thoughput assay to detect novel modulators of synaptic efficacy in neuronal cultures

Authors: *R. NEFF[1], C. LINDWALL-BLOM[2], Å. JÄGERVALL[2], B. BALANA[1], M. KARLSSON[2], P. KARILA[2], T. LOVENBERG[1];
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Abstract: In their native environment ion channels involved with fast synaptic neurotransmission are embedded in an intricate protein matrix. This scaffolding, signal transduction, modulatory, and neurotransmitter release machinery plays a critical role in determining the timing and efficacy of the signals that ion channels generate. Traditional high throughput screening approaches, however, primarily rely on heterologous expression systems which lack the ability to replicate synaptic complexity. Here, in a 96 well format, we used Cellaxess Elektra® electric field stimulation to excite cultured mouse cortical neurons loaded with the calcium fluorophore Calcium 5 in an imaging-based microplate reader. By simultaneously stimulating a discrete subset of the neurons in each well we recorded transient, synaptically mediated calcium elevations in neurons privileged from the stimulating field. These responses were mediated by AMPA and NMDA receptors, as evidenced by concentration dependent block by NBQX and MK-801, respectively. Calcium responses in neurons directly stimulated by the electric field, however, persisted in the presence of these compounds. In contrast, the voltage-gated sodium channel blocker tetracaine blocked calcium responses both distant from and within the electric field. In addition we have profiled other modulators of synaptic transmission to further characterize the assay including the NMDAR anagonists Ro 25-6981 and PEAQX, the mGluR5 modulator VU29, and the AMPA modulator LY404187. Together, these data demonstrate a novel, moderate throughput platform for assaying synaptic ion channel activity in a more physiologically relevant context.

Disclosures: **R. Neff:** A. Employment/Salary (full or part-time):; Janssen R&D, LLC. **C. Lindwall-Blom:** A. Employment/Salary (full or part-time):; Cellectricon AB. **Å. Jägervall:** A. Employment/Salary (full or part-time):; Cellectricon AB. **B. Balana:** A. Employment/Salary (full or part-time):; Janssen R&D, LLC. **M. Karlsson:** A. Employment/Salary (full or part-time):; Cellectricon AB. **P. Karila:** A. Employment/Salary (full or part-time):; Cellectricon AB. **T. Lovenberg:** A. Employment/Salary (full or part-time):; Janssen R&D, LLC.

Poster

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Topic: B.07. Synaptic Transmission

Support: BMBF Grant 01GQ1104

Title: Ionic current dynamics through the ultrastructure of dendritic spines

Authors: *D. PATIRNICHE[1], M. BREIT[2], T. WEINKAUF[3], M. STEMMLER[1], S. PHAN[4], E. BUSHONG[4], M. H. ELLISMAN[4], G. QUEISSER[2], A. V. M. HERZ[1];
[1]BCCN Munich, Martinsried-Planegg, Germany; [2]GCSC Frankfurt, Frankfurt a. Main, Germany; [3]KTH, Stockholm, Sweden; [4]NCMIR, UCSD, San Diego, CA

Abstract: The electrical role of dendritic spines is still a topic of intense debate today, more than a hundred years since these structures were first described. Time-dependent variations in spine morphology are likely related to changes in synaptic efficacy. Precise and direct electrophysiological measurements of morphology modulating current initiated at the post-synaptic density are, due to the small size of the dendritic spines, so far unavailable. Optical techniques that relying on signals based on different bio-physical processes than ionic current propagation provide, at best, just a coarse estimate. Using the smallness of the spine to our advantage, we simulate electro-chemical signal transduction on 2D idealized spine-geometries within the full non-linear Poisson-Nernst-Planck framework. The inner volume of spines is densely tiled by highly branched filamentous actin, which, with its electrostatic charges, causes ions to condensate in its vicinity. Within the condensation-profile (known as the electrical double-layer) cations, the main charge carriers for depolarizing phenomena, will propagate with great ease due to the high local concentration. Thus, a significant fraction of the current within the volume of the spines is constrained to flow in nano-slits along charged structures. Consistent biophysical boundary conditions as well as accurate ion-channel kinetics are enforced to guarantee physiological input-output relationships of our model. Micro-second time-step dynamics allows us to investigate how the ultrastructual features of the geometry shape the electrical properties of the dendritic spines. We present preliminary 3D simulations of ionic

current propagation in topologically-consistent segmentations of the filamentous actin network extracted from electron microscopy tomograms of cerebellar dendritic spines.

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Poster

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Title: Beta-neurexin proteins at the synapse: Functional link with the endocannabinoid signaling system

Authors: *G. R. ANDERSON[1], J. AOTO[1], K. TABUCHI[2], C. FÖLDY[1], J. COVY[1], A. YEE[1], D. WU[1], S.-J. LEE[1], L. CHEN[1], R. C. MALENKA[1], T. C. SÜDHOF[1];
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Abstract: Alpha- and beta-neurexins are presynaptic cell-adhesion molecules that have been implicated in the development of autism and schizophrenia neuropsychiatric disorders. Each of the three neurexin genes expressed in mammals contains two distinct promoters. The first promoter sits 5' upstream and initiates transcription of the full length neurexin known as alpha-neurexin. The second promoter is positioned internally within the neurexin gene and serves to initiate transcription of the short, truncated neurexin known as beta-neurexin. While the elimination of alpha-neurexins in neurons has previously been shown to have an essential role in maintaining release probability at both inhibitory and excitatory synapses, the role of beta-neurexins in neurons however is unknown. Here, we find that beta-neurexins are expressed at 10 to 100-fold lower levels than their alpha-neurexin counterparts. Yet despite being only a minor form of the neurexin molecule pool, we find that conditional knockout of beta-neurexins with continued expression of alpha-neurexins dramatically decreases the release probability at a subset of excitatory synapses in the hippocampus. Mechanistically, we find evidence for altered endocannabinoid signaling being present at these synapses upon loss of beta-neurexins. Moreover, deletion of beta-neurexins in CA1 hippocampal neurons impaired contextual fear

memory, suggesting that beta-neurexin-dependent endocannabinoid signaling is behaviorally significant. Thus, our data show that beta-neurexins control synaptic strength in a subset of excitatory synapses, revealing an unexpected role for beta-neurexins in the endocannabinoid-dependent regulation of neural circuits.

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Poster

209. Synaptic Transmission: Pharmacology

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Title: Possible involvement of Dopamine D1-D2 receptor heteromers in enhancement of excitatory synaptic transmission by SKF38393 in CA1 of the hippocampus

Authors: N. RIZVI, *A. C. ARAI;
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Abstract: Formation of heteromers has been reported among G-protein coupled receptors (GPCRs) as well as between GPCRs and ionotropic receptors. In many such cases, cooperativity of agonists exerts a rather complex functional regulation of each receptor and it may also change the coupling of the underlying biochemical cascade. Heteromers of D1- and D2-like dopamine receptors have been identified in the striatum and shown to play a significant role in regulating striatal function. In the present study, we show that SKF38393 (SKF), a D1/D5 dopamine receptor agonist, induces a lasting enhancement of excitatory postsynaptic potentials (EPSPs) in area CA1 of the hippocampus, possibly through activation of dopamine D1-D2 heteromers. SKF (20 μM) increased EPSP amplitude without significantly altering paired-pulse facilitation or waveform, and the enhancement persisted after termination of the drug. The effect of SKF was blocked by administration of an antagonist of D1/D5 receptors (SCH 23390) as well as by a selective antagonist of D2 receptors (sulpiride), but with the interesting difference that the former reversed only a late phase of the enhancement while the latter produced significant inhibition at all time points. These results suggest that combined activation of both D1/D5 and D2 receptors is required for the subsequent enhancement of synaptic transmission. Moreover, the synaptic enhancement appears to involve a non-conventional biochemical cascade in that it was not dependent on activation of adenylate cyclase or protein kinase A, but was abolished by an inhibitor of phospholipase C. Most importantly, the SKF-induced synaptic enhancement was greatly attenuated by application of a peptide which mimics the putative site of interaction between D1 and D2 receptors and according to published studies disrupts dopamine receptor

heteromers. These findings suggest that dopamine receptor heteromerization has a profound influence on synaptic function in hippocampal area CA1.

Disclosures: **N. Rizvi:** None. **A.C. Arai:** None.

Poster

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Title: Role of OculoCerebroRenal Lowe syndrome protein (OCRL) in synaptic function

Authors: ***N. E. MOHAMMED**, M. FERNÁNDEZ-MONREAL;
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Abstract: The enzyme OCRL is one of the main Inositol-5-phosphatases that regulate the levels of cellular Phosphatidylinositol-(4,5)-biphosphate. Mutations in OCRL gene cause Lowe Syndrome, an X-linked rare disease characterised by eye alterations, renal dysfunction and mental disease.Research in cell lines and fibroblasts from Lowe syndrome patients have shown that OCRL is mainly localized in Golgi, and have evidenced its participation in protein trafficking between plasma membrane and intracellular compartments. However, its function in the Central Nervous System is mostly unknown. We aim to characterized OCRL distribution and function in brain using molecular biology, biochemistry and fluorescent microscopy. We have found that OCRL expression increases during development in the hippocampus in parallel with synaptic proteins, which suggest a role in synaptic function regulation. In addition, we have localized OCRL protein in synapses of hippocampal neurons *in vitro*. On the other hand, we have investigated the cellular defects in neurons depleted on OCRL by using lentivirus driving the expression of shRNA for OCRL. We have found that young neurons deficient in OCRL presented alterations on actin cytoskeleton. Interestingly, OCRL depletion did not affect synaptic density in cultured hippocampal neurons. Furthermore, electrophysiological recordings on organotypic slices infected with the shRNA lentivirus showed that basal transmission was not affected by OCRL depletion. Finally, we have found that OCRL was involved in several forms of synaptic plasticity. These results are in agreement with the role of phosphoinositide metabolism in synaptic function, and could explain some of the neuropathological features of Lowe syndrome.

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Poster

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Title: Dopamine release in the prefrontal cortex: implications for the stimulant emission tomography paradigm

Authors: *D. HERNAUS[1], M. MEHTA[2];
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Abstract: Background: PET has been essential in revealing the functional properties of the dopamine (DA) system *in vivo* in humans. These PET paradigms typically utilize either an experimental task[1] or stimulants[2] (e.g. amphetamine challenge) to probe DA release. Although the striatum has often been the focus of attention, PET studies have recently started to investigate DA function in the PFC. However, one important observation is that stimulants make use of an indirect, primarily non-synaptic, route to increase extracellular PFC DA levels[3]. This contrasts with psychological tasks, which rely on an endogenous synaptic mechanism throughout the brain[1; 4]. A pertinent question therefore is: are task- and stimulant-induced PFC DA release captured equally well with PET? Given its more direct mechanism of action, we predicted that task-induced PFC DA release would be more uniformly captured with PET than stimulant-induced PFC DA release. Methods: We first review the mechanisms of task-induced (i.e. endogenous) and stimulant-induced PFC DA release. This is followed by a systematic review and meta-analysis of all PET studies investigating PFC DA function using tasks and stimulants. The aim was to investigate how reliably task- and stimulant-induced PFC DA release is detected with emission tomography. Results: Stimulant-induced PFC DA release was only detected with high-affinity radioligands (FLB 457; not fallypride), greater sample size (n>20) and/or PFC regions that showed the highest DA innervation (anterior cingulate). Task-induced PFC DA release was consistently detected across radioligands (FLB 457 & fallypride), sample sizes and PFC regions. The normalized BPND values were considerably higher for tasks (mean=1.23; SD=.31) than stimulants (mean=.59; SD=.36) (p=.005). Conclude: Although the magnitude of stimulant-induced PFC DA release measured with microdialysis is greater than task-induced PFC DA release (>200% vs. ≈20%), this is not reflected in the PET literature. Task-induced PFC DA release measured with PET is similar to/greater than stimulant-induced PFC

DA release. We suggest that this discrepancy may stem from the fact that stimulants increase PFC DA release outside the vicinity of the D2/3-bound ligand, leading to an underestimation of stimulant-induced PFC DA release measured with PET. In contrast, task-related DA release occurs via a direct route that is more uniformly captured with PET. These results indicate that task and stimulant-induced PFC DA release should be regarded as separate phenomena; detection of one does not guarantee the other.

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Poster

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Title: Quantifying the number of presynaptic voltage-gated calcium channels that are activated at different times during the action potential

Authors: M. SCARNATI, S. CLARKE, *K. G. PARADISO;
Cell Biol. and Neurosci., Rutgers Univ., Piscataway, NJ

Abstract: Small changes in calcium current in a presynaptic terminal will affect synaptic transmission. We examined the number of voltage-gated calcium channels that are opened at different times during the action potential (AP) to better understand how the recruitment of calcium channels during the AP affects calcium entry and subsequent synaptic transmission. During an AP, the number of calcium channels that are open and the driving force for calcium entry are continually changing, making it difficult to measure the number of open calcium channels at different times. To address this, we designed an approach using voltage jumps at different times during an AP waveform to determine the number of calcium channels activated at each time point. Although calcium channels begin to activate during the depolarization of the AP, the majority of calcium entry occurs during the repolarization. This reflects both the time required for the channels to reach an open state, and the changing driving force for calcium entry. During the repolarization, calcium channels continue to open, and the driving force for calcium entry steadily increases which results in the majority of calcium entering during the AP repolarization. Therefore, the duration and shape of the repolarization phase of the presynaptic AP strongly affect calcium entry and therefore synaptic transmission. To determine the amount of calcium channel activation at different times during the AP, we did patch-clamp recordings in calyx of Held nerve terminals and blocked voltage-gated sodium and potassium channels to isolate the presynaptic calcium channel response. Using an AP-like stimulation, we used voltage

jumps to negative potentials during the repolarization phase to determine the relative number of calcium channels that are open at each time point tested. For an AP-like stimulation, we find ~25% of the voltage-gated calcium channels that will open during the AP are open at the peak of the AP. The number of open calcium channels steadily increase during the repolarization phase until the AP reaches approximately 0 mV. When the AP stimulation reaches ~ -10 mV, the number of open channels steadily decreases, and when the stimulus ends at -80 mV, ~20 to 25% of the calcium channels are still open. We also find that 90% of the presynaptic voltage gated channels that are opened by an AP-like stimulus are open when the stimulus is between +20 and -28 mV. Shorter APs shift the peak activation to a later time in the repolarization, and longer APs shift it earlier in the repolarization phase. We also tested the postsynaptic target neuron of the calyx of Held to test the effects of different calcium channel kinetics calcium channel activation during APs.

Disclosures: **M. Scarnati:** None. **S. Clarke:** None. **K.G. Paradiso:** None.

Poster

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Topic: B.07. Synaptic Transmission

Title: MHCI regulates NMDAR-mediated synaptic transmission through cell-autonomous postsynaptic signaling mechanisms

Authors: *K. K. FRIETZE[1], C. M. TYLER[2], L. M. BOULANGER[1,2];
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Abstract: Proteins of the major histocompatibility complex class I (MHCI) are known for their role in the adaptive immune response. MHCI proteins are also expressed by healthy neurons, and are critical for normal brain development and plasticity. Hippocampal synaptic transmission mediated by N-methyl-D-aspartate receptors (NMDARs) is enhanced in mutant mice that express reduced levels of cell-surface MHCI. In addition, NMDAR-dependent hippocampal synaptic plasticity is altered in MHCI-deficient mice, and NMDAR- and hippocampus-dependent forms of learning and memory are impaired. MHCI is expressed in the postsynaptic density of hippocampal neurons, where NMDARs are found, but the molecular mediators of MHCI's effects on NMDARs are unknown. MHCI's effects on NMDAR-dependent synaptic transmission and plasticity have been studied using non-conditional β2m-/-TAP-/- knockout mice, which lack the obligatory MHCI light chain β2m and the MHCI peptide transporter TAP1. These double-mutant mice express reduced levels of MHCI on the surface of most cells throughout life, and have severe immune impairments, including a complete lack of CD4-8+ T cells. Thus changes in NMDAR-dependent synaptic transmission and plasticity in these mice

could be an indirect effect of compromised immune function, or reflect a non-immune role for MHCI in other cell types. Here we show that acutely disrupting MHCI function in individual WT neurons is sufficient to mimic the effects of genetic MHCI knockdown on synaptic transmission. To block MHCI function, we infused competitor peptides derived from the cytoplasmic domain of MHCI into individual WT CA1 pyramidal neurons in acute hippocampal slices via the patch pipette. Infusing MHCI competitor peptides significantly disinhibited NMDAR- but not AMPAR- mediated EPSCs, mimicking the phenotype of β2m-/-TAP-/- mice. Infused peptides likely compete with endogenous MHCI for intracellular binding partners, because they do not affect synaptic transmission in neurons that lack endogenous MHCI. These studies suggest that MHCI cell-autonomously regulates postsynaptic NMDAR function, and identify an unexpected signaling role for the cytoplasmic domain of MHCI at neuronal synapses.

Disclosures: K.K. Frietze: None. **C.M. Tyler:** None. **L.M. Boulanger:** None.

Poster

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Title: TLR3 activation inhibits synaptic activity in primary hippocampal cultures via multiple mechanisms

Authors: L. RITCHIE[1], *T. J. BUSHELL[2];
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Abstract: Toll like receptors (TLRs) belong to a family of pattern recognition receptors that recognise broadly shared molecules found on pathogens referred to as pathogen associated molecular patterns (PAMPs). TLRs are well known for their involvement in innate immunity however despite their presence in the CNS, our knowledge of their function in the CNS is limited. Therefore in the present study, we investigated the cellular localisation of TLR3 and the consequence of its activation on synaptic activity. Primary hippocampal cultures were prepared using P1-2 rats and used experimentally between 10-14 DIV. Standard whole cell patch clamp electrophysiology recordings in voltage and current clamp were used to monitor ion channel function and synaptic activity, respectively. In agreement with previous studies, immunocytochemical experiments revealed TLR3 expression in neurons, astrocytes and oligodendrocytes within our primary hippocampal cultures. Synaptically driven spontaneous action potential (AP) firing was significantly reduced by the acute application (5min) of the TLR3 specific activator, poly I:C (25μg/ml and 200μg/ml). Furthermore chronic poly I:C application (1 hr) resulted in a dramatic reduction in AP firing (1μg/ml and 25μg/ml) in comparison to controls. In agreement with this, poly A:U, another TLR3 activator, resulted in a

significant reduction in AP firing when applied chronically (25µg/ml) whereas the TLR4 activator, LPS, was without effect. Investigations were carried out to determine the mechanisms underlying these effects. Acute application of poly I:C (200µg/ml) significantly reduced the frequency and amplitude of miniature excitatory postsynaptic currents (mEPSCs) however no effect on sodium and potassium channel function was observed. In contrast, chronic application of poly I:C (25µg/ml) resulted in a significant reduction in mEPSC amplitude and peak sodium current. Furthermore chronic application of poly I:C (25µg/ml) resulted in a significant reduction of surface AMPAR expression in comparison to controls when investigated using immunostaining. These data imply that TLR3 activation modulates hippocampal synaptic activity. Further investigations regarding the mechanisms and signalling pathways underlying these effects are on-going and will give further insight into the role TLR3 plays in modulating synaptic activity and how this contributes to virally-mediated behavioural changes.

Disclosures: L. Ritchie: None. **T.J. Bushell:** None.

Poster

209. Synaptic Transmission: Pharmacology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 209.19/B30

Topic: B.07. Synaptic Transmission

Support: Italian Minister of Health GR-2011-02350639

Title: Optogenetic stimulation reveals distinct modulatory properties of thalamostriatal vs corticostriatal glutamatergic inputs to striatal fast-spiking interneurons

Authors: *G. SCIAMANNA[1,2], G. MANDOLESI[2], G. PONTERIO[2], P. BONSI[2], A. PISANI[1]; [1]Dept. Systems Med., Univ. of Rome TorVergata, Roma, Italy; [2]Lab. Neurophysiol. and Plasticity, Fondazione Santa Lucia, Rome, Italy

Abstract: Parvalbumin-containing fast-spiking interneurons (FSIs) exert a powerful GABAergic inhibition on striatal medium spiny neurons (MSNs), playing a critical role in timing striatal output. However, how glutamatergic inputs modulate their firing activity is still unexplored. In the present study by means of combined optogenetic and electrophysiological approach, we provide evidence for a differential modulation of cortico- vs thalamo-striatal synaptic inputs to FSIs in transgenic mice carrying light-gated ion channels channelrhodopsin-2 (ChR2) in glutamatergic fibers. Corticostriatal synapses show a postsynaptic facilitation, whereas thalamostriatal synapses present a postsynaptic depression. Moreover, thalamostriatal synapses exhibit more prominent AMPA-mediated currents than corticostriatal synapses. Furthermore, during current-evoked firing activity, simultaneous corticostriatal stimulation increases bursting activity. Conversely, thalamostriatal fiber activation shifts the canonical burst-pause activity to a

more prolonged, regular firing pattern. Finally by means of viral-injected animals carrying ChR2 in GABAergic fibers we characterized the inibitory input onto FSIs. Our findings demonstrate that cortical and thalamic glutamatergic input differently modulate FSIs firing activity through specific synaptic properties and represents an important step towards dissecting basal ganglia activity in both physiological and pathological conditions.

Disclosures: G. Sciamanna: None. **G. Mandolesi:** None. **G. Ponterio:** None. **P. Bonsi:** None. **A. Pisani:** None.

Poster

209. Synaptic Transmission: Pharmacology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 209.20/B31

Topic: B.07. Synaptic Transmission

Title: Investigating the role of the Gαs pathway in neurotransmission

Authors: *L. MANNING, A. MARTIN, O. BHETUWAL, J. RICHMOND;
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Abstract: The $G\alpha_s$ pathway has been studied as an important G-protein-coupled receptor-mediated modulator of neurotransmission. Specifically, cAMP and one of its primary targets, PKA, are implicated as molecular substrates for learning and memory in a variety of organisms. Previous work in *C. elegans* suggests that the $G\alpha_s$ pathway converges on the core $G\alpha_q$ pathway that is required for UNC-13-dependent priming of synaptic vesicles. However, the molecular mechanisms that underlie $G\alpha_s$ pathway regulation of synaptic function are not well understood. In *C. elegans*, the $G\alpha_s$ pathway is completely dependent on adenylyl cyclase (ACY-1) to increase intracellular levels of cAMP that can bind to the regulatory PKA subunit, KIN-2, releasing, and consequently activating, the catalytic subunit of PKA, KIN-1. A leading hypothesis is that cAMP-dependent PKA activity modulates neurotransmitter release by enhancing the readily releasable pool (RRP) of vesicles. To test this hypothesis, we are conducting an in-depth characterization of $G\alpha_s$ pathway mutants in *C. elegans*. Loss-of-function mutations in *acy-1* are predicted to reduce synaptic transmission, resulting in severe uncoordinated locomotory phenotypes. Conversely, gain-of-function mutations in this gene, which are expected to enhance transmission and confer hyperactivity in worm locomotion. Similarly, Loss-of-function mutations in *kin-2*, which result in constitutively active PKA, mimic the enhanced locomotion seen in *acy-1(g.o.f.)* animals. We first examined mutant sensitivities to the acetylcholinesterase inhibitor, Dylox, as a readout of synaptic function. We found *kin-2* and *acy-1(g.o.f.)* mutants to be Dylox-hypersensitive, where as *acy-1* mutants showed wild-type responses to the drug. Evidence from electrophysiological recordings at the neuromuscular junction (NMJ) of mutant worms suggests that mutations hyperactivating $G\alpha_s$ mildly enhance evoked release, and opposite

phenotypes are observed in *acy-1(l.o.f)* mutants. Ongoing experiments examining synaptic depression and release under low calcium conditions will test whether ACY-1 and PKA influence the RRP as predicted. Fluorescence and electron microscopic imaging provide additional insight into synaptic vesicle regulation by these proteins. Imaging both vesicular and non-vesicular synaptic makers suggests altered vesicle localization in $G\alpha_s$ mutants. Preliminary data from ultrastructural analysis suggests that there may be a change in docking of synaptic vesicles and the number of dense core vesicles (DCV) at *acy-1(lf)* and *kin-2* synapses, suggesting that the $G\alpha_s$ pathway also regulates a neuropeptide release pathway.

Disclosures: L. Manning: None. **A. Martin:** None. **O. Bhetuwal:** None. **J. Richmond:** None.

Poster

209. Synaptic Transmission: Pharmacology

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Topic: B.07. Synaptic Transmission

Support: DFG Hu797/7-1, 8-1 and CNMPB

Title: Alpha3 glycine receptors in glycinergic transmission to hypoglossal motoneurons

Authors: *S. HÜLSMANN[1], Y. KONO[2];
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Abstract: Glycinergic neurons provide an important mechanism to control excitation of hypoglossal motoneurons. Impairment of glycinergic inhibition can be deleterious such as in hyperekplexia or amyotrophic lateral sclerosis. Second messenger systems that change cyclic AMP and lead to phosphorylation of the alpha3 subunit (Glra3) of the glycine receptor have been shown to be potent modulators of synaptic inhibition. In this study we analyzed the role of Glra3 on glycinergic miniature synaptic inhibitory currents (mIPSCs) in the hypoglossal nucleus. We observed that glycinergic mIPSCs are normal in Glra3 -/- mice. However we found a slight increase of the glycinergic mIPSC amplitude in older Glra3 -/- mice. Modulation of synaptic transmission by cAMP-mediated pathways was diminished in Glra3 -/- mice. Forskolin (10 μM) induced an increase of mIPSC frequency in both Glra3 +/+ and Glra3 -/- mice. However, in the second postnatal week the forskolin-induced increase of mIPSC frequency was significantly larger in Glra3 +/+ as compared to Glra3 -/- mice indicating that glycine release in the hypoglossal nucleus can be modulated via presynaptic α3 containing glycine receptors.

Disclosures: S. Hülsmann: None. **Y. Kono:** None.

Poster

209. Synaptic Transmission: Pharmacology

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Topic: B.07. Synaptic Transmission

Support: Ministry of Education, Culture, Sports, Science and Technology, and the Ministry of Health and Welfare of Japan (22390109 to K.F.; 20790398 to S.M.)

Title: Sigma-1 receptor stimulation improves depressive-like behaviors and adult hippocampal neurogenesis in CaMKIV null mice

Authors: *S. MORIGUCHI[1], H. SAKAGAMI[2], K. FUKUNAGA[1];
[1]Tohoku Univ. Grad Sch. Pharm Scis, Sendai, Japan; [2]Kitasato Univ. Sch. Med., Sagamihara, Japan

Abstract: Sigma-1 receptor (Sig-1R) is molecular chaperone regulating calcium efflux from endoplasmic reticulum to mitochondria in neurons (Shioda et al, 2012). We previously documented that impaired cognition and depressive-like behaviors in olfactory bulbectomized mice are improved by chronic oral administration of dehydroepiandrosterone (DHEA) (Moriguchi et al., 2011; PLoS One 2013). It is reported that calcium/calmodulin-dependent protein kinase IV (CaMKIV) is involved in adult hippocampal neurogenesis, in which fluoxetine induces CaMKIV-dependent CREB phosphorylation in the hippocampal dentate gyrus (Song et al., 2013). In fact, CaMKIV null mice showed the depressive-like behaviors and reduced neurogenesis assessed by bromodeoxyuridine (BrdU)/NeuN incorporation in the dentate gyrus. In the present study, we demonstrated that chronic stimulation of Sig-1R by treatment with SA4503 or fluvoxamine for 14 days improve depressive-like behaviors observed in CaMKIV null mice. By contrast, paroxetine without affinity for Sig-1R failed to improve the depressive-like behaviors. Reduction of BrdU/NeuN-positive cells or Akt (Ser-473) phosphorylation in the hippocampal dentate gyrus in CaMKIV null mice was significantly restored by chronic administration of Sig-1R agonists. Taken together, Sig-1R stimulation ameliorates depressive-like behaviors in CaMKIV null mice by increased adult hippocampal neurogenesis through activation of Akt.

Disclosures: S. Moriguchi: None. **H. Sakagami:** None. **K. Fukunaga:** None.

Poster

210. Synaptic transmission: Modulation

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Topic: B.07. Synaptic Transmission

Support: Gertrude Ribble Research Fellowship

DAAD - RISE

Title: Acute and chronic effects of inhibiting mTOR by rapamycin on development, behvaior, and physiology in *Drosophila*

Authors: *S. J. POTTER[1,2], R. S. POTTER[2], S. L. E. BLÜMICH[3], R. L. COOPER[2]; [2]Biol., [1]Univ. of Kentucky, Lexington, KY; [3]Vet. Sci., Univ. of Leipzig, Leipzig, Germany

Abstract: Rapamycin is a compound that can specifically block mTOR signaling and is therefore used in experimental biology. It is being utilized clinically as an immunomodulator after transplantation procedures and treatment for some forms of cancer. Due to its many possible effects on different molecular pathways, it could have any number of impacts on synaptic transmission. This issue has not, however, been addressed in a developing system. We hope to address it by feeding second and third instar *Drosophila* larvae varying concentrations of rapamycin and monitoring larval stages, pupation, and survival. Typical larval behavioral assays being examined are mouth hook movement while eating and body wall movement while crawling on apple juice agar plates. Behaviors in the adults fed rapamycin include climbing, righting response, and movement assays. The results to date suggest 2nd instar larvae are more susceptible to rapamycin as compared to 3rd instar, based on a higher death rate. Adults fed rapamycin climb less over time and tend to fall off the wall when climbing. Dose-response studies are being established. This study is significant as we are starting to address the acute and long-term action of inhibiting the mTOR pathway on neuronal function and potential mechanisms to account for altered physiological function.

Disclosures: S.J. Potter: None. **R.S. Potter:** None. **S.L.E. Blümich:** None. **R.L. Cooper:** None.

Poster

210. Synaptic transmission: Modulation

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Program#/Poster#: 210.02/B35

Topic: B.07. Synaptic Transmission

Title: Functional and morphological analysis of Neuronal Ca^{2+}-binding protein 2 (NECAB2)

Authors: *A. METHNER[1], T. SCHACHT[2], K. FRAUENKNECHT[3], V. WUELLNER[2], S. RITZ[4], L. MAJEWSKI[5], L. GREMER[6], J. KUZNICKI[5];
[1]Johannes Gutenberg Univ. Mainz, Mainz, Germany; [2]Neurol., [3]Neuropathology, Univ. Med., Mainz, Germany; [4]IMB, Mainz, Germany; [5]The Intl. Inst. of Mol. and Cell Biol., Warsaw, Poland; [6]Inst. für physikalische Biologie, Heinrich Heine Univ., Duesseldorf, Germany

Abstract: NECAB2 is a **Ne**uronal **Ca**$^{2+}$-**b**inding protein characterized by the combination of EF-hand Ca^{2+}-binding domains, two coiled-coil domains (CC) and a monooxygenase (ABM) domain. It is expressed exclusively in the brain with strongest expression in the striatum where it co-localizes and interacts with metabotropic glutamate and adenosine receptors increasing their basal constitutive signaling. High concentrations of Ca^{2+} drastically reduce the ability of these proteins to interact with NECAB2. Prokaryotic homologs of NECAB2 possess monooxygenase activity and are involved in the detoxification of reactive oxygen species, suggesting a similar function in eukaryotes distinct from the interaction with membrane receptors. A structural analysis of prokaryotic NECAB2 homologs also suggests that the active enzyme is a homodimer. NECAB2 is indeed highly expressed in the striatum, the habenulae, and, with a predominant neuropil pattern, in the hippocampus. It is highly enriched in synaptic microsomes and colocalizes with presynaptic and postsynaptic marker proteins. Recombinant NECAB2 binds Ca^{2+} and dimerizes and oligomerizes similar to its prokaryotic orthologues. Ca2+ binding increases dimerization of mammalian NECAB2. We generated mutants of NECAB2 which lack distinguishable domains (CC1, CC2 or ABM) or have a non-functional EF hand (by mutating Glu residues to Ala) to further address the different functions of NECAB2. We found that the CC1 domain is responsible for the interaction with the metabotropic glutamate receptor 5 and homodimerization. A thorough analysis of the phenotype of NECAB2-deficient mice, which are viable, is currently underway and might be ready by the time of the annual meeting. Our data suggests that the increase in the cytosolic Ca^{2+} concentration causes homodimerization of NECAB2 in the cytosol, resulting in subsequent receptor desensitization in a negative feedback loop. The dimerization probably activates its monooxygenase activity, which probably serves to process reactive oxygen species and protons generated in active, stimulated neurons

Disclosures: **A. Methner:** None. **T. Schacht:** None. **K. Frauenknecht:** None. **V. Wuellner:** None. **S. Ritz:** None. **L. Majewski:** None. **L. Gremer:** None. **J. Kuznicki:** None.

Poster

210. Synaptic transmission: Modulation

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Topic: B.07. Synaptic Transmission

Support: FAPESP 2014/16711-6

CAPES

Title: Connexin 36 in hippocampus of rats submitted to neonatal anoxia

Authors: *S. H. TAKADA[1], J. M. IKEBARA[1], D. S. CARDOSO[1], E. SOUSA[1], G. S. V. HIGA[2], A. H. KIHARA[1];
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Abstract: Neonatal anoxia is the main cause of death in the perinatal period, especially in premature infants. When surviving the anoxic insult, most of these individuals have motor deficits, behavioral, sensory and /or cognitive impairments that persist throughout life and, for these reasons, neonatal anoxia is considered a major public health concern. Although extensively studied in different models of neonatal oxygen deprivation, the mechanisms of hippocampal neuronal death resulting from oxygen deprivation remains to be elucidated, particularly the injury that occurs secondary to the initial hippocampal cell death. Hypotheses about the involvement of connexin (Cxs) channels in the gap junctions (GJ) in the spreading of death caused by hypoxia-ischemia have been proposed, but in other models of deprivation of blood or oxygen. The aim of this study was to identify possible changes in the expression of Cx 36 caused by anoxic insult in neonatal rat. Eight litters of Wistar rats (Rattus norvegicus) were used for anoxia and sham groups. Anoxia was performed in P1/P2 neonates according to described system of neonatal anoxia composed by 25 minutes of 100% nitrogen gas exposure at 37°C (Takada et al., 2011). 24 hours after neonatal anoxia, the PCR analysis showed a decreased genic expression of Cx36 in anoxia treated animals ($p<0.05$). In the western blotting analysis, the protein levels of Cx36 was decreased too ($p=0.0096$). Interestingly, the density of immunolabelled hippocampal cells for Cx36 did not present differences in all hippocampal subfields. These results allow further investigation of Cx36 involvement in hippocampal cell death caused by anoxic insult and provide support for therapeutic approaches related to gap junctions functioning.

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Poster

210. Synaptic transmission: Modulation

Location: Hall A

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Support: USDoEd GAANN Grant

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Title: Synchronizing cortical dynamics via gap junctions between excitatory neurons

Authors: *J. KILE[1], G. KOVACIC[2], D. CAI[3];
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Abstract: Brain networks are known to give rise to global oscillations that are linked to synchronized neuronal activity, which has been shown to contribute to cognitive processes such as perception, motor performance, learning and memory. Electric coupling through gap junctions may facilitate the emergence of synchronized oscillations, and influence their properties. Gap junctions between inhibitory neurons in the mammalian cerebral cortex have been well studied, but gap junctions between excitatory, pyramidal neurons have only recently been discovered experimentally. In this study, we closely follow experimental data to construct a detailed, comprehensive model with both synaptic and electric coupling for both excitatory and inhibitory neurons using a modified version of the Hodgkin-Huxley equations. We have successfully replicated data for the interaction between pairs of electrically connected excitatory neurons, and pairs of electrically connected inhibitory neurons, using our mathematical model. Our realistic, computational network model includes 25% inhibitory neurons and 75% pyramidal cells coupled both electrically and synaptically. We use a 5% coupling probability for gap junctions to occur among neighboring pyramidal cells, and a 50% coupling probability for gap junctions between interneurons less than 80μm apart, mimicking the medial prefrontal cortex, and visual cortex, of rats and ferrets. We describe a uniform, external input to this small patch of the cortex, modeling the influences of neurons from other parts of the brain, in the form of a Poisson spike train. The neurons are placed on a grid to capture the highly structured spatial properties of a network containing both synaptic and gap-junction connections, and to ensure that the probability of neurons being coupled is dependent on their location within the network. Using this network model, we examine the resulting dynamical regimes from the inclusion of both electric and synaptic connections, with a specific interest in the emergence and properties of synchrony. We find that the addition of gap junctions increases the synchrony in the model network.

Disclosures: J. Kile: None. **G. Kovacic:** None. **D. Cai:** None.

Poster

210. Synaptic transmission: Modulation

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Program#/Poster#: 210.05/B38

Topic: B.07. Synaptic Transmission

Title: Sensitivity of presynaptic pH on synaptic transmission: Differences in evoked and spontaneous release

Authors: *W. P. PIEDADE[1,2], F. KOCH[3], Z. MAJEED[4], E. BRAILOIU[5], S. L. E. BLÜMICH[6], R. PUTMAN[7], R. L. COOPER[2];
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Abstract: The presynaptic terminals package transmitter into vesicles based on a proton gradient. We address issues related to altering this gradient in influencing synaptic responses. We are addressing two issues: (1) the influence of pH_i on vesicular packaging of neurotransmitter; (2) response of glutamate receptors on postsynaptic targets with altering extracellular and intracellular pH. These investigations are being addressed at the model crayfish and *Drosophila* neuromuscular junctions (NMJs). These two projects are interrelated as transmission at glutamatergic synapses is retarded in the presence of CO_2 which cannot be fully accounted for by a reduced pH_i within the presynaptic nerve terminal or within the postsynaptic muscle fiber since the EPSPs increase in amplitude with rebound acidification after a pulse of NH4Cl. High (40 mM) proprionic acid acidifies both the pre- and post-synaptic cells. When used the frequency and amplitude of mini's increases despite a slight membrane depolarization. However, evoked transmission is blocked. Examining low pH on mammalian glutamatergic neurons with Fura 2 (Ca2+ indicator) in culture indicated Ca2+ release from ER as a potential mechanism to explain some of the observations for the increase in frequency of minis. The use of high [CO_2] containing saline blocks evoked and mini's as well as the sensitivity of glutamate receptors. These NMJs are glutamatergic and the evoked (non-spiking) synaptic potentials and spontaneous (quantal) events are readily measured. Addressing the mechanisms underlying these observed phenomena may help in understanding synaptic depression after high frequency stimulation and feedback process in synaptic transmission. These studies tackle fundamental principles which are likely present in glutamatergic neurons for all animals.

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Poster

210. Synaptic transmission: Modulation

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Program#/Poster#: 210.06/B39

Topic: B.07. Synaptic Transmission

Title: Functional interaction between presynaptic nicotinic and NMDA receptors on dopaminergic and noradrenergic nerve terminals of the rat central nervous system

Authors: M. GRILLI[1], G. OLIVERO[2], C. PADOLECCHIA[2], J. CHEN[2], A. PITTALUGA[2], *M. MARCHI[2];
[1]DiFAR, [2]Univ. of Genova, Genova, Italy

Abstract: It is well known that cross-talk between receptors represent an important mechanism of neuromodulation and plasticity. Although, these interactions have been mostly localized post-synaptically, receptors cross-talk which involves common intracellular pathways have been reported to occur also at the presynaptic level (for a recent review see Marchi et al 2015). Neuronal nicotinic acetylcholine receptors (nAChRs) in the CNS are located mostly presynaptically and have been implicated in facilitating release of neurotransmitter. It has been shown that dopaminergic and noradrenergic axon terminals in the nucleus accumbens and hippocampus possess nAChRs mediating enhancement of dopamine (DA) and noradrenaline (NA) release respectively. We investigated whether nAChRs and N-methyl-D-aspartic acid (NMDA) receptors interact on the same nerve endings using rat synaptosomes pre-labelled with [3H]DA or [3H]NA. The *in vitro* short-term pre-exposure of synaptosomes (10 min) to different concentrations of acetylcholine (from 0.01μM to 10 μM) caused a significant reduction (maximal effect: -54.0 % at 10 μM) of the 100 μM NMDA-evoked [3H]DA overflow in the rat nucleus accumbens . This inhibitory effect was abolished when synaptosomes were pretreated with acetylcholine plus atropine (0.1 μM) and completely counteracted when nerve endings were pretreated in the presence of the selective antagonist DHβE indicating that the changes of the NMDA-dependent [3H]DA release reported was dependent to the activation of a β2* nAChR subtype. Similarly, the pre-exposure of synaptosomes to nicotine (from 0.01μM to 30 μM) caused a significant reduction of the 100 μM NMDA-evoked [3H]NA overflow in the rat hippocampus. Nicotine pre-incubation failed to modify the 10 μM 4-aminopyridine-induced [3H]NA overflow suggesting that the changes in the exocytotic machinery of release does not account for the nicotine-induced modifications of the NMDAR function. The pre-exposure of synaptosomes to nicotine or acetylcholine also caused a marked reduction of the nicotine-induced [3H]DA or [3H]NA overflow (-85.0% and -91.2 % respectively) suggesting that these nAChRs could undergo an agonist-induced receptor desensitization. To conclude our results show that the NMDA receptor function can be dynamically and negatively regulated in neurons in response to a brief incubation with different nicotinic agonists. Marchi et al., (2015) Front. Pharmacol., doi: 10.3389/fphar.2015.00089

Disclosures: **M. Grilli:** None. **G. Olivero:** None. **C. Padolecchia:** None. **J. Chen:** None. **A. Pittaluga:** None. **M. Marchi:** None.

Poster

210. Synaptic transmission: Modulation

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Brain & Behavior Research Foundation (NARSAD) grant

Title: Maternal deprivation induces hyperexcitablity and synaptic abnormalities of lateral habenula glutamatergic neurons

Authors: *M. E. AUTHEMENT, F. NUGENT;
Pharmacol., Uniformed Services Univ., Bethesda, MD

Abstract: Adverse early life experiences increase the risk of cognitive impairment and later life mental health disorders such as depression, schizophrenia and substance abuse. Maternal deprivation (MD) is one particularly potent early life stressor that impairs brain dopamine (DA) and serotonin (SER) systems, both of which are implicated in the aforementioned disorders. The lateral habenula (LHb) exerts strong inhibitory influence over the brain monoaminergic systems, namely DA and SER systems. Here we investigated whether a single 24 hour episode of MD promotes dysfunction of DA and SER systems through changes in the activity of the LHb. Using a combination of whole-cell and cell-attached electrophysiology in rat brain slices, we show that MD induces a robust hyperexcitability of glutamatergic LHb neurons. We also examine the contribution of both excitatory and inhibitory synaptic transmission onto glutamatergic LHb neurons to determine whether MD-induced hyperexcitability is due to an imbalance of excitation and inhibition. Overall, MD-induced hyperexcitablity of LHb neurons and synaptic abnormalities may be the mechanistic link between early-life stress and an increased later-life propensity for mental health disorders involving dysfunction of brain DA and SER systems.

Disclosures: M.E. Authement: None. **F. Nugent:** None.

Poster

210. Synaptic transmission: Modulation

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Title: Connexin36 expression in major centers of the auditory system in mouse and rat: evidence for neurons forming purely electrical synapses and morphologically mixed synapses

Authors: *M. E. RUBIO[1], J. I. NAGY[2];
[1]Otolaryngology, Univ. of Pittsburgh Med. Sch., Pittsburgh, PA; [2]Dept. of Physiol. and Pathophysiology, Fac. of Med., Univ. of Manitoba, Winnipeg, MB, Canada

Abstract: Electrical synapses formed by gap junctions composed of connexin36 (Cx36) are widely distributed in the mammalian central nervous system. Here, we used immunofluorescence methods to document the expression of Cx36 in the cochlear nucleus and along upstream nuclei of the auditory pathway of rat and mouse. Labelling of Cx36 visualized exclusively as Cx36-puncta was densely distributed primarily on the cell body and initial dendrites of neuronal populations in the ventral cochlear nucleus, and was abundant in superficial layers of the dorsal cochlear nucleus. Other auditory centers displaying Cx36-puncta included the medial nucleus of the trapezoid body (MNTB), regions surrounding the lateral superior olivary nucleus, the dorsal nucleus of the medial lemniscus, the nucleus sagulum, all subnuclei of the inferior colliculus, and the auditory cerebral cortex. In EGFP-Cx36 transgenic mice, EGFP reporter was detected in neurons located in each of auditory center that harboured Cx36-puncta. In the ventral cochlear nuclei and the MNTB, many neuronal somata were heavily innervated by nerve terminals containing vesicular glutamate transporter-1 (vglut1) and Cx36 was frequently localized at these terminals. Cochlear ablation caused a near total depletion of vglut1-positive terminals in the ventral cochlear nuclei, with a commensurate loss of labelling for Cx36 around most neuronal cell bodies, but preserved Cx36-puncta at somatic neuronal appositions. The results suggest that electrical synapses formed by Cx36-containing gap junctions occur in most of the widely distributed centers of the auditory system. Further, it appears that morphologically mixed chemical/electrical synapses formed by nerve terminals are abundant in the ventral cochlear nucleus, including those at endbulbs of Held formed by cochlear primary afferent fibers, and those at calyx of Held synapses on MNTB neurons.

Disclosures: **M.E. Rubio:** None. **J.I. Nagy:** None.

Poster

210. Synaptic transmission: Modulation

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Title: Interaction between Aβ and membrane cholesterol underscores presynaptic transmission

Authors: *R. M. LAZARENKO[1], A. KAMALOVA[2], Q. ZHANG[3];
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Abstract: Synaptic dysfunction is known to precede the clinic symptoms of Alzheimer's disease (AD) and if the neurodegeneration to be prevented - that is the time point of intervention. Ample evidence supports the notion that β-Amyloid peptides (Aβs) are genetically and pathologically associated with AD. Furthermore, Aβs alter the structure and function of neuronal synapses via mechanisms that remain unclear. Recent structural studies revealed that the C-terminal fragment (C99) of Amyloid Precursor Protein (APP) can directly bind to cholesterol via six key amino acid resides, which all reside in Aβ40, the most common form of Aβs. In neonatal as well as mature brain, cholesterol is essential for synaptogenesis and synaptic plasticity. In particular, cholesterol plays an important role in the origination and maintenance of synaptic vesicles. Therefore, we set up to investigate how APP and Aβ interact with synaptic membrane cholesterol and how such interaction affects synaptic transmission. By using TopFluor cholesterol (TFC) as a fluorescent surrogate for cholesterol, we monitored in real time how Aβ or APP affects the distribution of cholesterol in the membranes of cultured hippocampal neurons and consequently regulates evoked or spontaneous synaptic transmission. Our results indicate that the application of Aβ40 or the direct manipulation of synaptic membrane cholesterol causes profound changes to spontaneous release of synaptic vesicles. We find that Aβ40 sequesters and enriches cholesterol on the plasma membrane. The magnitude of the effect depends on the levels of endogenous APP or Aβ40 level and is higher in APP-overexpressing cells. These results collectively suggest that soluble Aβ40 stabilizes cholesterol in the neuronal membranes, which facilitates synaptic transmission. We thus propose that the abnormality in Aβ species or amount along with the unbalanced cholesterol homeostasis impairs presynaptic integrity and leads to synaptic dysfunction or neurodegeneration.

Disclosures: R.M. Lazarenko: None. **A. Kamalova:** None. **Q. Zhang:** None.

Poster

210. Synaptic transmission: Modulation

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Topic: B.07. Synaptic Transmission

Support: NIH Grant R01NS 072238

Title: Simulation of synchronization and reverberation of excitation in a network of neurons interconnected through a 16-state gating model of gap junction channels

Authors: *F. BUKAUSKAS[1,2], K. MACIUNAS[2], M. SNIPAS[3,2];
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Abstract: Gap junction (GJ) channels formed of connexin (Cx) proteins provide a direct pathway for electrical cell-cell interaction in neuronal networks. We combined Hodgkin-Huxley equations of membrane excitability with a gating model of GJ channels to simulate the spread of excitation in neuronal networks coupled by gap junctions. The model includes changes of junctional conductance (g_j) induced by transjunctional voltage, unitary conductances and I-V rectification of *fast* and *slow* gates, and allows analysis of synchronization of weakly coupled neuronal networks and changes in g_j at macroscopic and single channel levels. We show that inhomogeneity of I-V rectification can cause unidirectional action potential transfer resulting in reverberation of excitation in the 2-D network composed of more than 100 cells in response to a single depolarizing electrical stimulus and can be terminated by a single depolarizing or hyperpolarizing electrical stimulus if applied in a relatively narrow 'window' of the reverberation cycle. The model accounts for the influence of dynamically modulatable electrical synapses in shaping network responses and the formation of reverberation, which, as proposed earlier, may be important for the development of a short-term memory and its consolidation into a long-term memory.

Disclosures: F. Bukauskas: None. **K. Maciunas:** None. **M. Snipas:** None.

Poster

210. Synaptic transmission: Modulation

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Program#/Poster#: 210.11/B44

Topic: B.07. Synaptic Transmission

Support: RO1 MH96899

Title: Effect of endogenous estradiol on the lateral amygdala synapses

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Abstract: Evidence suggest that changes in estradiol levels exert a profound effect on anxiety-like symptoms and affective behaviors. Endogenous estradiol has been shown to enhance fear extinction, an inhibitory learning believed to play an important role in relieving anxiety- and trauma-related symptoms which are also risk factors for affective disorders. However, the mechanism underlying these effects is largely unknown. A recent study from our group showed

that endogenous estradiol favors synaptic potentiation in the medial prefrontal cortex, a potential mechanism in fear extinction. Here, we studied whether endogenous estradiol affects excitatory synaptic transmission in the lateral nucleus of the amygdala (LA), which serves as the sensory interface of the amygdala, a key brain region involved in fear memory processing. Comparing diestrus mice (low estradiol) with proestrus mice (high estradiol), we observed an increase in the spontaneous glutamatergic transmission in the principal neurons of LA during periods of high estradiol. Our results suggest that endogenous estradiol modulates excitatory synapses in the lateral amygdala which might play a role in the regulation of fear behavior.

Disclosures: A. Manganaro: None. **I. Ninan:** None.

Poster

210. Synaptic transmission: Modulation

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Topic: B.07. Synaptic Transmission

Support: FAPESP 2013/08211-0

Title: Magnetic nanoparticles induce hyperalgesia in rats

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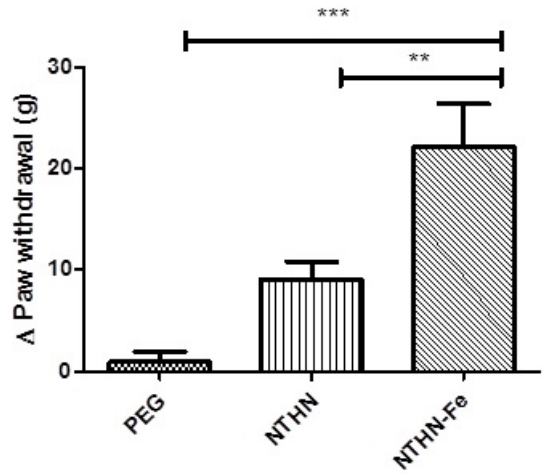
Abstract:



The interaction between living organisms and nanoparticles has been growing, thus raising more concerns about its effects and consequences. This work aims at investigating the consequences of the administration of high magnetic (NTHN-Fe) and low magnetic (NTHN) nanoparticles in the subarachnoid space of rats. Rats that received 0,5 ug/uL (polyethyleneglycol as vehicle at a total volume of 20 uL) of highly magnetic nanoparticles showed a time-dependent decrease in the paw withdrawal threshold in the Electronic Von Frey test when compared to animals that received low-magnetic nanoparticles, such effect was present even when dexamethasone (1 mg/Kg) was administered (i.p) 1 hour prior to the nanoparticles. The hyperalgesia was set 20 minutes after administration and gradually decreased until the 24th hour. These data are very intriguing because the highly magnetic nanoparticles NTHN-Fe induced a strong hyperalgesia while NTHN induced it weakly (figure 1). Since dexamethasone was pre-injected and nevertheless the hyperalgesia was not reverted, the hyperalgesia was not due to an inflammation caused by the nanoparticles. According to the cytometry assay, dorsal root ganglion (DRG) cells incubated with the nanoparticles (NTHN-Fe) for 24 hours showed higher granulocity compared to non-incubated cells (53,8 and 45,4 respectively). This data may suggest that the nanoparticles are internalized by the cells. The strong hyperalgesia caused by highly magnetic nanoparticles may be due to a magnetic effect which depends on the spacial orientation of the nanoparticles in the extra-cell space and once the cells internalize the nanoparticles, the effect is gone. We, however, cannot assure how and why this effect is seen. More efforts are being made to assess this very intriguing phenomenon.

Disclosures: **L. Manzo:** None. **H. Ceragioli:** None. **I.J.M. Bonet:** None. **C.A. Parada:** None.

Poster

210. Synaptic transmission: Modulation

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Topic: B.07. Synaptic Transmission

Title: Exploring the roles of electrical and chemical synapses in a *C. elegans* chemotaxis circuit

Authors: *M. DOBOSIEWICZ[1], J. LARSCH[2], C. BARGMANN[1];
[1]The Rockefeller Univ., New York, NY; [2]Max Planck Inst. of Neurobio., Munich, Germany

Abstract: Neurons communicate through chemical synapses, which propagate directed signals through neurotransmitter release, and through electrical synapses, in which current and small molecules can pass bidirectionally between two neurons. In the nervous systems of *C. elegans*, the only organism with a complete anatomical wiring diagram, there are 600 predicted electrical synapses as well as ~7000 chemical synapses, and most neurons have both types of connections. Despite the prevalence of both electrical and chemical synapses, their relative contributions in transmitting information are poorly understood. *C. elegans* senses the attractive odor diacetyl

using the AWA sensory neuron. AWA forms chemical synapses and electrical synapses onto several interconnected interneurons, which in turn connect to command and motor neurons to drive the animal's chemotaxis toward diacetyl. We are studying the AWA circuit using a high-throughput microfluidic device that allows us to deliver odor stimuli in precise patterns while simultaneously recording calcium levels in single neurons as a proxy for neural activity (Larsch et al., 2013). We find that AWA responds to odor in a complex, concentration-dependent manner, but one downstream interneuron gives a stereotyped response to odor increases, regardless of their magnitude. This interneuron may thus be responsible for signaling any change in odor concentration, while information about absolute concentration may be transmitted through other neurons in the circuit. AWA connects to this normalizing interneuron exclusively with electrical synapses. We have used synaptic mutants and transgenic animals to selectively manipulate electrical and chemical synapses and explore the consequences on circuit dynamics. Our initial results suggest that the electrical and chemical synaptic components of the circuit account for distinct temporal components of the odor response.

Disclosures: M. Dobosiewicz: None. **J. Larsch:** None. **C. Bargmann:** None.

Poster

210. Synaptic transmission: Modulation

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Topic: B.07. Synaptic Transmission

Support: NIH NS 059072

NIH NS 070084

Title: Synaptic mechanisms underlying suppression of neuronal activity induced by Cortical spreading depression (CSD)

Authors: *P. S. SURYAVANSHI, P. M. SAWANT-POKAM, K. C. BRENNAN;
Univ. of Utah, Salt Lake City, UT

Abstract: Cortical spreading depression (CSD) is a self-propagating wave of neuronal and glial depolarization. Apart from its association with several forms of brain injury, CSD is widely considered to be the physiological correlate of migraine aura, a form of sensory hallucination preceding migraine attacks. CSD causes long lasting changes in sensory processing - sharpening of sensory maps, potentiation of response and blunted adaptation. However precise synaptic and cellular mechanisms underlying these network changes are not fully understood. In this study, we used *in vivo* whole cell current clamp techniques to test effects of CSD on evoked and spontaneous post-synaptic potentials (PSPs) from layer 2/3 neurons in mouse somatosensory cortex. Upon CSD induction, the membrane potential rapidly depolarized resulting in of action

potentials followed by complete silencing of neuronal activity. Although rheobase was increased 5 minutes after CSD induction, other measures of excitability such as slope of the frequency current curve and input resistance were not affected by CSD. The frequency and amplitude of spontaneous PSPs were suppressed from 5 minutes up to 30 minutes post-CSD. CSD induced modification of excitatory and/or inhibitory synaptic activity could be responsible for reduction in PSPs we observed. To uncover the precise synaptic mechanisms, we used *in-vitro* whole cell voltage-clamp recordings to examine changes in spontaneous and miniature excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs). We observed reduction in the frequency of sEPSCs and mEPSCs at 5 and 30 minutes post-CSD, thus providing evidence for pre-synaptic changes in the release probability at glutamatergic synapses. Additionally, *in-vitro* experiments showed an increase in the amplitude of sIPSCs and mIPSCs at 5 and 30 minutes post-CSD, suggesting possible post-synaptic modification of GABA receptors which thus further affects the output of pyramidal neurons. This is the first study to perform electrophysiological characterization of CSD *in-vivo*. Together with the *in-vitro* experiments, we propose novel synaptic mechanisms underlying the effects of CSD on sensory networks.

Disclosures: **P.S. Suryavanshi:** None. **P.M. Sawant-Pokam:** None. **K.C. Brennan:** None.

Poster

210. Synaptic transmission: Modulation

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Topic: B.07. Synaptic Transmission

Title: Activating transcription factor 4 (ATF4) gates gabaergic neurotransmission by regulating GABABRs levels

Authors: *C. CORONA, S. PASINI, J. LIU, L. GREENE, M. SHELANSI;
Dept. of Pathology and Cell Biol., Columbia Univ., New York, NY

Abstract: Activating Transcription Factor 4 (ATF4) is a member of the ATF/cAMP responsive element binding protein (CREB) family described to be involved in learning and memory processes. In this regard, we previously reported that specific hippocampal ATF4 downregulation induces synaptic plasticity and memory deficits accompanied by a reduction in glutamatergic functionality. Here we extend our studies to address the role of ATF4 in GABAergic neurotransmission. Our electrophysiological data, obtained after long-term knockdown of ATF4 levels in cultured rat hippocampal neurons by lentiviral infection, show that ATF4 downregulation significantly increases the frequency without affecting the amplitude of miniature inhibitory post-synaptic currents (mIPSCs). These findings, along with the observation that GABAA receptors (GABAARs) levels are not changed upon ATF4 downregulation, led us to hypothesize a role for ATF4 in controlling GABA release. The metabotropic GABAB

receptors (GABABRs) are one of many neuromodulatory receptors that can effectively influence the release of neurotransmitters. We therefore next queried whether modulating ATF4 levels would in turn influence the expression of GABABRs. Western immunoblotting analysis revealed that altering ATF4 levels significantly affects the expression of the subunit R1 of GABABRs. Specifically, we found that ATF4 downregulation strongly reduces (by about 80%) the expression of GABAB R1, whereas simultaneous co-infection with shATF4 and a rescue construct which overexpress ATF4 (ATF4add) produces a 3-fold increase in the levels of GABAB R1. Noteworthy, differently from the effect of ATF4add, transfecting with a transcriptionally inactive mutant form of ATF4 (ATF4add/mut) is unable to rescue both the electrophysiological and biochemical phenotypes observed upon ATF4 downregulation. This suggests that ATF4 needs to retain its transcriptional activity to influence GABABRs expression as well as mIPSCs frequency. However, qRT-PCR analysis revealed that ATF4 does not directly regulate GABABR transcription, indicating an indirect mechanism of regulation. Finally, we found that specific pharmacological manipulations of GABABR function mimic the effects of alteration of ATF4 levels on the frequency of mIPSCs. This further supports the idea that our observed effects of ATF4 on inhibitory neurotransmission are due to regulation of presynaptic GABABRs and consequently, to the capability to release GABA. In conclusion, our data favor a model in which ATF4 regulates GABAergic function by modulating presynaptic GABABRs.

Disclosures: C. Corona: None. **S. Pasini:** None. **J. Liu:** None. **L. Greene:** None. **M. Shelansi:** None.

Poster

210. Synaptic transmission: Modulation

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Topic: B.07. Synaptic Transmission

Support: NIH NCRR R01DK047320

NIH NIMHD G12MD007601

Title: Genetic deletion of selenoprotein M alters gender specific hippocampal synaptic plasticity

Authors: *E. D. NGUYEN-WU[1], M. A. REEVES[2], M. J. ROBLES[1], A. C. HASHIMOTO[1], M. W. PITTS[1], F. P. BELLINGER[1], M. J. BERRY[1];
[1]Cell and Mol. Biol., Univ. of Hawaii At Manoa, John A. Burns Sch. of Med., Honolulu, HI;
[2]Biologic Inst., Redmond, WA

Abstract: Selenium (Se) is a trace element that is integral for life and known to have protective effects against oxidative damage. Severe dietary selenium deficiency is associated with neurological dysfunction. Se is incorporated into selenoproteins as selenocysteine (Sec), the 21st

amino acid. Selenoprotein M (SelM) is an endoplasmic reticulum (ER) localized protein abundantly expressed in the brain relative to other tissues. Reeves et al. (2010) previously demonstrated that SelM modulates calcium release from intracellular stores and has a protective effect against oxidative stress in SH-SY5Y neuronal cells. Studies in an Alzheimer's disease mouse model have shown that mutation in the presenilin-2 gene leads to suppression of SelM. Here, we investigate the role of SelM in synaptic physiology in hippocampal slices of SelM knockout (SelM KO) and wild type mice. We demonstrate that SelM KO male mice are deficient in long-term potentiation (LTP), a cellular model for learning and memory, in the CA1 region of the hippocampus. Notably, we reveal this deficit in synaptic plasticity is gender specific, as SelM female KO mice have normal LTP. We also found an overall increase in synaptic efficacy, shown by an augmented input-output relationship of the field excitatory postsynaptic potential (fEPSP) in response to increasing stimulation of the Schaffer collateral fibers. We observed no difference in paired pulse facilitation (PPF), arguing against any presynaptic changes. Our findings strongly demonstrate an important gender-specific role for selenoprotein M in maintaining normal hippocampal function and facilitating synaptic plasticity. These data provide a foundation for further studies on the role of SelM in neurological function and neurodegenerative disorders such as Alzheimer's disease.

Disclosures: E.D. Nguyen-Wu: None. **M.A. Reeves:** None. **M.J. Robles:** None. **A.C. Hashimoto:** None. **M.W. Pitts:** None. **F.P. Bellinger:** None. **M.J. Berry:** None.

Poster

210. Synaptic transmission: Modulation

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 210.17/B50

Topic: B.07. Synaptic Transmission

Title: The role of cAMP in presynaptic synaptic vesicle recruitment at high and low output neuromuscular junctions

Authors: *R. S. POTTER[1], S. POTTER[1], W. WU[2], R. L. COOPER[1];
[1]Univ. of Kentucky, Lexington, KY; [2]The Univ. of Texas MD Anderson Cancer Ctr., Houston, TX

Abstract: Synaptic efficacy among neurons communicating to other neurons or to targets, such as skeletal muscle, is a dynamic process throughout development and for established synapses. The ability of synaptic function to increase or decrease in regulating the appropriate range of synaptic transmission is important in maintaining correct neural responses. Subtle changes in synaptic modulation can have pronounced acute and chronic effects. Vesicles are distributed inside a nerve terminal as a readily releasable pool (RRP) and a reserve pool (RP). The ability to mobilize the RP is known to be regulated by various second messengers (i.e. cAMP, IP3, PKA)

depending on the type of preparation. Few studies have examined the differences in mobilizing the RP by cAMP following synaptic depression induced by high frequency stimulation; the same goes for synapses which are deemed high or low in synaptic efficacy. We are examining the crayfish and larval *Drosophila* neuromuscular junctions (NMJs) and addressing these hypothesis: (1) Low output synapses will show a greater degree of synaptic enhancement due to activation of cAMP as compared to high output synapses; (2) after induction of high frequency evoked depression, little recruitment of RP vesicle will occur in either synapse type; and (3) enhancing the cAMP production will lead to enhanced synaptic depression in the low output synapses as compared to high output synapses. Activation of cAMP by application of forskolin, an activator of adenylate cyclase, was used. Low output NMJs increased by 127.8% with prior 1hr incubation and only 36.16% without incubation of forskolin (N=5, P< 0.05). A 56.29% (n = 5) increase occurred after depression without incubation. Studies are underway with high output crayfish and *Drosophila* NMJs. These studies are significant as the results will inform us which types of synapses may be modulated by pharmacological agents for therapeutic targets.

Disclosures: **R.S. Potter:** None. **S. Potter:** None. **W. Wu:** None. **R.L. Cooper:** None.

Poster

210. Synaptic transmission: Modulation

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: B.07. Synaptic Transmission

Title: Intrinsic circuitry of the lateral central amygdala

Authors: ***S. HUNT**, P. SAH;
Queensland Brain Inst., St Lucia, Australia

Abstract: The amygdala is a region of the brain that is responsible for the processing and memory of emotions. Understanding the neural circuitry of this area is essential to grasping how it achieves this function and may aid in developing treatments for a range of anxiety disorders. Two main subregions of the amygdala, the basal and lateral nuclei (BLA), and the central nucleus (CeA), play key roles in the acquisition and expression of emotions respectively. While the BLA has been extensively studied, the CeA has only recently gained interest, and as such, its intrinsic circuitry is under intense study. Cells in the CeA are mainly inhibitory GABAergic (gamma-aminobutyric acid) cells and single unit recordings of the activity of specific cell populations suggest that these cells form strong local inhibitory connections. These local connections are central to the proposed circuitry underlying the behavioural roles of the CeA. However, our understanding of these local connections at the cellular level is in its infancy; the strength and physiological role of individual connections are largely unknown. We have made dual whole-cell recordings in the lateral CeA to determine the physiological properties and

strength of local inhibitory connections. Current-clamp recordings showed three types of discharge properties: regular spiking (RS), late firing (LF) and fast spiking (FS). In paired recordings, 32 out of 94 (≈ 34%) were connected, of which 29 connected pairs were unidirectional and three were bidirectional. Connections were on average 22 ± 5 pA when voltage-clamped at -40 mV, and were inhibited by picrotoxin, indicating that these were indeed GABAergic connections. In these pairs, the presynaptic cell was RS (47%), LF (35%) or FS (18%) whereas the postsynaptic cell was mostly LF (70%). Finally, the inhibitory connection was sufficient to halt firing in the postsynaptic cell. These results confirm that cells in the lateral portion of CeA form local inhibitory connections, which are capable of silencing the postsynaptic cell.

Disclosures: S. Hunt: None. **P. Sah:** None.

Poster

210. Synaptic transmission: Modulation

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Program#/Poster#: 210.19/B52

Topic: B.07. Synaptic Transmission

Support: NIH Grant MH099054

Title: Mechanisms of serotonergic 2A-receptor-mediated excitation in callosal projection neurons in the mouse prefrontal cortex

Authors: *E. K. STEPHENS, A. T. GULLEDGE;
Physiol. & Neurobio., Geisel Sch. of Med. At Dartmouth, Lebanon, NH

Abstract: Serotonin (5-HT) selectively excites subpopulations of pyramidal neurons in the neocortex via activation of $5HT_{2A}$ (2A) receptors. Classically, 2A and other G_q-coupled excitatory responses are thought to result from turning off potassium conductances, including those mediated by KCNQ channels. However, the precise mechanism underlying the 2A-dependent excitation in the cortex has not yet been established. We tested several potential mechanisms of serotonergic excitation in callosal projection neurons (COM neurons) in layer 5 of the mouse medial prefrontal cortex. Under baseline conditions, focally applied 5-HT increased the rate of action potential generation during suprathreshold DC somatic current injections. To confirm that potassium conductances are involved in serotonergic excitation, we measured 2A-excitation in control conditions and after reducing extracellular potassium from 3 mM to 0.5 mM. Surprisingly, increasing the driving force for potassium failed to enhance 2A-mediated excitatory responses (mean percent change in serotonergic excitation from baseline levels was -28 ± 22 %; n = 11, p = 0.24), even as the KCNQ channel blocker XE 991 (10 or 20 μM) reduced serotonergic excitation (by 32 ± 9%; n = 10, $p < 0.05$). These data suggest that serotonergic

excitation is mediated by combined suppression of KCNQ channels and activation of nonspecific cation channels permeable to potassium, similar to the G_q-mediated cholinergic excitation observed in these same neurons (see, for instance, Haj-Dahmane and Andrade, J. Neurosci., 1996). Indeed, we found that activation of muscarinic receptors with bath-applied carbachol (50 μM) occluded 2A-mediated excitation, confirming that both cholinergic and serotonergic excitation utilize the same G_q-pathway and effector mechanisms in COM neurons. Finally, we found that 2A-mediated excitation was not dependent on intracellular calcium, as responses were enhanced above control levels (by 176%), rather than reduced, when internal calcium was chelated with BAPTA (10 mM in patch pipette; $n = 12$, $p < 0.05$). Our results point to a role for multiple ionic effectors, including a nonspecific cation conductance and KCNQ-mediated M-current, working together to mediate serotonergic and cholinergic excitation in prefrontal pyramidal neurons.

Disclosures: E.K. Stephens: None. **A.T. Gulledge:** None.

Poster

210. Synaptic transmission: Modulation

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Topic: B.07. Synaptic Transmission

Support: NSERC Grant RGPIN05881

Title: Peripheral nerve injury promotes emergence of acute, neuron-type-specific depressant actions of gabapentin in substantia gelatinosa and primary somatosensory cortex

Authors: *S. ALLES, N. BUKHANOVA, M. BANDET, I. WINSHIP, P. A. SMITH; Neurosci. and Mental Hlth. Inst., Univ. of Alberta, Edmonton, AB, Canada

Abstract: The effectiveness of the α2δ ligand gabapentin (GBP) in treatment for neuropathic pain is thought to develop over periods of 3-5 days. Acute mechanisms may also contribute as clinically-relevant doses (100 mg/kg) of intraperitoneal (IP)-injected GBP significantly increase paw withdrawal threshold to Von Frey filaments in rats subject to sciatic chronic constriction injury (CCI) within 30 min of injection. We used *ex vivo* whole-cell recording and confocal Ca2+ imaging of spinal cord slices and *in vivo* cortical Ca2+ imaging to identify the neuronal correlates of these acute drug actions. Excitatory substantia gelatinosa neurons were identified by their delay firing pattern and inhibitory neurons by their tonic firing pattern. When “neuropathic” rats (subject to 7-14 days of sciatic CCI) received an IP injection of 100 mg/kg GBP 30 min prior to euthanasia, excitatory drive to excitatory neurons was decreased compared to control saline-injected neuropathic rats. This involved a decreased frequency and amplitude of spontaneous excitatory post-synaptic currents (sEPSC). By contrast, excitatory drive to inhibitory neurons

increased as a result of increased sEPSC frequency. In excitatory neurons from neuropathic animals, rates of action potential discharge in response to depolarising current were decreased by prior GBP administration. These changes, which were not observed in sham-operated animals, led to an overall decrease in dorsal horn excitability as monitored by a decrease in K+-evoked Ca2+ responses. All experiments were blinded. *In vivo* imaging studies of neuropathic rat cortex in response to vibrotactile stimulation showed that there is a significant reduction in the neural mass (% of responsive neurons x mean normalized fluoresence) 10 min, 20 min and 30 min following IP injection of 100mg/kg GBP. This novel result implies that GBP is influencing cortical responses and may be affecting pain per se within 10 min of injection. In sham animals a significant effect is only observed after 20 min and 30 min of GBP injection. Most previous studies of the gabapentinoids have failed to identify any major acute effects since these studies have been mainly carried out on naïve animals or with no reference to effects on specific cell types. Elucidation of the mechanisms of these acute, cell-type specific actions of gabapentinoids may provide a basis for development of more effective therapeutic approaches.

Disclosures: S. Alles: None. **N. Bukhanova:** None. **M. Bandet:** None. **I. Winship:** None. **P.A. Smith:** None.

Poster

210. Synaptic transmission: Modulation

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Topic: B.07. Synaptic Transmission

Support: Whitehall Foundation

Brain and Behavior Foundation, Haddie Investigator

Title: Mechanisms of long-term depression at electrical synapses in the thalamic reticular nucleus

Authors: J. SEVETSON, *J. S. HAAS;
Dept. of Biol. Sci., Lehigh Univ., Bethlehem, PA

Abstract: Long-term depression (LTD) of electrical synapses in the thalamic reticular nucleus (TRN) has been demonstrated following paired activity and following activation of metabotropic glutamate receptors by stimulation of corticothalamic afferents, but the interactions and downstream mechanisms of these two paradigms for LTD induction remain unclear. Using dual whole-cell recordings, we demonstrate that these two stimuli induce LTD by separable pathways. LTD following application of the mGluR agonist ACPD occludes further paired bursting in pairs of coupled TRN neurons, as do the stimuli in reverse order. We show that burst-induced LTD depends on calcium influx via T-type and L-type channels and calcium flux in in the intracellular

environment. However, ACPD-induced LTD can be induced independently of those sources of calcium. Together, these results provide support for a mechanistic model whereby activity-induced depression is mediated by calcium entry and dynamics, while afferent activity-induced depression is not; we hypothesize that the two induction mechanisms converge at a shared downstream pathway.

Disclosures: J. Sevetson: None. **J.S. Haas:** None.

Poster

210. Synaptic transmission: Modulation

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Topic: B.07. Synaptic Transmission

Support: Grant 25861365

Title: Underlying mechanisms of intravenous administration of lidocaine on synaptic transmission in spinal dorsal horn neurons: An *in vitro* patch-clamp recording

Authors: M. KURABE[1], H. FURUE[2], *H. BABA[1], T. KOHNO[1];
[1]Niigata Univ, Sch. Med., Niigata, Japan; [2]Dept. of Information Physiol., Natl. Inst. for Physiological Sci., Okazaki, Japan

Abstract: Intravenous administration of lidocaine (IVL) has been used clinically for neuropathic pain or postoperative pain management. Many investigators have considered that voltage-gated sodium channel is a primary target for analgesic action of lidocaine. However, plasma concentration follwoing IVL is 1-5 μM, that concentration of lidocaine is not enough to block sodium channel. Moreover, several studies suggest that lidocaine has other potential targets (e.g., glycine transporter 1, muscarinic receptors) other than sodium channel. Therefore, the analgesic mechanisms of IVL have not been fully elucidated. We hypothesized that IVL would have an action on synaptic transmission in spinal dorsal horn neurons. We examined the effect of IVL using *in vivo* patch-clamp recordings in substantia gelatinosa neurons. Under urethane anesthesia, lumbar laminectomy was performed at the level of Th12-L2, and the animal was then placed in a stereotaxic apparatus. Blind whole-cell patch-clamp recordings were made with a resistance of 8-12 M Ω electrode. In the voltage-clamp mode (Holding potential = - 70 mV), IVL dose-dependently decreased the frequency, but not the amplitude, of spontaneous excitatory post synaptic currents (sEPSCs). IVL also decreased the frequency without changing the amplitude of miniature EPSCs in the presence of tetrodotoxin. We next examined the effect of IVL on inhibitory post synaptic currents (IPSCs) at a holding potential of 0 mV. IVL did not change the frequency and amplitude of spontaneous IPSCs. Furthermore, IVL decreased the area under the curve of currents induced by peripheral noxious pinch stimuli. However, bath-applied lidocaine

on the spinal cord surface did not affect the frequency and the amplitude of sEPSCs. Our results suggest that IVL inhibits the excitatory synaptic transmission presynaptically in spinal dorsal horn neurons, resulting in antinociceptive effect. Considering that intravenous, but not bath-applied, lidocaine affected the sEPSCs, lidocaine may not directly modulate excitatory synaptic transmission in the dorsal horn.

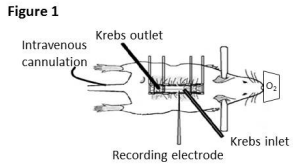

Figure 1. Schematic diagrams of in vivo rat preparation

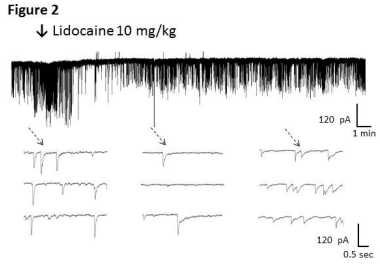

Figure 2. Continuous chart recording of sEPSCs before, during and after intravenous administration of lidocaine (10 mg/kg). The neuronal activity in this figure shows a typical response to lidocaine.

Disclosures: **M. Kurabe:** None. **H. Furue:** None. **H. Baba:** None. **T. Kohno:** None.

Poster

210. Synaptic transmission: Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 210.23/B56

Topic: B.07. Synaptic Transmission

Support: NIH Grant NS080967

Brain Research Foundation BRFSG-2014-13

Title: Parkin is necessary for excitatory synaptic transmission in hippocampus

Authors: G. P. CORTESE, C. VOLLMER, *C. WAITES;
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Abstract: The E3 ubiquitin ligase Parkin is highly expressed in glutamatergic neurons of the cortex and hippocampus, and is reported to interact with and/or ubiquitinate multiple synaptic proteins. Such findings suggest that Parkin could be an important regulator of glutamatergic synaptic transmission. However, few studies have examined Parkin's role in this context. Here, we have used shRNA-mediated knockdown combined with single-cell and paired-cell recordings to investigate the contribution of Parkin to excitatory synaptic transmission in hippocampal

neurons. Remarkably, we find that knockdown of Parkin leads to decreased spontaneous and evoked postsynaptic currents, as well as an increased synaptic failure rate. Moreover, AMPA- and NMDA receptor-mediated currents are dramatically reduced in knockdown neurons, indicating that Parkin is an important regulator of postsynaptic glutamate receptor expression and/or function. This study demonstrates Parkin's critical role in glutamatergic synaptic transmission, and highlights the need for additional studies to define the molecular mechanisms underlying these phenotypes.

Disclosures: G.P. Cortese: None. **C. Vollmer:** None. **C. Waites:** None.

Poster

210. Synaptic transmission: Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 210.24/B57

Topic: B.07. Synaptic Transmission

Title: Regulation of GABA release by a synapsin llla phospho-switch

Authors: *S.-H. SONG[1,2], G. J. AUGUSTINE[1,2];
[1]Lee Kong Chian Sch. of Med., Singapore, Singapore; [2]Ctr. for Functional Connectomics, KIST, Seoul, Korea, Republic of

Abstract: We have examined the function of a MAPK phosphorylation site in synapsin IIIa, S470, in regulating GABA release from interneurons. The peak amplitude of electrically evoked inhibitory postsynaptic currents (IPSCs) was reduced in cultured hippocampal neurons from synapsin triple knockout (TKO) mice compared to neurons from wild-type (TWT) mice. This phenotype was rescued by synapsin IIIa, as well as by synapsin IIIa containing a schizophrenia-associated mutation in the MAPK phosphorylation site (S470N; Biol. Psych. 55: 118). As previously reported (Nature Comm. 4:1512), synapsins influence the time course of GABA release: IPSCs decayed more slowly in TKO neurons than in TWT neurons. Deconvolution analysis (J. Neurosci. 24: 6127) indicated that this is due to a prolongation of quantal GABA release following a stimulus, with the time constant of release slowing from 96+4.9 msec in TWT to 124+10 msec in TKO neurons. This desynchronization of GABA release was rescued by S470N (82+5.3 msec), but not by wild-type synapsin llla (127+9.7 msec). Thus, MAPK phosphorylation of synapsin IIIa controls the kinetics of GABA release but not the peak rate of GABA release. Because synapsin llla rescued the defect in peak rate, but not in release kinetics, there was a 2-fold increase in the total number of GABA quanta released in response to a stimulus. The S470 phosphorylation site also regulates the response to BDNF. While BDNF (100 ng/ml) had little effect on the frequency of miniature IPSCs (mIPSCs) in TWT neurons, there was a pronounced (1.4-fold) increase in mIPSC frequency in TKO neurons. Synapsin llla rescued the effect of BDNF on mIPSC frequency, but S470N did not. This indicates that

synapsin IIIa is a negative regulator of BDNF signaling and this action is also under the control of MAPK via phosphorylation of S470. In summary, synapsin llla serves unique roles in regulating GABA released in response to both electrical and chemical stimuli, with these roles controlled by MAPK phosphorylation of S470. These novel functions, and their disruption by S470N, could implicate synapsin IIIa in the etiology of schizophrenia.

Disclosures: S. Song: None. **G.J. Augustine:** None.

Poster

210. Synaptic transmission: Modulation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 210.25/B58

Topic: B.07. Synaptic Transmission

Support: Langer-Simon Award

Young Investigator Award, Brain and Behavior Research Foundation

Whitehall Foundation

Title: Investigating homeostatic plasticity at electrical synapses

Authors: *E. L. HECKMAN[1], S. FITTRO[1], Y. SONG[2], Y. BERDICHEVSKY[3], J. S. HAAS[1]; [1]Biol. Sci., [2]Bioengineering Program, [3]Dept. of Electrical and Computer Engin. and Bioengineering Program, Lehigh Univ., Bethlehem, PA

Abstract: Electrical synapses are specialized junctions that allow ionic current to flow directly between coupled neurons, thus allowing the rapid propagation of signals relevant to neural communication. They play a large role in synchronizing hippocampal oscillations and maintaining attention. Long-term depression of electrical synapses in the thalamic reticular nucleus (TRN) has been demonstrated following paired activity or activation of metabotropic glutamate receptors by stimulation of corticothalamic afferents. Whether electrical synaptic strength is regulated by homeostatic mechanisms that have been shown to up- or down-regulate synaptic strength in response to global activity modulation is unknown. Using dual whole-cell recordings in hippocampal slice cultures and acute horizontal slices containing the TRN, we investigated whether distributions of electrical synaptic strength varied after long-term exposure to tetrodotoxin used to block activity. Our results show that under these conditions, electrical synapses are not homeostatically plastic. However, coupling incidence was increased in acutely sliced TRN following exposure to tetrodotoxin; this was not true for coupling incidence in hippocampal cultures. These experiments gave us further insight into the modifiability and role of electrical synapses, which mediate interneuronal communication across the brain.

Disclosures: **E.L. Heckman:** None. **S. Fittro:** None. **Y. Song:** None. **Y. Berdichevsky:** None. **J.S. Haas:** None.

Poster

211. Long-Term Depression (LTD)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 211.01/B59

Topic: B.08. Synaptic Plasticity

Support: NIH-NINDS R21 NS085471

NIH 5 P30 RR032135

NIGMS 9 P30 GM103498

Title: Kv1.2 potassium channel role in Cerebellar learning and memory

Authors: ***S. C. MADASU**[1], M. L. SHIPMAN[2], J. T. GREEN[2], A. D. MORIELLI[3]; [1]Univ. of Vermont, Colchester, VT; [2]Dept. of Psychological Sci., [3]Dept. of Pharmacol., Univ. of Vermont, Burlington, VT

Abstract: Learning is regulated by synaptic and intrinsic plasticity. Synaptic plasticity in turn is regulated by ion channel function. Major contributors of neuronal excitability and intrinsic plasticity are voltage-gated ion channels. The regulation and surface expression of ion channels such as voltage-gated potassium channel Kv1.2 has been shown not only to govern Purkinje cell (PC) excitability in the cerebellum(1) but also cerebellum-dependent associative learning and memory such as eyeblink conditioning (EBC). Our lab has previously shown that inhibition of Kv1.2 in cerebellar cortex via infusion of the potent and selective Kv1.2 blocker tityustoxin-Kα facilitates EBC in rats (2). AMPAR endocytosis during mGluR1 stimulated long term depression (LTD) at parallel fiber-PC synapses has been proposed as a mechanism for EBC. mGluR1 knock out mice show impaired EBC and impaired LTD (3, 4). However, mutation studies in mouse that block AMPAR endocytosis and prevent LTD have no effect on EBC. (5). Thus, mGluR1, but possibly not AMPAR endocytosis, is important for cerebellar dependent learning. Here we show that mGluR1 stimulation via DHPG decreases surface expression of both Kv1.2 and AMPAR. We propose an alternative mechanism for mGluR1 involvement in EBC that involves modulation of surface expression of Kv1.2 in cerebellar cortex. References: 1. Khavandgar S, Walter JT, Sageser K, & Khodakhah K (2005) Kv1 channels selectively prevent dendritic hyperexcitability in rat Purkinje cells. *J Physiol* 569(Pt 2):545-557. 2. Williams MR, Fuchs JR, Green JT, & Morielli AD (2012) Cellular mechanisms and behavioral consequences of Kv1.2 regulation in the rat cerebellum. *J Neurosci* 32(27):9228-9237. 3. Kishimoto Y*, et al.* (2002) mGluR1 in cerebellar Purkinje cells is required for normal association of temporally contiguous stimuli in classical conditioning. *Eur J Neurosci* 16(12):2416-2424. 4. Ohtani Y*, et al.* (2014)

The synaptic targeting of mGluR1 by its carboxyl-terminal domain is crucial for cerebellar function. *J Neurosci* 34(7):2702-2712. 5. Schonewille M*, et al.* (2011) Reevaluating the Role of LTD in Cerebellar Motor Learning. *Neuron* 70(1):43-50.

Disclosures: S.C. Madasu: None. **M.L. Shipman:** None. **J.T. Green:** None. **A.D. Morielli:** None.

Poster

211. Long-Term Depression (LTD)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 211.02/B60

Topic: B.08. Synaptic Plasticity

Support: National Research Foundation of Korea Grant WCI 2009-003

Korea Institute of Science and Technology Institutional Program Project No. 2E24210

Title: Postsynaptic mechanism for progression of cerebellar long-term depression

Authors: *T. KIM, Y. YAMAMOTO, K. TANAKA-YAMAMOTO;
KIST, Seoul, Korea, Republic of

Abstract: Synapses can undergo changes in their efficacies by regulating the number of postsynaptic receptors, upon the external stimulus. Once the stimulus exceeds a certain threshold, synaptic efficacies are changed and maintained for long time, which is called long-term synaptic plasticity. Time course of long-term synaptic plasticity is characterized by stable baseline, induction of signaling activities, expression of changes in synaptic efficacies, and maintenance of resultant level of synaptic efficacies. Although many studies have discovered mechanisms of induction and expression, it remains unclear how synaptic plasticity can be maintained. In this study, we addressed this question by investigating one of the well-characterized long-term synaptic plasticity, long-term depression (LTD) at synapses between parallel fibers (PFs) and Purkinje cells (PCs) in cerebellar cortex. While synaptic transmission at PF-PC synapses is stably kept at the basal state, LTD is triggered when PF stimulation coincides with climbing fiber stimulation, and then is maintained. Experimental and theoretical studies showed that once net increase of intracellular calcium concentration reaches threshold to initiate PKC-MAPK positive feedback loop, activated PKC phosphorylates GluA2, the most abundant subunit of AMPAR in PC synapse, and consequently enhances the internalization of GluA2 by clatherin mediated endocytosis. These processes are responsible for LTD induction and expression, but not for LTD maintenance, as shown by our previous results. Early treatment of PKC inhibitor made LTD to be reversed back to basal level, whereas later treatment led two distinct responses; successful maintenance of LTD and restoration to baseline. This indicates that there is another sensitive bistable switch activated after PKC-MAPK positive feedback loop. The

current study demonstrates that Rab5-Rab7 conversion is the bistable switch responsible for the maintenance of LTD. Our computational model including the processes of induction and expression of LTD together with this conversion switch for sorting to late endosome successfully described the time course of LTD. Our experiments using dominant negative form of Rab7 (Rab7DN) showed that the conversion is indeed required for LTD. Further, photo-sensitive Rab7DN is newly developed and utilized to examine time course of LTD. Based on these results, we conclude that late endosome sorting of GluA2 works as the mechanism of LTD maintenance and there is variability in timing of each LTD progression phase.

Disclosures: **T. Kim:** None. **Y. Yamamoto:** None. **K. Tanaka-Yamamoto:** None.

Poster

211. Long-Term Depression (LTD)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 211.03/B61

Topic: B.08. Synaptic Plasticity

Title: Cerebellar LTD induced in mice having mutated GluA2 C-terminus resolves discrepancy between motor learning and synaptic plasticity

Authors: *K. YAMAGUCHI[1], S. ITOHARA[2], M. ITO[1];
[2]Lab. for Behavioral Genet., [1]RIKEN BSI, Wako Saitama, Japan

Abstract: Long-term depression (LTD) of synaptic transmission between cerebellar parallel fiber (PF) and Purkinje cell has been considered to be essential for motor learning. As a molecular mechanism underlying LTD, it has been shown that coincidental activation of PFs and climbing fiber (CF) induces a sharp increase in [Ca2+]in, which activates PKCα. Then, PKCα phosphorylates Ser-880 at GluA2 C-terminus, and thereby detaches AMPA receptors containing the phosphorylated GluA2 from GRIP (a scaffold protein). Finally, the destabilized AMPA receptors are internalized via endocytosis. Mutated GluA2 lacking the C-terminal 7 amino acids (GluR2α7) and that having replaced Lys -882 to Ala (GluR2K882A) are not phosphorylated via PKCα, and LTD was not induced in knockin mice having these mutant GluA2 (Steinberg et al., 2006). Recently, motor learning ability of these mice lacking LTD was demonstrated to be normal (Schoneville et al., 2011), and these authors claimed that PF-PC LTD was not essential for cerebellar motor learning. However, LTD-inducibility was examined under only limited conditions. Here, we reassessed LTD-inducibility in these KI mice by using several types of protocols in slice of 3- to 6-month-old KI mutants, which were gift from Prof. R.L. Huganir. First, simultaneous stimulation of PF and CF at 1Hz for 5 min was applied under current-clamp condition. This pattern of conjunctive stimulation (CJ-stim) induced LTD in wild type (WT), but not in the two types of KI mice. Second, PFs were stimulated twice with 50 ms intervals and the second PF stimulus was synchronized with a single CF stimulus. This protocol induced LTD in

WT (EPSC-amplitude: 82.8±5.0%) and K882A (80.8±3.4%). EPSC-amplitudeof α7 (95.3±4.9%) was significantly larger than that of WT ($p<0.05$, Tukey-Kramer post hoc test) or K882A ($p<0.05$). Third, the CF stimulus was replaced with a single depolarizing pulse (50 ms) applied through the whole-cell patch pipette containing Cs+-based internal solution. This protocol induced LTD in WT (69.5±3.7%, n=5), K882A (71.3±3.6, n=7) and D7 (76.2±6.8, n=5). Thus, LTD is inducible in both mutant GluR2 KI mice under certain CJ-stim conditions. The discrepancy between motor learning and LTD raised in these mutant mice should be resolved.

Disclosures: **K. Yamaguchi:** None. **S. Itohara:** None. **M. Ito:** None.

Poster

211. Long-Term Depression (LTD)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 211.04/B62

Topic: B.08. Synaptic Plasticity

Support: CDMRP grant#W81XWH-08-2-0136 to MJF

Title: Role of the temporal pattern of conditioning stimulation on induction of synaptic plasticity in visual cortex after mild traumatic brain injury (mTBI)

Authors: *D. KALIKULOV, Q. S. FISCHER, M. J. FRIEDLANDER;
Virginia Tech. Carilion Res. Inst., Roanoke, VA

Abstract: Traumatic brain injury can be accompanied by deficits of learning, memory and their underlying physiological processes - synaptic plasticity. We investigated a range of temporal patterns of conditioning stimulation of afferents on the induction of synaptic plasticity in visual cortex from control rats and from rats who had received mild impact TBI. Whole cell recordings were made from individual layer 2/3 pyramidal neurons in response to stimulation of layer 4 in acute slices of primary visual cortex from rats at 10-12 weeks postnatal age, including those who received an mTBI under anesthesia 2-3 weeks prior. Conditioning stimulation consisted of either a continuous train of 900 pulses at 10 Hz for 90s with an interstimulus interval (ISI) distribution coefficient of variation (CV) of 0.0 (perfectly regular ISIs), 0.2 (slightly irregular ISIs), or 1.0 (highly irregular ISIs) or of a discontinuous train of 9 bursts of 100 pulses each at 10 Hz with a pause between each burst. Baseline postsynaptic responses were evoked at 0.1 Hz for 10 minutes prior to the conditioning stimulus and for 20 minutes after the end of conditioning stimulus. We successfully recorded from 112 control and 101 mTBI cells. In controls, 10Hz continuous stimulation induced LTD in 58% (CV=1), 35% (CV=0.2) and 55% (CV=0) of the neurons. The incidence of LTP increased following increasing regularity of the conditioning stimulus (0%, 24% and 32% for CV=1, 0.2, 0, respectively). In mTBI rats, 10Hz continuous stimulation induced LTD in 42% (CV=1) , 61% (CV=0.2), and 44% (CV=0); and LTP in 29% (CV=1),

11%(CV=0.2) and 23% (CV=0) of the neurons. The plasticity outcomes were significantly different among certain different CV groups for mTBI: 10Hz, 90s, CV=0.2 vs CV=1 ($P<0.05$, t-test), and for certain groups between control vs. mTBI: 10 Hz, CV=1, 90s ($P<0.05$, t-test). In controls, discontinuous 10Hz stimulation produced LTD in 63%(CV=0), 56%(CV=0.2) and 82% (CV=1); and LTP in 16%(CV=0), 33% (CV=0.2) and 18% (CV=1) of the neurons. The distribution of plasticity outcomes for the CV=1 group was significantly different for control: 10Hz, 90s vs 900s ($P<0.005$, KS-test). In mTBI rats, the incidence of LTD increased with increasing conditioning stimulus regularity (53%, 44% and 33% for CV=0, 0.2, 1, respectively), while the incidence of LTP increased with increasing conditioning stimulus irregularity (20%, 28%, and 47% for CV=0, 0.2, 1, respectively). The distribution of plasticity outcomes for CV=1 stimulation was significantly different for control vs mTBI: 10Hz, 900sec ($P<0.005$, KS-test). Thus, stimulus pattern should be considered in selecting therapeutic approaches to chronic brain stimulation after mTBI.

Disclosures: D. Kalikulov: None. **Q.S. Fischer:** None. **M.J. Friedlander:** None.

Poster

211. Long-Term Depression (LTD)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 211.05/B63

Topic: B.08. Synaptic Plasticity

Support: CDMRP grant # W81XWH-08-2-0136 to MJF

Title: Effect of interstimulus interval regularity and mild traumatic brain injury (mTBI) on synaptic plasticity induced by 1Hz stimulation in visual cortex

Authors: *Q. S. FISCHER, D. KALIKULOV, M. J. FRIEDLANDER;
Virginia Tech. Carilion Res. Inst., Roanoke, VA

Abstract: Typical protocols for inducing synaptic plasticity use stimulus patterns with constant interstimulus intervals (ISIs), while neurons *in vivo* receive synaptic input with irregular ISIs. A study of more physiologically salient stimulation patterns is important to understand the role that stimulus temporal pattern may play in synaptic plasticity induction. Here we evaluate how stimulus regularity influences synaptic plasticity induction in the visual cortex of 10-12 week-old normal rats and those which received a mTBI 2-3 weeks prior. We applied a defined pattern of stimulation to layer 4 in acute slices and made whole-cell recordings of evoked postsynaptic potentials (PSPs) from 116 layer 2/3 pyramidal cells. Conditioning stimulation consisted of 900 pulses at 1Hz with 1 of 3 different patterns of ISI regularity defined by the coefficient of variation (CV): regular (CV=0), slightly irregular (CV=0.2), or highly irregular (CV=1). PSP peak amplitudes evoked by 0.1Hz stimulation were measured before and after conditioning

stimulation, and the post- to pre-conditioning ratio was used to assess plasticity. In controls, long-term depression (LTD) occurred in 68% of cells for CV=0 (n=25) and 75% of cells for CV=0.2 (n=20) stimulation, but just 44% of cells for CV=1 stimulation (n=23). Plasticity (LTD or long-term potentiation=LTP) did not occur in 16% of cells for CV=0 or CV=0.2 stimulation, increasing to 39% of cells for CV=1 stimulation (remaining cells showed LTP). Notably, the distribution of plasticity outcomes was significantly different for CV=0.2 vs. CV=1 stimulation ($P<0.05$, KS test). In mTBI rats, LTD occurred in 93% of cells for CV=0.2 stimulation (n=15), but just 44% of cells for CV=1 (n=18) and 40% of cells for CV=0 (n=15) stimulation. Plasticity did not occur in 47% of cells for CV=0 stimulation, and 28% of cells for CV=1 stimulation (remaining cells showed LTP). Notably, the distribution of plasticity outcomes for CV=0.2 stimulation was significantly different from both CV=0 ($P=0.02$, KS-test) and CV=1 ($P=0.03$, KS-test) stimulation. We also compared the evoked PSP half width, rise time, decay rate, and latency before and after conditioning stimulation. In controls, CV=0.2 stimulation induced a significant increase in rise time and latency ($P<0.02$, t-test), while CV=1 stimulation induced a significant decrease in half width and decay rate ($P<0.03$, t-test). In contrast, cells from mTBI rats showed no change in these parameters for any stimulation pattern. These results suggest that stimulus regularity modifies the efficacy of synaptic plasticity induction and that mTBI can alter this interaction.

Disclosures: **Q.S. Fischer:** None. **D. Kalikulov:** None. **M.J. Friedlander:** None.

Poster

211. Long-Term Depression (LTD)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 211.06/B64

Topic: B.08. Synaptic Plasticity

Support: Basic Science Research Program (2014-055260) through the National Research Foundation of Korea funded by the Korea government (MSIP).

Title: Layer-specific involvement of endocannabinoid signaling in cholinergic-induced long-term depression of layer 2/3 pyramidal neurons in rat visual cortex

Authors: K. JOO[1], K.-H. CHO[1], H.-J. JANG[1,2], *D.-J. RHIE[1,2];
[1]Coll Med. Catholic Univ. Korea, Seoul, Korea, Republic of; [2]Catholic Neurosci. Inst., Seoul, Korea, Republic of

Abstract: Endocannabinoid, acting as a retrograde signal, is involved in some forms of long-term synaptic depression (LTD) in the cortex. In our previous study, the activation of muscarinic receptors induced cholinergic-dependent LTD in the visual cortex. Cholinergic facilitation of long-term potentiation (LTP) and LTD plays a important role of learning, memory and sensory

processing. In this study, we investigated whether endocannabinoid signaling is involved in muscarinic LTD (mLTD). In addition, we studied layer-specific involvement of endocannabinoid signaling on mLTD because modulation of synaptic transmission in different layers is important in the pathway-specific control of cortical information flow. We recorded excitatory postsynaptic potentials with whole-cell patch-clamp technique by alternating stimulation applied to layer 1 and layer 4 (every 20 s each) in layer 2/3 pyramidal neurons of the rat primary visual cortex. Application of muscarine (10 μM, 10 min) evoked LTD at both layers to similar extents (~70% of the baseline), which was inhibited by D-AP5 (50 μM), an N-methyl-D-aspartate (NMDA) receptor antagonist. Application of the non-competitive NMDA receptor antagonist MK801 (1 mM) into the pipette blocked the mLTD at both layers, as well. mLTD evoked by layer 4, but not layer 1, stimulation showed an increase in paired-pulse ratio of EPSPs, implying that mLTD at layer 4 is presynaptically expressed. Bath application of AM251 (5 μM), an inverse agonist of cannabinoid (CB1) receptors, blocked mLTD at layer 4 only. Hence, NMDA receptor-dependent induction of mLTD was expressed presynaptically via endocannabinoid signaling at layer 4 whereas mLTD at layer 1 was expressed in postsynaptic neurons. Our results suggest that layer-specific involvement of endocannabinoid systems in cholinergic-induced synaptic plasticity might be important for the pathway-specific modulation of information processing in the neocortex. This study was supported by the Basic Science Research Program (2014-055260) through the National Research Foundation of Korea funded by the Korea government (MSIP).

Disclosures: **K. Joo:** None. **K. Cho:** None. **H. Jang:** None. **D. Rhie:** None.

Poster

211. Long-Term Depression (LTD)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 211.07/B65

Topic: B.08. Synaptic Plasticity

Title: Abnormal Retina -Specific Segregation at the dLGN of the Flailer - a myosin 5a mutant mice

Authors: *S. PANDIAN, M. CONSTANTINE-PATON;
McGovern Inst. for Brain Res., MIT, Cambridge, MA

Abstract: MyosinVa (MyoVA), is a widely distributed vesicular-cargo-binding actin motor known to deliver the major scaffold complex for glutamate receptors to spine synapses. In Flailer (Flr) mutant mice the cargo-binding domain of myo5a is driven by the brain specific promoter of Gnb5 (Jones et al 2000). When this truncated myo5a is expressed in 1:1 ratio reproducible abnormal behavior are seen (eg; early seizure, anxiety, repetitive whole body grooming (Pandian et al in prep)). In visual cortex neurons these mice have abnormally high AMPAR miniature current frequencies and their eAMPA/eNMDA ratio is significantly larger than in WT strain. Flr

shows no LTD at the layer 4 to layer 2/3 synapses although LTP is normal (Yoshii et al 2013). In developing rodent LGN the ipsilateral input arrives later than the contralateral input and there is a competition between the left and right eye axons (Huberman et al., 2008) Thus we hypothesized that if the retinogeniculate pathway also lacked LTD the ipsilateral territory might be unable to terminate in its normal region because the earlier contralateral innervating projections could not undergo LTD. Here we report that when both eyes of Flr pups are differentially labeled with CTB (555, 647) they show abnormally small and displaced ipsilateral zone while the contralateral projection occupies a larger territory. Monocular enucleation before eye-specific segregation in Flr causes the contralateral projection to spread throughout the dLGN with lowest density of input in region normally occupied by the ipsilateral eye. The ipsilateral projection is abnormally large with a shift towards the medial side of the nucleus. The results are consistant with the hypothesis that NMDAR LTD is also defective in the LGN of Flr and prevents displacement of early contralateral inputs by the later arriving axons.

Disclosures: S. Pandian: None. **M. Constantine-Paton:** None.

Poster

211. Long-Term Depression (LTD)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 211.08/B66

Topic: B.08. Synaptic Plasticity

Support: NSERC

The Hearing Foundation of Canada

Title: Gating of synaptic plasticity in the rodent primary auditory cortex by 17β-estradiol

Authors: *C. N. SOUTAR[1], S. G. RODIER[2], S. A. CHEE[1], N. PUN[2], H. C. DRINGENBERG[2]; [1]Ctr. for Neurosci. Studies, [2]Dept. of Psychology, Queen's Univ., Kingston, ON, Canada

Abstract: 17β-estradiol (E2) is an estrogenic hormone primarily synthesized in the ovary. Hormonal E2 has been shown to exert profound effects on both structural and functional synaptic plasticity, as well as learning. Interestingly, in males and females of several species, E2 is synthesized and released by forebrain neurons, including those in sensory regions such as the primary auditory cortex (A1). Thus, in addition to its role as a circulating hormone, E2 may also function as a modulator of synaptic activity and plasticity in the forebrain. Here we examined the role of E2 in synaptic plasticity (long-term potentiation and long-term depression; LTP and LTD, respectively) in A1 of adult, male rats under urethane-anaesthesia. In control animals, theta-burst stimulation (TBS) of the medial geniculate nucleus (MGN) resulted in only modest LTP of field postsynaptic potentials (fPSPs) in A1, while low-frequency stimulation (LFS) failed to induce LTD, data reflecting the high degree of resistance to plasticity induction in the mature A1.

Surprisingly, during local application of E2 in A1, both TBS and LFS resulted in the induction of LTD. Conversely, A1 application of Fadrozole, an aromatase inhibitor that suppresses local E2 synthesis, enhanced TBS-induced LTP, indicating that E2 functions as a potent inhibitor of LTP. E2 also resulted in a suppression of baseline fPSP amplitude in A1. Together, our experiments suggest that E2 exerts significant modulatory effects on synaptic transmission and plasticity in the mature A1. These findings hold important implications for the regulation of auditory processing and learning in adult mammals. Supported by NSERC and The Hearing Foundation of Canada.

Disclosures: **C.N. Soutar:** None. **S.G. Rodier:** None. **S.A. Chee:** None. **N. Pun:** None. **H.C. Dringenberg:** None.

Poster

211. Long-Term Depression (LTD)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 211.09/B67

Topic: B.08. Synaptic Plasticity

Support: NIH Grant R15NS078645

BYU ORCA Grant

BYU Mentored Environment Grant

Title: Hippocampal stratum radiatum interneuron plasticity type corresponds with cell subtype and mGluR5 expression

Authors: *T. M. NUFER[1], C. B. MERRILL[3], L. N. FRIEND[2], Z. H. HOPKINS[2,4], J. G. EDWARDS[2];
[1]Physiol. and Developmental Biol., [2]Brigham Young Univ., Provo, UT; [3]Univ. of California, Irvine, Irvine, CA; [4]Univ. of Utah Sch. of Med., Salt Lake City, UT

Abstract: Changes in synaptic strength in hippocampal CA1 pyramidal cells are thought to be responsible for the acquisition and retention of short-term memory. This plasticity is modulated by inhibitory interneurons in the stratum radiatum which are composed of many subtypes including, among others, parvalbumin-containing axo-axonic cells, calretinin-containing interneuron-selective cells, and cholecystokinin/calbindin positive basket cells. While radiatum interneurons induce long-term depression (LTD), short-term depression, or lack of plasticity (McMahon & Kauer, 1997, Neuron), it is not known whether these types of plasticity correlate to any specific interneuron subtype. Using whole cell electrophysiology and real time quantitative PCR, we characterized the plasticity expressed by different hippocampal interneuron subtypes in correlation with their mRNA expression patterns to determine cell subtype using calcium binding

proteins. We also assessed the expression of endocannabinoid (eCB) biosynthetic enzymes including diacylglycerol lipase α, N-acyl-phosphatidylethanolamine-specific phospholipase D, and 12-lipoxygenase, as well as metabotropic glutamate receptor subunits known to mediate stratum radiatum interneuron LTD. We identified a correlation in that cells that exhibited long-lasting depression tended to express mRNA for at least one of the eCB biosynthetic enzymes and the metabotropic glutamate receptor subunit mGluR5. Cells that exhibited short-term depression tended to express mRNA for at least one of the eCB biosynthetic enzymes, but not mGluR5. This suggests that stratum radiatum interneuron plasticity can be predicted based on cell subtype and mGluR expression, and that these different types of plasticity may have some importance in hippocampal function.

Disclosures: T.M. Nufer: None. **C.B. Merrill:** None. **L.N. Friend:** None. **Z.H. Hopkins:** None. **J.G. Edwards:** None.

Poster

211. Long-Term Depression (LTD)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 211.10/B68

Topic: B.08. Synaptic Plasticity

Support: NIH K99/R00 number: R00NS076364

Title: Nanometer resolution imaging of Arc-mediated AMPA receptor endocytosis

Authors: *A. TAIBI[1], R. HOBSON[1], E. HUJBER[1], M. GUDHETI[2], E. JORGENSEN[1,3], J. SHEPHERD[1];
[1]Univ. of Utah, Salt Lake City, UT; [2]Univ. of Utah, Vutara Inc., Salt Lake City, UT; [3]Howard Hughes Med. Inst., Chevy Chase, MD

Abstract: The formation and storage of memory is mediated by a complex series of membrane and protein trafficking events at synapses. Activity Regulated Cytoskeletal protein (Arc) is necessary for long-term memory. *Arc* is an immediate early gene that is transcribed and translated in response to neural activity. Arc regulates multiple forms of synaptic plasticity, such as LTP, LTD, and homeostatic scaling, through the trafficking of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs). How Arc regulates these different forms of synaptic plasticity remains unclear. We hypothesize that Arc facilitates endocytosis of AMPA receptors at the lateral margin of post-synaptic spines on excitatory neurons. Arc may, however, exhibit nanoscale variations in temporal and spatial dynamics that are specific to certain patterns of activity. To investigate this hypothesis we are developing novel super-resolution imaging methods for single fluorophore tracking of tagged or endogenous synaptic proteins. Since confocal microscopy cannot visualize proteins at the nanometer-scale, we are employing 3-

dimensional fluorescence photoactivation localization microscopy (3D-fPALM). This imaging platform allows single molecule tracking and colocalization analyses with 10-30nm resolution. Initial imaging of endogenous PSD95, Arc, and MAP2 using Alexafluor conjugated antibodies showed Arc is localized to discrete clusters along dendrites while confocal imaging shows a diffuse and nonspecific pattern of localization. PALM imaging of Arc with internalized GluA1 shows that Arc clusters around internalized GluA1 receptors. In order to visualize these events within the context of synaptic ultrastructure, we are also combining this method with high-pressure freeze electron microscopy. We are using EM-stable small molecule organic dyes to visualize HALO and SNAP tagged proteins such as Arc and GluA1 after induction of chemical LTD. This imaging method will allow us to definitively localize the site of AMPAR endocytosis and endosomal trafficking within the synaptic ultrastructure during synaptic plasticity. This cutting-edge imaging approach will allow the characterization of postsynaptic endocytosis at an unprecedented temporal and spatial scale.

Disclosures: A. Taibi: None. **R. Hobson:** None. **E. Hujber:** None. **M. Gudheti:** None. **E. Jorgensen:** None. **J. Shepherd:** None.

Poster

211. Long-Term Depression (LTD)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 211.11/B69

Topic: B.08. Synaptic Plasticity

Title: Dynamic nanoscale organization of ampa receptors during atp-mediated long term depression

Authors: *B. COMPANS, E. TOULMÉ, D. CHOQUET, E. HOSY;
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Abstract: AMPA-type glutamate receptors (AMPAR) mediate most of the fast excitatory transmission. The number of AMPARs at the synapse is a key determinant of synaptic strength. Long term changes in the efficacy of the synaptic transmission are thought to underlie learning and memory. Two major forms of long lasting plasticity, Long-Term Potentiation (LTP) and Long-Term Depression (LTD), are characterized by a long lasting increase or decrease in synaptic strength, respectively. LTD can be induced by Low Frequency Stimulation (LFS) or by chemical activation of appropriate receptors (cLTD). Recently, it has been demonstrated that stimulation of P2X receptors (P2XR), by ATP treatment or by a noradrenergic-dependent glial release of endogenous ATP, induces AMPAR endocytosis and long lasting depression of AMPAR currents through activation of phosphatases or CamKII. The dynamic and the nanoscale organization of AMPAR have both been shown to play an important role in synaptic activity and could be modified during long lasting plasticity. Using super resolution microscopy coupled to

electrophysiology, it has been that AMPARs are concentrated in clusters measuring around 80 nanometer and containing around 20 receptors. Most of dendritic spines contain 1 or 2 nanodomains which are composed by 60% of the synaptic AMPARs and could be responsible of quanta excitatory post synaptic currents. The other part of AMPARs are free to diffuse in and out the dendritic spines. Synaptic activity increases the percentage of mobile AMPAR and it is known that AMPAR lateral diffusion is important for responses in frequency of neurons. Here, we report the effect of ATP-mediated cLTD on nanoscale AMPAR dynamic organization. Single particle tracking microscopy (UPAINT) and d-STORM are applied to decipher the variation of AMPAR lateral diffusion and synaptic organization, respectively. AMPAR mobility seems to not be affected by a pharmacological activation of P2XRs whereas it organization is affected. Indeed, ATP induces a long lasting decrease of surface AMPAR number, and changes of the nanodomain organization. We observed a decrease of 40% of the number of surface AMPAR. This decrease is not observed with phospho-mutants of GluA1 subunit of AMPAR. We shown that ATP-mediated LTD correspond to a decrease of AMPAR number at synapses, leading to a reorganization of AMPARs without affecting their dynamic properties.

Disclosures: **B. Compans:** None. **E. Toulmé:** None. **D. Choquet:** None. **E. Hosy:** None.

Poster

211. Long-Term Depression (LTD)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 211.12/B70

Topic: B.08. Synaptic Plasticity

Support: AMPARzeta

Nano-Dyn-Syn

MODIFSYN

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Title: Pin1 binding to PSD-95 regulates excitatory synapses

Authors: ***J. Y. DELGADO**[1], G. G. TURRIGIANO[1], D. CHOQUET[2];
[1]Brandeis Univ., Waltham, MA; [2]CNRS, Bordeaux, France

Abstract: Phosphorylation dependent peptidyl-propyl cis/trans isomerization by the peptidyl-prolyl cis/trans Isomerase 1 (Pin1) plays key roles in cell cycle progression, the pathogenesis of cancer, and age-related neurodegeneration. Most of our knowledge about Pin1 function is restricted to non-synaptic proteins and whether Pin1 regulates excitatory synapses is unknown.

We identified the Postsynaptic Density Protein 95 (PSD-95) as a novel Pin1 binding partner. Pin1 binds and isomerises phosphorylated threonine 19 and serine 25 at the N-terminus domain of PSD-95. Pin1 binding to phospho-threonine 19 prevents PP2A mediated dephosphorylation and ubiquitination of PSD-95. Pin1 down regulation increases the amount of ubiquitinated PSD-95 and decreases the number of PSD-95 positive dendritic spines, the amounts of surface AMPA receptors, and the frequency of mEPSCs. The decrease in mEPSC frequency by Pin1 down regulation was fully rescued by overexpressing wild type PSD-95. Thus, proline directed phosphorylation of PSD-95, Pin1 binding and peptidyl-prolyl isomerization regulates excitatory synapses by increasing PSD-95 protein lifetime at synapse.

Disclosures: **J.Y. Delgado:** None. **G.G. Turrigiano:** None. **D. Choquet:** None.

Poster

211. Long-Term Depression (LTD)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 211.13/B71

Topic: B.08. Synaptic Plasticity

Support: NSF PHY 1205919

Title: Neuronal network dynamics of n-methyl-d-aspartate-induced chemical long term depression in cultured rat hippocampal neurons

Authors: ***S. DJEMIL**[1], X. CHEN[1,2], R. DZAKPASU[1,2];
[1]Dept. of Pharmacol. and Physiol., [2]Physics, Georgetown Univ., Washington, DC

Abstract: During learning and memory, synapses, the conduits through which neurons “talk” to each other, can either be strengthened or weakened via long-term potentiation (LTP), or long-term depression (LTD) respectively. It is generally understood that both forms of synaptic plasticity, LTP and LTD, must be tightly regulated for correct memory storage to occur; therefore, the interference with the regulation of one or both would lead to incorrect memory storage. Since its discovery, many insights about LTD have been gained via studies performed at the single cell level. However, effects of LTD on neuronal network dynamics have yet to be explored. Within the hippocampus, chemical LTD (cLTD) can be induced via the activation of N-Methyl-D-aspartate (NMDA) receptors using NMDA. To assess the effects of cLTD on network activity, we utilize multielectrode arrays (MEA), an electrophysiological system that allows for simultaneous extracellular recordings from 59 electrodes. Preliminary data show that NMDA results in a decrease in spontaneous network activity of embryonic rat hippocampal cultures that is dose dependent, stable and long-lasting. Further experiments using pharmacological agents are needed to assess whether this effect can be attenuated.

Disclosures: **S. Djemil:** None. **X. Chen:** None. **R. Dzakpasu:** None.

Poster

211. Long-Term Depression (LTD)

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Topic: B.08. Synaptic Plasticity

Support: ANR-12-BSV4-0021-01

ANR-13-JSV4-0002

programme ATIP, Ville de Paris Programme Emergence

Title: Actomyosin contraction mediates presynaptic long-term synaptic plasticity

Authors: M. H. MCFADDEN[1], H. XU[2], Y. CUI[2], R. A. PISKOROWSKI[3], V. CHEVALEYRE[3], L. VENANCE[2], *Z. LENKEI[1];
[1]ESPCI-ParisTech, Paris, France; [2]Ctr. for Interdisciplinary Res. in Biol., Col. de France, Paris, France; [3]Univ. Paris Descartes Sorbonne Paris Cité, Paris, France

Abstract: Long-term synaptic plasticity is critical for adaptive function of the brain, but presynaptic mechanisms of functional plasticity remain poorly documented. Here, we show that changes in synaptic efficacy during one of the most widespread forms of long-term presynaptic plasticity, i.e. endocannabinoid-mediated long-term depression (eCB-LTD), require contractility of the neuronal actomyosin cytoskeleton. First, we measured the effect of CB1R activation on synaptic vesicle release at individual axonal boutons by expressing synaptophysin-pHluorin (SpH) in rat hippocampal cultures. While CB1R activation led to a marked reduction in vesicle release as compared to control conditions, pharmacological inhibition of either non-muscular myosin II (NMII) or of its major activating kinase, Rho-associated protein kinase (ROCK), prevented this effect, as well as the increased number of silent boutons observed under CB1R activation. We then tested whether activity-dependent forms of plasticity mediated by endogenous cannabinoids (eCB) could engage similar mechanisms, by investigating two well-described eCB-mediated forms of plasticity both at inhibitory synapses in the hippocampus and at excitatory corticostriatal synapses. At both types of synapse, both NMII and ROCK inhibition prevented long-term forms of eCB-mediated synaptic depression, while short-term forms, such as depolarization-induced suppression of inhibition or of excitation, i. e. DSI or DSE, remained unaffected. Collectively, these results show that the long-term, but not short-term, blockade of the presynaptic release machinery under CB1R activation relies on ROCK-mediated actomyosin contraction, providing a novel mechanistic link relating changes in synaptic efficacy to one of the most widespread forms of presynaptic plasticity in the brain.

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Poster

211. Long-Term Depression (LTD)

Location: Hall A

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Topic: B.08. Synaptic Plasticity

Support: MRC RA2346

BBSRC G32301

Title: Convergence of synaptic pathophysiology in the hippocampus of Fmr1 knockout and Syngap1 heterozygous mice

Authors: *S. BARNES[1], A. D. JACKSON[1,2], E. K. OSTERWEIL[1], P. C. KIND[1,2], D. J. A. WYLLIE[1];
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Abstract: Genetically distinct causes of intellectual disability (ID) and autism spectrum disorder (ASD) may converge on common neuropathological mechanisms. For example, modulators of metabotropic glutamate receptor (mGluR)-dependent protein synthesis, for which ERK-MAPK is a key regulator, prevent and/or reverse many of the phenotypes associated with mouse models of Fragile X Syndrome (FXS) and Tuberous Sclerosis. Recently it was found that heterozygous *de novo* mutations in *SYNGAP1*, a negative regulator of the Ras/ERK pathway, results in ID with co-occurring ASD and epilepsy in approximately 4% of affected individuals, suggesting a similar prevalence to FXS. We have previously shown that, like *Fmr1*$^{-/y}$ mice, *Syngap*$^{+/-}$ mice show enhanced mGluR-dependent LTD that is independent of new protein synthesis. (Barnes et al., 2013; SFN abstract). Furthermore introduction of the *Fmr1* mutation in to *Syngap*$^{+/-}$ mice occludes a further increase in mGluR-LTD in *Syngap*$^{+/-}$*Fmr1*$^{-/y}$ double mutants indicating that mutations in *Fmr1* and *Syngap1* converge on a similar expression mechanism for this form of synaptic plasticity. We now show that direct measurements of protein synthesis in acute hippocampal slices from *Syngap*$^{+/-}$ mice reveal an elevation in basal protein synthesis that is saturated downstream of mGluR1/5 activation (Veh: WT 100 ± 7%; *SG*$^{+/-}$ 135 ± 7%; DHPG: WT 138 ± 14%; *SG*$^{+/-}$ 148 ± 15%; $n = 6$, $p < 0.05$). This is accompanied by an increase in steady-state activity levels of ERK (p-ERK/Total: WT 100 ± 7%; *SG*$^{+/-}$162 ± 15%, $n = 9$, $p = 0.004$) and p-S6 (Ser$^{235/236}$/S6: WT 100 ± 5%; *SG*$^{+/-}$ 121 ± 8%, $n = 8$, $p = 0.03$). Importantly, increased translation rates in *Syngap*$^{+/-}$ hippocampal slices can be corrected to WT levels by inhibiting mGluR5 (*SG*$^{+/-}$: Veh 125 ± 4%; CTEP 102 ± 8%; $n = 9$, $p = 0.06$), Ras (*SG*$^{+/-}$: Veh 142 ± 9%; Lovastatin 106 ± 9%; $p < 0.05$, $n = 7$) or ERK (*SG*$^{+/-}$: Veh 130 ± 9%; U0126 94 ± 9%; $p < 0.05$, $n = 9$). Furthermore we show that inhibition mTOR can reduce protein synthesis rates (*SG*$^{+/-}$: Veh 124 ± 6%; Rapamycin 109 ± 8%; $p < 0.05$; $n = 8$), normalize the magnitude of mGluR-LTD and restore the protein synthesis dependency of this form of LTD in the *Syngap*$^{+/-}$

hippocampus ($SG^{+/-}$: Veh 62 ± 5%, n = 15; Rapamycin 73 ± 5 %, n = 12; Rapamycin + Anisomycin 95 ± 4%, n = 9; $p < 0.05$). Together these findings raise the intriguing possibility that therapeutic strategies used in the treatment of FXS may also be of benefit for individuals with *Syngap1* haploinsufficiency.

Disclosures: S. Barnes: None. **A.D. Jackson:** None. **E.K. Osterweil:** None. **P.C. Kind:** None. **D.J.A. Wyllie:** None.

Poster

211. Long-Term Depression (LTD)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 211.16/B74

Topic: B.08. Synaptic Plasticity

Support: NMRC/CBRG/0014/2013

Title: G9a governs metabotropic glutamate receptor dependent LTD (mGluR-LTD) by regulating N-ethylmaleimide-sensitive factor/GluR2 (NSF-GluR2) dependent trafficking of postsynaptic AMPA receptors

Authors: *M. SHARMA, S. GOPINADHAN, S. SAJIKUMAR;
Physiology, YLL Sch. of Med., Natl. Univ. of Singapore, Singapore, Singapore

Abstract: Synaptic plasticity mechanisms such as long -term potentiation (LTP) and long -term depression (LTD) are the cellular correlates of learning and memory. N-methyl-D-aspartate receptor dependent LTD (NMDAR-LTD) and metabotropic glutamate receptor dependent LTD (mGluR LTD) are the two forms of LTD that contribute to memory mechanisms at cellular level. The role of translation and transcription in LTP and LTD is well studied but the role of transcriptional regulators in memory is less well understood. G9a, a methyltransferase, has been reported to regulate the gene transcription in the hippocampus during memory consolidation. G9a controls a prominent Histone H3 lysine 9 dimethylation (H3K9me2). In the present study, we have investigated the role of G9a on mGluR-LTD in CA1 region of acute rat hippocampal slices using long-term functional plasticity methods and elucidated the mechanism of regulation of mGluR-LTD. Our preliminary results show that G9a inhibition prevents mGluR-LTD and increases the levels of protein kinase M zeta (PKMζ), a critical plasticity protein (PRP) necessary for the maintenance of LTP. Associated with the increased level of PKMζ, the phosphorylated GluR2 subunits of AMPA receptors were also high. Our results suggest that G9a is a negative regulator of plasticity that sets the stage for mGluR-LTD induction and maintenance by inhibiting PKMζ, thereby regulating the N-ethylmaleimide-sensitive factor/GluR2 (NSF-GluR2) -dependent trafficking of postsynaptic AMPA receptors.

Disclosures: M. Sharma: None. **S. Gopinadhan:** None. **S. Sajikumar:** None.

Poster

211. Long-Term Depression (LTD)

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Program#/Poster#: 211.17/B75

Topic: B.08. Synaptic Plasticity

Support: Swiss contribution SH7/2/18

PhD fellowship of the Boehringer Ingelheim Fonds

Title: Endocannabinoid-dependent plasticity in excitatory and inhibitory spinal dorsal horn neurons

Authors: *C. VON SCHOULTZ[1,2], S. G. WOODHAMS[3], I. KATONA[3], H. U. ZEILHOFER[1,2];
[1]Inst. of Pharmacol. and Toxicology, Univ. of Zurich, Zurich, Switzerland; [2]Inst. of Pharmaceut. Sci., Swiss Federal Inst. of Technol. (ETH) Zurich, Zurich, Switzerland; [3]Hungarian Acad. of Sci., Inst. of Exptl. Med., Budapest, Hungary

Abstract: The superficial spinal dorsal horn serves a critical function in the relay and filtering of incoming nociceptive signals. Plastic changes in synaptic transmission between primary nociceptors and second order dorsal horn neurons have been implicated in many pathological pain conditions. Such plastic changes include among others long-term depression (LTD) or long-term potentiation (LTP) of synaptic transmission. Both forms of plasticity can be NMDA receptor-dependent, but in particular LTD can also involve other signaling molecules such as endocannabinoids (eCBs). In the present study, we were particularly interested in eCB-dependent synaptic plasticity in the spinal dorsal horn, because of the well-established role of eCBs in pain control. Using acute spinal cord slices and whole-cell patch-clamp recordings, we have previously demonstrated the presence of eCB-dependent primary nociceptor LTD in second order neurons of the superficial dorsal horn. The occurrence of this LTD was reduced by about half both in cannabinoid $(CB)_1$ receptor-deficient mice and in the presence of the NMDAR antagonist APV, suggesting a certain degree of heterogeneity in nociceptor LTD. To gain further insights into this heterogeneity, we investigated eCB-LTD separately in excitatory or inhibitory dorsal horn neurons visualized in “glutamatergic” vGluT2::eGFP or “GABAergic” $Gad67^{eGFP}$ transgenic mice, respectively. Although LTD occurred in both neuronal subpopulations, LTD in inhibitory neurons was resistant both to deletion of CB_1 and to blockade of NMDA receptors. Accordingly, neither deletion of NAPE-PLD nor of DGL-α, synthesizing enzymes of the major eCBs anandamide and 2-AG, respectively, could prevent LTD in inhibitory interneurons. In glutamatergic neurons, LTD was strongly reduced in CB_1 receptor-deficient mice and by pretreatment with APV, suggesting that NMDA and CB_1 receptors might act in series to induce primary nociceptor LTD in glutamatergic dorsal horn neurons. Our results demonstrate that the mechanisms of synaptic plasticity at primary nociceptor synapses differ between synapse types

and that this difference is at least partially determined by the neurotransmitter phenotype of the postsynaptic neuron.

Disclosures: C. Von Schoultz: None. **S.G. Woodhams:** None. **I. Katona:** None. **H.U. Zeilhofer:** None.

Poster

211. Long-Term Depression (LTD)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 211.18/B76

Topic: B.08. Synaptic Plasticity

Support: R01 AA015167

U01 AA016651

Title: Operant ethanol self-administration affects the magnitude of LTD in D1 Medium spiny neurons in nucleus accumbens shell but not core

Authors: *T. A. MEYERS, R. A. MANGIERI, R. MORRISETT;
Univ. of Texas At Austin, Austin, TX

Abstract: Passive ethanol administration has been shown to induce alterations in synaptic plasticity within the nucleus accumbens (NAc) in the occlusion of NMDAR-dependent long-term depression (LTD) of D1 medium spiny neurons (MSN). It has also been shown that the core and shell subregions of the NAc encode different aspects of drug responding and have distinct dopamine responses to reinforcers. However, the effect on accumbal plasticity following operant ethanol self-administration has not been extensively studied and therefore, this study sought to examine the effects of volitional ethanol consumption via operant self-administration on accumbal plasticity in both the core and shell subregions of the NAc. Initially, male Drd1a-tdTomato(+) mice received limited access two bottle choice within their home cages of 15% ethanol (15E) or tap water (mean ethanol consumption: 2.08 ± 0.05 g/kg/session). They next were trained to respond for 15E over 15 daily operant sessions; training then continued for a minimum of 10 additional sessions with a fixed ratio (FR) 4 lever press requirement (mean consumption: 1.33 ± 0.18 g/kg/session). Whole cell patch clamp electrophysiology was performed to investigate plasticity induction of excitatory postsynaptic currents (EPSCs) within Tom(+) MSNs of both the core and shell twenty-four hours following the final operant session (final session consumption: 1.64 ± 0.22 g/kg). The induction of LTD in core neurons was apparently normal (~60% baseline amplitude), and there was no correlation between ethanol consumption and the magnitude of LTD ($R = 0.086$, $p=0.872$). In contrast, shell recordings showed a significant inverse relationship between ethanol consumption and magnitude of LTD (Spearman's $R=-0.928$, $p<0.01$). Taken together, these results indicate that the dose of ethanol

self-administered during operant sessions plays a role in accumbal plasticity in the shell, but not core of the NAc.

Disclosures: **T.A. Meyers:** None. **R.A. Mangieri:** None. **R. Morrisett:** None.

Poster

211. Long-Term Depression (LTD)

Location: Hall A

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Program#/Poster#: 211.19/B77

Topic: B.08. Synaptic Plasticity

Support: NIAAA Division of Intramural Clinical and Biological Research

Title: Dopamine D2 receptors located on indirect pathway MSNs are not needed for high frequency stimulation-induced striatal long-term synaptic depression

Authors: *S. M. AUGUSTIN, M. I. DAVIS, D. M. LOVINGER;
NIAAA/Laboratory For Integrative Neurosci., Rockville, MD

Abstract: The dorsal striatum is a critical brain region for the integration of synaptic signals from cortex, thalamus, and midbrain. Plasticity at these afferent synapses contributes to action control and learning. The best understood form of synaptic plasticity in the striatum is endocannabinoid (eCB)-mediated long-term depression (LTD), which requires dopamine (DA) D2 receptors. Within striatum, there are different neuronal populations of which 90 percent are medium spiny projection neurons (MSNs) divided into two subpopulations, the “indirect” pathway MSNs (iMSNs) and “direct” pathway MSNs (dMSNs). The remaining 10 percent of striatal neurons are interneurons, including cholinergic interneurons (ChI). Both iMSNs and ChIs contain dopamine (DA) D2 receptors. For almost a decade, the site of the DA D2 receptors needed for the induction of LTD has been highly debated. Two different mechanisms for D2 involvement in LTD induction have been proposed; one involving the D2 receptors on the iMSNs and the other involving the D2 receptors on ChIs. Experimental evidence for these mechanisms is based on pharmacological experiments that could suffer from non-specific effects. Thus, the use of sophisticated gene-targeting strategy to selectively delete DA D2 receptors in the iMSNs and ChIs will allow for the dissection of DA D2 receptor roles in high frequency stimulation (HFS) induced eCB-mediated LTD in the dorsal lateral striatum. Mice carrying a “floxed” D2 allele were bred with mice expressing the Cre recombinase under the control of the iMSN-active A2A promoter to delete D2 receptors from iMSNs. There was a loss of D2 immunoreactivity in the MSNs, revealing somata of putative cholinergic interneurons in the iMSN knockout mice compared to control mice (D2 f/f) in dorsal striatum. In field potential recordings, HFS induces LTD of a similar magnitude in brain slices obtained from mice that lack DA D2 receptors on iMSNs in comparison to D2 f/f. This form of LTD was blocked by a DA

D2-class receptor antagonist and a cannabinoid receptor type 1 antagonist in both genotypes (DA D2 iMSN knockout and control). Whole cell recordings from iMSNs and dMSNs show that HFS induces LTD in both MSN subtypes, although DA D2 receptors are expressed on only iMSNs. HFS can induce a DA D2 receptor dependent LTD at synapses onto MSNs in the DA D2 iMSN knockout and control mice in whole cell recordings. These data indicate that D2 receptors on iMSNs are not necessary for LTD induction by HFS, and ongoing work will further assess this conclusion and allow us to determine the cellular locus of the D2 receptors that are most important for eCB mediated LTD induced using different stimulus protocols.

Disclosures: S.M. Augustin: None. **M.I. Davis:** None. **D.M. Lovinger:** None.

Poster

212. Spike Timing-Dependent Plasticity (STDP)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 212.01/B78

Topic: B.08. Synaptic Plasticity

Title: Normalization of excitatory-inhibitory balance by cortical spike-timing-dependent plasticity

Authors: *J. A. D'AMOUR[1], R. C. FROEMKE[2];
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Abstract: Synapses are plastic and can be modified by changes of spike timing. While most studies of long-term synaptic plasticity focus on excitation, inhibitory plasticity may be critical for controlling information processing, memory storage, and overall excitability in neural circuits. Here we examine spike-timing-dependent plasticity (STDP) of inhibitory synapses onto layer 5 neurons in slices of young mouse auditory cortex (P10-25), together with concomitant STDP of excitatory synapses. Pairing pre- and postsynaptic spikes potentiated inhibitory inputs irrespective of precise temporal order within ~10 msec (162.2±15.3% n=34 for short positive spike timing intervals, and 133.7±15.8% n=26 for short negative intervals). This was in contrast to excitatory inputs, which displayed an asymmetrical STDP time window (132±6.2% n=46 for short positive intervals, and 81.9±2.8% n= 30 for negative intervals). These combined synaptic modifications both required NMDA receptor activation, and synergistically acted to normalize the excitatory-inhibitory ratio of events paired together with postsynaptic spiking. Finally, subthreshold events became suprathreshold, and the time window between somatically recorded excitation and inhibition became more precise, suggesting that these combined modifications also enforce spike-timing reliability. These findings demonstrate that cortical inhibitory plasticity requires interactions with co-activated excitatory synapses to properly regulate excitatory-inhibitory balance.

Disclosures: **J.A. D'Amour:** None. **R.C. Froemke:** None.

Poster

212. Spike Timing-Dependent Plasticity (STDP)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 212.02/B79

Topic: B.08. Synaptic Plasticity

Support: NIH grant R01 MH087631

Title: Modulation of homosynaptic and heterosynaptic plasticity by adenosine receptors in rat visual cortex *in vitro*

Authors: ***N. M. BANNON**, M. CHISTIAKOVA, M. VOLGUSHEV;
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Abstract: In the rat neocortex, pairing presynaptic stimulation with postsynaptic bursts of spikes can lead to plasticity at both paired 'homosynaptic' inputs and at unpaired, 'heterosynaptic' inputs (which experience postsynaptic spiking but not presynaptic activation). Heterosynaptic plasticity can also be induced by intracellular tetanization: bursts of spikes in the postsynaptic cell induced in the absence of presynaptic stimulation (mimicking conditions experienced at unpaired inputs). The outcome of homo- and hetero-synaptic plasticity induced by these protocols is weight-dependent. Synapses with a high release probability (low initial paired-pulse ratio; PPR) tend to depress, while synapses with a low release probability (high PPR) tend to potentiate. Adenosine, which is released by neurons and glia in an activity-dependent manner, is an endogenous regulator of presynaptic release in the neocortex acting primarily via A1 receptors. We asked if manipulation of adenosine receptors can modulate long-term plasticity by altering baseline synaptic strength. To test this, we made *in vitro* whole-cell recordings from layer 2/3 pyramidal neurons in slices of rat visual cortex and studied synaptic responses evoked with stimulating electrodes placed in layer 4. We induced long-term plasticity by intracellular tetanization or a pairing protocol, with postsynaptic activity consisting of three trains (1/min) of ten bursts (1 Hz) of five pulses (5 ms, 100 Hz, 0.4-1.5 nA). We compared outcome of these plasticity protocols under conditions of normal artificial cerebral spinal fluid (control), and with 20uM adenosine or 30nM of the selective A1R antagonist 8-Cyclopentyl-1,3-dipropylxanthine (DPCPX) added to the bath. Adenosine suppressed synaptic responses and increased the baseline PPR, suggesting a reduction in release probability, while antagonism of A1 receptors decreased the PPR. Concurrent with changes in PPR were changes in plastic outcome predicted by the established weight-dependence relationship. In control solution, intracellular tetanization induced potentiation in 21% of cases, and depression in 29% of cases. Under adenosine, 44% of inputs exhibited potentiation and only 19% exhibited depression. Further, blocking endogenous adenosine tone with DPCPX restricted the range of initial synaptic strengths so that the weight-

dependent relationship was no longer observable. Thus, adenosine receptor manipulation modulates the outcome of weight-dependent plasticity by altering the baseline synaptic strength, and an endogenous adenosine tone is responsible for the full range of synaptic strengths which underlie this weight-dependence.

Disclosures: **N.M. Bannon:** None. **M. Chistiakova:** None. **M. Volgushev:** None.

Poster

212. Spike Timing-Dependent Plasticity (STDP)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 212.03/B80

Topic: B.08. Synaptic Plasticity

Title: History dependence of calcium dynamics at the larval fruit fly neuromuscular junction

Authors: ***M. CURRAN**, J. KRANS;
Western New England Univ., Springfield, MA

Abstract: Calcium (Ca^{2+}) is an essential ion for both pre- and post-synaptic function. Its actions as a second messenger and its contribution to synaptic plasticity are well described. Less is known about the history dependence of changes in free Ca^{2+} at the neuromuscular junction (NMJ), especially post-synaptically (i.e. muscle). However, previous research has shown that the decay rate of [Ca^{2+}] in muscle attenuates with constant rate synaptic activation over short intervals (< 1 min); Ca^{2+} is available for longer periods. It is also known that basal [Ca^{2+}] increases upon long-term stimulation of muscle (hours to days). Changes in [Ca^{2+}] that occur over intermediate time scales (~minutes) are not well described. Moreover, rarely is the NMJ activated in a physiologically relevant manner (e.g. rhythmic oscillation of synaptic activation). We investigated changes in [Ca^{2+}] in larval bodywall muscles of *D. melanogaster*. We employed a genetically encoded Ca^{2+} sensitive fluorophore (GCaMP5) to estimate the rate of calcium sequestration (decay of free Ca^{2+}) immediately following various stimulus trains. The NMJ was activated by multiple trains of stimuli, each of which was frequency modulated (FM) in a sinusoidal pattern to reflect peristaltic NMJ activation. Immediately after stimulation, [Ca^{2+}] was estimated by computing the change in relative fluorescence of select regions from each video (Stacks function, ImageJ). This decay in fluorescence – 0 to 0.5 s post stimulus train - was well fit by an exponential model (R= 0.98 +/- 0.002 SEM, n=28). We used this metric to evaluate the history dependence of post-synaptic [Ca^{2+}] decay rate. We presented multiple trains of stimuli separated by an inter-train interval (ITI). We hypothesized that history dependence would diminish with longer ITIs. When three stimulus trains were presented, 120 s apart from each other, the rate of fluorescence decay after each stimulus train decreased: stimulus train 2 exhibited 25% less decay than train 1, and train 3 was about 40% less decay than train 1. However, upon presentation of the fourth stimulus train the decay rate returned to it's initial

value (+/-5% decay rate of train 1). This return to initial decay rate upon presentation of the fourth train may represent a loss of history dependence, and was observed in all other ITIs. The time at which this 'reset' , or loss of history dependence, occurred was ~6 min. after initiation of the multi-train stimulus protocol. Interestingly, we also observed that decay rates changed less when ITI was short (ITIs = 45, 90, 120; N= 14) than when ITI was longer (ITIs = 150, 165, 180; N=14; $p < 0.01$), suggesting a maximal dependence upon history at ~ 180 s post stimulus initiation.

Disclosures: **M. Curran:** None. **J. Krans:** None.

Poster

212. Spike Timing-Dependent Plasticity (STDP)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 212.04/B81

Topic: B.08. Synaptic Plasticity

Title: Astrocytic glutamate uptake gates spike-timing-dependent plasticity and counteracts spurious plasticity

Authors: S. VALTCHEVA, *L. VENANCE;
Ctr. for Interdisciplinary Res. In Biol. (INSERM 1050/CNRS 7241), Paris, France

Abstract: Astrocytes constitute the major sink for released glutamate. Glutamate uptake is mainly operated by a high-affinity glutamate transporter, the excitatory amino acid transporter type-2 (EAAT2), expressed by astrocytes. Astrocytic glutamate uptake is crucial in shaping synaptic transmission by limiting glutamate spillover and therefore acting on the strength and the timing of synaptic inputs. Spike-timing-dependent plasticity (STDP) is a synaptic Hebbian learning rule, which relies on the precise order and timing of paired activity on either side of the synapse. EAAT2 is highly expressed in the striatum where it controls corticostriatal transmission and short-term plasticity (Goubard et al., 2011). The corticostriatal long-term plasticity provides a fundamental mechanism for the function of the basal ganglia in procedural learning. We investigated the impact of astrocytic glutamate uptake via EAAT2 on corticostriatal STDP. Here we show that proper function of EAAT2 is crucial for the temporal contingency necessary for STDP expression. Indeed, when EAAT2 was transiently blocked, STDP was replaced by another form of synaptic plasticity (GluN2B-mediated LTP) that invades the temporal windows in which STDP do not normally occur. When random patterns of pre- and postsynaptic activity were applied during EAAT2 transient blockade, plasticity could still be induced. It indicates that this form of plasticity depends neither on the timing nor on the order of pre- and postsynaptic activity and could be triggered even by highly uncorrelated events. In addition, this plasticity also occurred for unpaired activity consisting in postsynaptic spiking without presynaptic stimulation. Furthermore, overexpression of EAAT2 by ceftriaxone precluded STDP. We unravel a new role

of astrocytic glutamate uptake in synaptic plasticity. Indeed, astrocytic glutamate uptake favors the time-based coding at corticostriatal synapses over frequency-based and thus operates as a selector of learning rules. The novelty of our findings is that glutamate uptake selects the temporal coding paradigm at corticostriatal synapses. In this context, our report places astrocytes as a key player in the establishment of genuine Hebbian plasticity by gating STDP and in counteracting spurious plasticity.

Disclosures: **S. Valtcheva:** None. **L. Venance:** None.

Poster

212. Spike Timing-Dependent Plasticity (STDP)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 212.05/B82

Topic: B.08. Synaptic Plasticity

Support: NSF-IGERT in Systems Neuroengineering (DGE-1069104)

Title: Optimization of deep brain stimulation parameters using computational models of the basal ganglia with spike-time dependent plasticity

Authors: ***L. GRADO**, M. JOHNSON, T. NETOFF;
Univ. of Minnesota, Minneapolis, MN

Abstract: Deep brain stimulation (DBS) of the basal ganglia is a widely used and effective treatment for patients with medication-refractory Parkinson's disease (PD). Current theories of DBS mechanisms propose that DBS suppresses pathological oscillations (15-35 Hz) that dominate the basal ganglia and filter information flow through the basal ganglia. Importantly, the effects of DBS are not instant, often taking minutes or more before an effect manifests. This time scale suggests DBS may induce a change in network architecture through synaptic plasticity, destabilizing oscillations in the network. In this study, we implemented spike-time dependent plasticity (STDP) mechanisms in a computational network model of the basal ganglia that exhibits emergent pathological 34 Hz oscillations following dopaminergic denervation (Hahn & McIntyre, 2010, J Comput Neurosci). The network model consists of biophysical single compartment models of STN, GPe, and GPi neurons. The neurons are organized into functional columns with excitatory connections from STN to GPe and GPi, and inhibitory connections from GPe to STN and GPi, driven by cortical beta rhythms. STDP was added to each synapse type present in the model: inhibitory-inhibitory, inhibitory-excitatory, and excitatory-inhibitory. For each STDP type, a separate curve was constructed relating the time between pre and post synaptic spike times and synaptic potentiation/depression. Simulations of the network model with STDP following DBS onset showed that these high-beta oscillations dissipate over time, fading out over the course of minutes. The model results also suggest that it may be possible to

automatically tune stimulation settings to take advantage of long-term potentiation and/or long-term depression within the network through reinforcement learning algorithms.

Disclosures: **L. Grado:** A. Employment/Salary (full or part-time):; University of Minnesota. **M. Johnson:** A. Employment/Salary (full or part-time):; University of Minnesota. **T. Netoff:** A. Employment/Salary (full or part-time):; University of Minnesota.

Poster

212. Spike Timing-Dependent Plasticity (STDP)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 212.06/B83

Topic: B.08. Synaptic Plasticity

Support: BFU2012-33413

Title: The encoding of episodic memory via spike-timing-dependent plasticity: a computational model

Authors: ***P. THEODONI**, A. ROXIN;
Computat. Neurosci. Group, Ctr. De Recerca Matemàtica, Bellaterra (Barcelona), Spain

Abstract: It is well known that the hippocampus plays a major role in the formation and retrieval of episodic memories. In rodents, hippocampal cells called place-cells exhibit activity which is selective to the spatial location of the animal in a given environment. As the animal traverses the environment, a particular pattern of place-cell activity will be generated, corresponding to the specific trajectory taken. In this way, cells with spatially adjacent place fields will also fire in temporal proximity, potentially leading to changes in their recurrent synaptic weights via spike-timing-dependent plasticity (STDP) mechanisms [1]. Here we model this process with a computational model in which we consider both pairwise and triplet STDP rules in a recurrent network of hippocampal place cells. We study how the exploration of the animal over time can lead to the formation of low-dimensional attracting manifolds in the neuronal network, each one of which encodes the memory of a specific environment. For example, the traversal of a 1D ring-like environment leads, via STDP, to a so-called ring attractor [2]. Once formed, the presence of the attractor can explain the dynamics of so-called hippocampal replay, via spontaneous re-activation of spatially localized bump states [3]. In fact, the temporal compression of replay can be related to the details of the underlying STDP rule. Interestingly, once the memories of several distinct environments have been encoded, the correlation of replay activity with any given memory can be modulated by the level of external input. [1] Bi and Poo, J. Neurosci. 1998. [2] Romani and Tsodyks, PloS Comp. Biol. 2010. [3] Romani and Tsodyks, Hippocampus 2015. This work was funded by a grant from the Spanish Ministry of Economics and Competitiveness BFU2012-33413.

Disclosures: **P. Theodoni:** None. **A. Roxin:** None.

Poster

212. Spike Timing-Dependent Plasticity (STDP)

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Topic: B.08. Synaptic Plasticity

Support: 973 program, 2011CBA00400

Strategic Priority Research Program, XDB02020001

Title: Potentiation of synaptic connections among cortical neuronal groups via coactivation - an *in vitro* test of Hebb's cell

Authors: *D. ZHANG[1,2], X. YAN[3,2], M.-M. POO[3,2];
[1]Inst. of Neuroscience,Shangai Inst. For Bi, Shanghai, China; [2]CAS center for excllence in brain science, Shanghai, China; [3]Shanghai institutes for biological sciences,CAS, Shanghai, China

Abstract: Hebb proposed in 1949 that correlated firing of neurons strengthens their synaptic connections, and the perceptual memory is formed by establishing specific assembly of neurons with strengthened interconnections. Neuronal signals in the brain are often conveyed among co-activated neuronal groups rather than single neurons. We have tested the extended form of Hebb's cell assemble hypothesis by studying potentiation of interconnections among groups of cortical neurons in anesthetized mice. Cortical layer 2/3 pyramidal neurons were transfected with associated adenovirus coding for channelrhodopsin2 (ChR2), and coactivation of several groups of neurons was accomplished by simultaneous application of laser spots at multiple cortical locations. Laser spot-induced neuronal firing was detected by multiunit recording at co-activated locations to determine the strength of interconnections among coactivated neuronal groups. We found that coactivation induced persistent potentiation of connections among neuronal groups that depended on activation of N-methyl-D-aspartic acid (NMDA) subtype of glutamate receptors, consistent with activity-induced long-term potentiation (LTP) between individual neurons. This was confirmed by *in vivo* whole-cell recording of laser-evoked synaptic potentials in cortical neurons. Further studies showed that this potentiation was saturable and depended on the pattern of co-activation, with burst stimulation more effective than tonic stimulation at the same total number of stimuli. Similar coactivation-induced potentiation was observed in both visual and motor cortices. These results support the idea that Hebb's hypothesis could be applied to the assembly of neuronal groups by correlated activity, a situation more relevant to conditions of signal processing in the brain.

Disclosures: **D. Zhang:** None. **X. Yan:** None. **M. Poo:** None.

Poster

212. Spike Timing-Dependent Plasticity (STDP)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 212.08/B85

Topic: B.08. Synaptic Plasticity

Support: NIH grant MH087631

Title: An interplay between homosynaptic and heterosynaptic plasticity enforces synaptic competition and allows re-learning

Authors: *M. A. VOLGUSHEV[1], M. CHISTIAKOVA[1], V. ILIN[2], R. GOZ[1], J.-Y. CHEN[3], M. BAZHENOV[3];
[1]Dept Psychol, Univ. Connecticut, Storrs Manfld, CT; [2]Univ. Connecticut, Storrs, CT; [3]Univ. of California, Riverside, CA

Abstract: Homosynaptic Hebbian type plasticity provides a cellular mechanism of learning and refinement of connectivity during development in a variety of biological systems. However, Hebbian-type learning rules impose positive feedback on synaptic weight changes, and make learning systems with plastic synapses prone to runaway dynamics. An additional mechanism is necessary for preventing the runaway dynamics of synaptic weights and activity. We propose that this homeostatic role is played by heterosynaptic plasticity - changes that do not require activation of a pre-synapse, and thus can occur at synapses which were not active during the induction of homosynaptic plasticity. Here we show that in layer 2/3 pyramidal neurons in slices from rat visual cortex induction of homosynaptic plasticity with STDP-protocol is accompanied by heterosynaptic changes in un-paired synapses. The direction and magnitude of heterosynaptic plasticity correlated with initial paired-pulse ratio (PPR): inputs with initially high PPR tended to potentiate, while inputs with initially low PPR tended to depress or do not change. Because PPR is inversely related to release probability, this correlation suggests that heterosynaptic plasticity had a normalizing effect on synaptic strength. Using computer simulations we demonstrate that heterosynaptic plasticity with the observed experimental properties can indeed effectively prevent runaway dynamics of synaptic weights imposed by STDP learning rules. We further show that this form of heterosynaptic plasticity enhances synaptic competition in a model neuron and facilitates segregation of weights of synapses which differ by details of plasticity rules, frequency of presynaptic firing and level of correlation of activity of presynaptic neurons. Because heterosynaptic plasticity prevents saturation of synaptic weights but keeps them in the operation range, it also enables learning of new patterns on the background of existing memories (distributions of synaptic weights). We conclude that heterosynaptic plasticity is complementary to homosynaptic Hebbian-type plasticity and represents a necessary cellular component for homeostatic regulation of synaptic weights and maintaining the ability of neurons with plastic synapses for continuing learning.

Disclosures: **M.A. Volgushev:** None. **M. Chistiakova:** None. **V. Ilin:** None. **R. Goz:** None. **J. Chen:** None. **M. Bazhenov:** None.

Poster

212. Spike Timing-Dependent Plasticity (STDP)

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Program#/Poster#: 212.09/B86

Topic: B.08. Synaptic Plasticity

Support: Ministerio de Economía y Competitividad BFU2012-38208

Proyecto Excelencia Junta de Andalucía CVI-7290

Title: Presynaptic induction and expression of spike timing-dependent long-term depression in the CA1 region of the hippocampus

Authors: *A. RODRIGUEZ-MORENO[1], Y. ANDRADE-TALAVERA[1], P. DUQUE-FERIA[1], O. PAULSEN[2];
[1]Univ. Pablo de Olavide, Seville, Spain; [2]Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Spike timing-dependent plasticity (STDP) is a model of synaptic plasticity that may underlie learning and memory. The aim of our research was to investigate the signalling pathway for the induction of spike timing-dependent long-term depression (t-LTD) in the hippocampus. Whole-cell recordings were made from individual CA1 cells in hippocampal slices prepared from P12-P18 mice. Two independent afferent pathways (Schaffer collaterals) were activated alternately by extracellular stimulation. We have previously shown in the hippocampus that a post-before-pre pairing protocol (pairing postsynaptic action potentials with EPSPs at 0.2 Hz) produced robust input-specific t-LTD and that the induction of this form of LTD was completely blocked by D-AP5, by the broad spectrum mGluR antagonist MCPG, as well as by group I mGluRs selective antagonists, phospholipase C (PLC) inhibitors and by the CB1 receptor antagonist AM251. The blockade of postsynaptic NMDARs by application of MK-801 (1 mM) through the patch pipette did not affect the induction of t-LTD (76 ± 8 %, n = 12), but abolished the induction of t-LTP induced by a pre-before-post pairing protocol (104 ± 7 %, n = 9, vs 152 ± 8 % in control experiments, n = 14). We have carried out pre-post and post-pre pairings in the same cell treated with MK-801. t-LTP was not observed after pre-post pairing (103 ± 6 %, n = 6, but subsequent post-pre pairing in the same pathway induced robust t-LTD (71± 7 %, n = 6). We have also determined that this t-LTD requires astroglial signalling as it is completely prevented by loading astrocytes with 20 mM BAPTA (129 ± 7 %, n = 5 vs interleaved slices 54 ± 6 %, n = 5). Fluctuation, failures and paired-pulse ratios analysis all indicated a presynaptic locus of expression of this t-LTD. These results show that whereas t-LTP induction depends on postsynaptic NMDARs, the induction of t-LTD is independent of postsynaptic activation of

NMDARs and likely requires presynaptic NMDA receptors. The results suggest that the induction and expression of t-LTD at CA3-CA1 synapses are presynaptic.

Disclosures: **A. Rodriguez-Moreno:** None. **Y. Andrade-Talavera:** None. **P. Duque-Feria:** None. **O. Paulsen:** None.

Poster

212. Spike Timing-Dependent Plasticity (STDP)

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Topic: B.08. Synaptic Plasticity

Support: NRF Grant 2013-R1A1A2053280

Title: $A\beta_{1\text{-}42}$ impairs metabotropic glutamate receptor-gated spike-timing dependent long-term potentiation of hippocampal CA3-CA1 synapse

Authors: ***J. LEE**, J. KWAG;
Dept. of Brain and Cognitive Engin., Korea Univ., Seoul, Korea, Republic of

Abstract: Alzheimer's disease is a neurodegenerative disease causing memory deficit, characterized by abnormal accumulation of amyloid-β oligomers ($A\beta_{1\text{-}42}$) in the hippocampus. We have recently reported that $A\beta_{1\text{-}42}$ impairs the induction of spike-timing dependent long-term potentiation (tLTP), which is considered a physiologically more realistic way to induce hippocampal LTP. Activation of metabotropic glutamate receptors (mGluR) is known to gate and enhance the hippocampal tLTP at the CA3-CA1 excitatory synapse but the effect of $A\beta_{1\text{-}42}$ on mGluR-gated tLTP is unclear. Therefore we investigated the effect of $A\beta_{1\text{-}42}$ on induction of mGluR-gated tLTP using whole-cell patch-clamp *in vitro*. tLTP was induced by pairing presynaptic CA3 input with postsynaptic CA1 pyramidal cell spike with a time window of +10 ms, 200 times at 1 Hz. Hippocampal slices were incubated in artificial cerebrospinal fluid (ACSF) containing 200 nM $A\beta_{1\text{-}42}$ or 0.02% dimethyl sulfoxide for 20 minutes prior to pairing for $A\beta_{1\text{-}42}$ or vehicle experiments, respectively. Group I mGluR agonist, dihydroxyphenylglycine (DHPG; 50 μM), or mGluR5 blocker, 2-methyl-6-(phenylethynyl)pyridine (MPEP; 10 μM) were dissolved in ACSF to modulate the activation of mGluR. tLTP induction protocol at CA3-CA1 synapse in the presence of DHPG could induce mGluR-gated tLTP reliably in the test pathway in vehicle experiment while mGluR-mediated long-term depression (LTD) was induced in the control pathway (test: 157 ± 25%, control: 79 ± 15%, paired *t*-test, $p < 0.05$, n = 5). In $A\beta_{1\text{-}42}$-treated slices, the induction of mGluR-gated tLTP failed in test pathway but LTD was induced in both the test and control pathways (test: 60 ± 15%, control: 66 ± 13%, paired *t*-test, $p = 0.72$, n = 6). Interestingly, stronger mGluR-mediated LTD was induced by $A\beta_{1\text{-}42}$ (vehicle control: 79 ± 15%, $A\beta_{1\text{-}42}$ control: 66 ± 13%). These results suggest that $A\beta_{1\text{-}42}$ impairs mGluR-gated tLTP

and enhances mGluR-mediated LTD. To confirm the role of mGluR, we repeated the experiments with MPEP. MPEP abolished both the mGluR-gated tLTP in the test pathway and mGluR-mediated LTD in the control pathway in vehicle experiments (test: 98 ± 34%, control: 97 ± 27%, paired *t*-test, $p = 0.96$, n = 6) while $A\beta_{1\text{-}42}$ induced strong LTD in both pathways (test: 24 ± 13%, control: 20 ± 7%, paired *t*-test, $p = 0.72$, n = 5). These results suggest that mGluR5 mediates the mGluR-gated tLTP induction and that $A\beta_{1\text{-}42}$ facilitates LTD. We demonstrate that $A\beta_{1\text{-}42}$ disrupts the induction of mGluR-gated tLTP at hippocampal CA3-CA1 synapse and enhances LTD induction. Further investigation on the interaction between $A\beta_{1\text{-}42}$ and mGluR is needed to better understand the $A\beta_{1\text{-}42}$-mediated impairment of synaptic plasticity.

Disclosures: J. Lee: None. **J. Kwag:** None.

Poster

212. Spike Timing-Dependent Plasticity (STDP)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 212.11/B88

Topic: B.08. Synaptic Plasticity

Title: Retroactive effect of dopamine on spike timing-dependent plasticity

Authors: *Z. BRZOSKO, W. SCHULTZ, O. PAULSEN;
Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Spike timing-dependent plasticity (STDP) is a physiologically relevant form of Hebbian learning in which the order and precise timing of presynaptic and postsynaptic spikes determine the sign of synaptic change: pre–before-post spike pairings induce timing-dependent long-term potentiation (t-LTP) whereas post–before-pre pairings induce long-term depression (t-LTD). The quantitative rules of STDP are influenced by neuromodulators, including dopamine (DA). In light of the potential role of DA in reward learning, here we examined whether DA modulates STDP not only when applied during, but also – more importantly - when applied *after* the pairing event. The slope of excitatory post-synaptic potentials (EPSPs), evoked by extracellular stimulation of Schaffer-collateral-CA1 pathway, was monitored during whole-cell recordings of CA1 pyramidal cells in mouse hippocampal slices (postnatal days 12-18). Plasticity was induced by a pairing protocol that involved 100 pairings of a single postsynaptic spike and a single presynaptic EPSP at 0.2 Hz. In accordance with previous findings[1], we found that the presence of DA *during* the coordinated spiking activity widens the spike time window for induction of t-LTP. Crucially, we also found that DA applied *after* the post-before-pre pairing protocol (with the spike preceding the EPSP by 20 ms) converts t-LTD into t-LTP. This implies that DA can act retroactively to allow negative spike pairings to induce t-LTP. This effect of DA was activity-dependent, demonstrating that DA is capable of acting specifically on active inputs. In addition, the observed DA-induced conversion of t-LTD into t-LTP depends on the timing of

application following the post-before-pre protocol. Delayed application of DA (10 or 30 minutes after t-LTD pairing protocol) failed to convert t-LTD into t-LTP, suggesting that DA can convert t-LTD into t-LTP when acting within a short time window following the induction protocol. Finally, we explored possible mechanisms underlying the DA-induced conversion of t-LTD into t-LTP and showed that this effect is mediated in part through the activation of the cAMP/PKA cascade and requires synaptic NMDA receptors. Together our work demonstrates a retroactive effect of DA on STDP. This supports the concept of a slowly decaying synaptic *eligibility trace*, which is committed to memory by the occurrence of reward. It therefore provides a possible mechanism for associating specific experiences with behaviorally distant, rewarding outcomes.
1. Zhang, J.C., Lau, P.M., & Bi, G.Q. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 13028–13033 (2009).

Disclosures: **Z. Brzosko:** None. **W. Schultz:** None. **O. Paulsen:** None.

Poster

212. Spike Timing-Dependent Plasticity (STDP)

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 212.12/B89

Topic: B.08. Synaptic Plasticity

Support: NSF-DMS-1313225

Title: Stable assembly training and reinforcement through spike timing

Authors: ***G. K. OCKER**[1], B. DOIRON[2];
[1]Neurosci., [2]Mathematics, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Spike timing-dependent plasticity (STDP) leverages the temporal correlations between pre- and post-synaptic activity to shape synaptic strengths. It is a candidate mechanism for the formation of Hebbian assemblies, linking network structure to stimulus representation. Theories explaining the emergence of such macroscopic structure have either 1) relied on synaptic plasticity driven primarily by neurons' firing rates rather than precise spike timing or 2) have neglected internally generated spike-time correlations. We have recently developed a theory for spike timing-dependent plasticity in recurrent networks. This theory accounts for plasticity driven by spike-time correlations generated both by external inputs and by intra-network connectivity. We describe macroscopic structure at the level of average connection strengths while taking into account bidirectional connectivity between neurons. Using this theory we show that internally generated spike-time correlations can reinforce a diversity of macroscopic structures, including homogenous, clustered and feedforward architectures. External inputs can promote any of these, depending on their spatial profile. Furthermore, we study how rate-based homeostatis rules can stabilize the learned network structures and spiking activity. Such homeostatic rules control individual neurons'

firing rates. In contrast to previous studies, our network exhibits homogenous firing rates so that only precise spike-time correlations control plasticity, separating mechanisms supporting the formation of network structure from those responsible for homeostatic rate control.

Disclosures: G.K. Ocker: None. **B. Doiron:** None.

Poster

212. Spike Timing-Dependent Plasticity (STDP)

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Topic: B.08. Synaptic Plasticity

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BMBF grant (No. 01GQ1406 – Bernstein Award 2013 to H.C.)

Title: Toward a unifying plasticity model in extended dendrites

Authors: *C. EBNER[1,2], C. CLOPATH[3], P. JEDLICKA[4], H. CUNTZ[1,2];
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Abstract: A wide variety of stimulation protocols have unraveled the complex relations governing synaptic plasticity. Neuronal firing rates, the precise timing of pre- and postsynaptic spikes and the location of synaptic inputs along the dendritic tree are just a few examples that modulate synaptic plasticity. Several computational models of synaptic plasticity exist that reproduce different aspects of the data obtained in these experiments. Here, we adapt a phenomenological voltage-based spike timing-dependent plasticity (STDP) rule (1) and combine it with a detailed compartmental model of a L5 pyramidal cell (2). The aim of this study was to investigate the mechanisms of plasticity in extended dendritic tree models that account for the exact attenuation of backpropagating action potentials and the genesis of dendritic calcium spikes. Our hypothesis based on simulations in (1) was that one unifying plasticity rule might exist that reconciles all stimulation procedures. We show that, in combination with the realistic dendritic morphology, the same STDP rule is able to account for a large number of experiments studying the dependence of synaptic plasticity on stimulation frequency, synapse location and stimulation timing. Consistent with experimental data, we find that low-frequency pairing using bursts of five pre- and postsynaptic spikes resulted in long-term potentiation (LTP) at positive (pre before post) and in long-term depression (LTD) at negative (post before pre) timings,

consistent with classical STDP assumptions, while the LTD component increases with distance from the soma (3, 4). We conclude that the voltage-based STDP rule (1) which does not vary with dendritic location is able to account for the observed LTP/LTD dendritic switch with distance from the soma (4). 1. C. Clopath, W. Gerstner, Front. Synaptic Neurosci. 2, 25 (2010). 2. E. Hay, S. L. Hill, F. Schürmann, H. Markram, I. Segev, PLoS Comput. Biol. 7, e1002107 (2011). 3. P. J. Sjöström, G. G. Turrigiano, S. B. Nelson, Neuron. 32, 1149-1164 (2001). 4. P. J. Sjöström, M. Häusser, Neuron. 51, 227-238 (2006).

Disclosures: **C. Ebner:** None. **C. Clopath:** None. **P. Jedlicka:** None. **H. Cuntz:** None.

Poster

212. Spike Timing-Dependent Plasticity (STDP)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 212.14/B91

Topic: B.08. Synaptic Plasticity

Support: JSPS Fellowship (DC2)

JST CREST

Title: GABA driven circuit formation through heterosynaptic spike-timing-dependent plasticity

Authors: *N. HIRATANI[1,2], T. FUKAI[1];
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Abstract: A cortical neuron receives thousands of synaptic inputs through its dendrite, and the spatial distribution of the synaptic inputs on the dendrite is thought to significantly influence neural computation. Recent experimental results revealed that the relative spike timings among neighboring synapses on a dendritic branch have significant influence on changes in synaptic efficiency of these synapses, in addition to the spike time difference between presynaptic and postsynaptic neurons. Especially, the timing of GABAergic input exerts a great impact on synaptic plasticity at nearby Glutamatergic synapses. Here, we derived a simple yet biologically plausible computational model of this heterosynaptic form of spike-timing-dependent plasticity (h-STDP), by extending a previous model of homosynapic STDP, and investigated its functional role. In particular, we explored its contribution to dendritic computation. The model reproduces the several effects of h-STDP observed in the hippocampal CA1 area and the striatum of rodents. The model further predicts that h-STDP causes the detailed balance between excitatory and inhibitory inputs on a dendritic branch to enrich information capacity of a neuron through dendritic computation.

Disclosures: **N. Hiratani:** None. **T. Fukai:** None.

Poster

212. Spike Timing-Dependent Plasticity (STDP)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 212.15/B92

Topic: B.08. Synaptic Plasticity

Support: Swiss National Science Foundation

Title: The NMDA-spike as a fundamental mechanism in timing-dependent plasticity at hippocampal CA3 recurrent synapses

Authors: *F. BRANDALISE[1], S. CARTA[2], F. HELMCHEN[2], U. GERBER[2];
[1]Univerity of Zurich, Brain Res. Inst., Zurich, Switzerland; [2]Brain Res. Inst., Univ. of Zurich, Zurich, Switzerland

Abstract: Hippocampal CA3 pyramidal cells receive thousands of inputs onto their dendrites. It was previously shown that under certain conditions these inputs can trigger active integration leading to dendritic spikes, thereby enhancing the computational power of an individual neuron (Major et al. 2013). In CA3 pyramidal cells mainly two types of dendritic spikes have been described: the sodium dendritic spike and the NMDA spike (Kim et al. 2012; Makara and Magee 2013). As previously shown both *in vitro* (Golding et al. 2002; Brandalise and Gerber 2014) and *in vivo* (Gambino et al. 2014; Chicon and Gan 2015) dendritic spikes can induce synaptic plasticity. However, it is unclear whether the NMDA-spike is the ultimate effector in timing-dependent plasticity, as previously suggested (Lisman and Spruston 2005; Schiess et al. 2012) or if it represents a mechanism distinct from other forms of associative plasticity, e.g. spike timing dependent plasticity (STDP). We performed experiments to search for a causal relationship between the occurrence of NMDA spikes and the induction of LTP. We therefore obtained simultaneous double patch recordings from the soma and a dendrite of a CA3 pyramidal cell and applied an input-timing-dependent protocol (ITDP) as previously described (Brandalise and Gerber 2014). Focal hyperpolarization of a specific branch achieved through the dendritic patch pipette prevented the generation of an NMDA spike in those cases where the majority of the stimulated inputs targeted that same branch. Consequently LTP was not induced, consistent with a causal relation between the dendritic NMDA spike and synaptic plasticity. In contrast, in experiments where the stimulated inputs targeted dendritic branches other than the recorded branch, focal hyperpolarization through the patch pipette did not block LTP induction. We then confirmed the importance of extrasynaptic NMDA receptors in generating dendritic spikes (Chalifoux and Carter 2011) using a specific blocker for glutamate re-uptake (TBOA 70 μM). In this case, weak pairing stimulation that in control conditions failed to induce supralinear events and consequently did not induce LTP, evoked an NMDA spike during TBOA perfusion and CA3 EPSPs recurrent were potentiated. In a third set of experiments combining electrophysiological recording with calcium imaging we confirmed that subthreshold pairing of a CA3 EPSP

followed by an MF EPSP after 10 ms (ITDP) can evoke NMDA spikes manifesting as local, branch specific calcium transients. In conclusion our data support a mechanism whereby locally evoked NMDA spikes at excitatory dendritic synapses suffice to generate STDP.

Disclosures: **F. Brandalise:** None. **S. Carta:** None. **F. Helmchen:** None. **U. Gerber:** None.

Poster

212. Spike Timing-Dependent Plasticity (STDP)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 212.16/B93

Topic: B.08. Synaptic Plasticity

Support: DBT Research Grant (BT/PR12531/BRB/10/747/2009)

Title: Optogenetic control of stimulus and background activity in spike-triplet and theta-burst plasticity in acute hippocampal slices

Authors: ***A. BHATIA**, U. S. BHALLA;
Neurobio., Natl. Ctr. For Biol. Sci. (NCBS), Bangalore, India

Abstract: We wish to understand plasticity mechanisms in the context of more natural stimuli and synaptic input. We addressed two aspects of this: the nature of the stimulus, and the presence of background activity. Of the known plasticity paradigms, Spike Timing Dependent Plasticity (STDP) seems physiologically more plausible. Unlike initial experiments done in hippocampal cultures (Bi and Poo, 1998), STDP has been shown not to elicit the same changes in acute hippocampal slices (Pike et al., 1999)(Buchanan and Mellor, 2007). This opens the possibility of some other rule existing in this system. In the current study we have examined spike triplets in adult mouse acute hippocampal slices. We further examined the influence of ongoing background activity on plasticity, using optogenetic stimulation to deliver pseudo-random input patterns as background to theta-burst stimulation. EPSPs were recorded from CA1 cells while optically stimulating CA3 region of CA3-cre mice injected with Channlelrhodopsin2(ChR2)-lox virus. Using optical stimuli makes the STDP and background activity specific to the CA3-CA1 pathway, allows us to titrate the number of inputs by scaling spot size and intensity and present rapidly varying patterned stimuli from a projector. We observed ~60% potentiation of EPSPs in case of post-pre-post triplet where the post spikes were separated by 10ms and pre spike happened between 5-7ms. 60 pairings were done in the induction protocol consisting of trains of 5 triplets at 5Hz, repeated 12 times at 0.1Hz. No change was seen when only post synaptic spike pairs were presented. We also observed robust theta-burst LTP in the presence of noisy background synaptic input which simulates background activity in awake animals. Our results show that spike triplets are able to induce plasticity in hippocampal slices, extending previous work in hippocampal cultures (Wang et al., 2005). Further, we show that plasticity using

conventional protocols can be elicited in the background of random activity. Together this preparation facilitates the investigation of synaptic plasticity in more in-vivo-like activity contexts.

Disclosures: **A. Bhatia:** None. **U.S. Bhalla:** None.

Poster

212. Spike Timing-Dependent Plasticity (STDP)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 212.17/B94

Topic: B.08. Synaptic Plasticity

Title: Long-term plasticity expressed in neuronal ensemble

Authors: ***H. KWON**[1,2], T. KIM[1,3];
[1]Max Planck Florida Inst., Jupiter, FL; [2]Max Planck Inst. of Neurobio., Martinsried, Germany; [3]MIT, Boston, MA

Abstract: During cortical circuit development in the mammalian brain, groups of excitatory neurons receiving similar sensory information form microcircuits. However, cellular mechanisms underlying cortical microcircuit development remain poorly understood. Here we implemented combined two-photon imaging and photolysis *in vivo* to monitor and manipulate neural activities in order to study processes underlying activity-dependent circuit changes. We found that repeated triggering of spike trains in a randomly chosen group of layer 2/3 pyramidal neurons in somatosensory cortex increased the probability that the members of this group exhibited correlated firing patterns in a long-term manner. The formation of this functionally correlated group of neurons was dependent on the time interval between spike trains, NMDA receptor, CaMKII activation. In addition, repetitive sequential trains of firing from a subset of neurons made functional circuit assembly uneven. Thus, our results demonstrate that the formation of functional microcircuits requires wide-ranging neural connectivity change whose directionality is essentially non-random.

Disclosures: **H. Kwon:** None. **T. Kim:** None.

Poster

212. Spike Timing-Dependent Plasticity (STDP)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 212.18/B95

Topic: B.08. Synaptic Plasticity

Support: NIA grant SC1AG046907

Title: Examining the region-specific changes in plasticity and excitability in medial prefrontal cortex

Authors: B. OWEN, *M. BENVENISTE;
Neurosci. Inst., Morehouse Sch. of Med., Atlanta, GA

Abstract: The medial prefrontal cortex (mPFC), composed of the prelimbic (PL) and infralimbic (IL) areas, is a region associated with both memory and depression. These regions form connections with the hippocampus, amygdala, and raphe nucleus, and the PL and IL areas were observed to undergo region-specific changes in excitability. While others have examined the excitability changes within a specific network (i.e. mPFC-amygdala, hippocampus-mPFC) with regards to memory and fear conditioning, little work has been done to examine how serotonin, whose signaling is reduced in depressive states, modulates these network changes *in vitro*. Here, we examine the plasticity changes in the connections between layer V cells in the PL and IL areas using protocols which can induce long-term potentiation (LTP) and changes in EPSP-spike (E-S) coupling. Whole-cell current clamp recordings have been made from 300 μm brain slices containing the PL and IL areas from 8 - 12-week-old male C57Bl/6 mice. EPSPs were evoked by placing a stimulating electrode in layer V approximately 100 - 300 μm from the recorded cell. Preliminary data indicates that IL neurons undergo E-S potentiation following theta burst stimulation but do not show LTP. Yet, changes in the rheobase measured 30 minutes after the induction protocol were not observed. In contrast, PL neurons exhibit LTP but do not undergo E-S potentiation. These results lay the groundwork for examination of how serotonin input could influence neuronal plasticity, and yield insight into how depressive states influence memory acquisition.

Disclosures: B. Owen: None. **M. Benveniste:** None.

Poster

212. Spike Timing-Dependent Plasticity (STDP)

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 212.19/B96

Topic: B.08. Synaptic Plasticity

Title: Cooperative coincidence detectors control mixed pre- and postsynaptic expression of spike-timing dependent plasticity at the cerebellar input stage

Authors: *F. PRESTORI[1], M. SGRITTA[1,2], E. D'ANGELO[1,3];
[1]Dept of Brain and Behavioral Sci., Univ. of Pavia, Pavia, Italy; [2]EBRI, Rome, Italy; [3]Brain Connectivity Ctr., IRCCS C.Mondino, Pavia, Italy

Abstract: Excitatory central synapses show a special form of persistent change, spike-timing dependent plasticity (STDP), in which long-term potentiation and depression (LTP and LTD) are related to the relative phase of occurrence of EPSPs and action potentials. At the cerebellar mossy fiber - granule cell synapse, LTP and LTD have been previously related to the duration and frequency of input busts but their EPSP-spike phase sensitivity was unknown. Here we show that EPSP-spike pairing on the 6Hz band can reliably induce STDP in this synapse. LTP was confined to the +5/+20 ms time-window, while LTD occurred at longer positive phases and at negative phases revealing a high temporal precision for LTP induction. STDP as a whole required NMDA receptor activation and calcium release from intracellular stores, but LTP also required mGluR activation and higher calcium levels. Importantly, STDP was 2-3 times larger than any forms of long-term synaptic plasticity previously reported at this same synapse (LTP:+61.4%± 20.2%, n=5, t<0.05; LTD:-50.6%±12.6%, n=5, t<0.05). While LTP and LTD induced by modulated burst duration and frequency were uniquely expressed by a release probability change, STDP showed a mixed pre- and postsynaptic expression attested by consistent changes in EPSC amplitude and coefficient of variation, EPSC paired-pulse ratio (PPR; LTP:-32.3%± 4.9%, n=5, t<0.001; LTD:+21.0%±14.9%, n=5, t<0.05) and minis amplitude (LTP:+23.4%± 9.9%, n=5, t<0.05; LTD:-16.1%±5.2%, n=5, t<0.05) and frequency (LTP:+18.1%± 8.7%, n=5, t<0.05; LTD:-30.7%±8.6%, n=5, t<0.05). Therefore STDP appears a powerful form of plasticity that binds LTP to the mossy fiber burst phase on the millisecond time-scale and could control granular layer functions binding it tightly to ongoing brain temporal dynamics.

Disclosures: F. Prestori: None. **M. Sgritta:** None. **E. D'Angelo:** None.

Poster

213. Oligodendrocytes: Myelination and Remyelination

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 213.01/B97

Topic: B.11. Glial Mechanisms

Support: NIH NS052741 (IAS)

NIH NS50465 (FGP)

NMSS RG4958 (IAS)

Title: The interplay between exercise and dietary fat modulates myelinogenesis in the adult spinal cord

Authors: *I. A. SCARISBRICK[1], H. YOON[1], A. KLEVEN[1], P. STARSKI[1], A. PAULSEN[1], J. WU[1], L. KLEPPE[1], Z. YING[2], F. GOMEZ-PINILLA[2];
[1]Physical Med. and Rehabil., Mayo Clin., Rochester, MN; [2]Integrative Biol. and Physiol., UCLA, Los Angeles, CA

Abstract: Proper myelination is crucial for transmission of information and myelination is likely modulated by the interaction between lipid components and axonal activity. Here we test the hypothesis that dietary saturated fatty acids alone, or in combination with exercise training, can influence myelin homeostasis in the adult spinal cord. To test this hypothesis, 9 week old adult C57BL6/J male mice were fed a diet enriched in fat (60% total fat, 20% from saturated fat) for a period of 7 weeks, provided access to free wheel running for a corresponding period, or provided access to both interventions in combination. We used quantitative Western blot, real time PCR and immunohistochemical approaches to quantify myelin proteins, oligodendrocyte progenitor cells (OPCs), mature oligodendrocytes, associated growth factor systems, and signaling cascades in the lumbosacral spinal cord of mice under these conditions compared to those with a sedentary lifestyle. Results demonstrate that the abundance of the major myelin membrane proteins, proteolipid (PLP) and myelin basic protein (MBP), as well as NG2, a marker for OPCs, were significantly elevated in the spinal cord after 7 weeks of exercise training in combination with high dietary saturated fats. Expression of MBP and PLP RNA, as well that for Myrf1, a transcription factor driving oligodendrocyte differentiation, were also differentially increased with exercise and/or high dietary saturated fats. In conjunction with these findings however, consumption of a high fat diet alone resulted in a reduction in NG2 and Nkx2.2-OPCs present in the spinal cord white matter of adult mice. A parallel decrease in mature CC-1+-oligodendroglia and those labeled for the pan-OPC and oligodendrocyte marker Olig2 was also seen with consumption of high fat in the context of a sedentary lifestyle. Of potential clinical significance, seven weeks of exercise training completely reversed the deleterious effects of a high fat diet on OPC and oligodendrocyte numbers. Exercise and dietary fatty acid-induced changes in myelinogenesis occurred in parallel with increases in the expression of spinal cord IGF-1 and IGF-1 receptor. Parallel increases in phosphorylated-AKT, a signaling intermediate involved in the myelinogenic effects of IGF-1, was also observed in response to consumption of high dietary saturated fat alone or in combination with exercise. Together these data support a model in which exercise in combination with high dietary saturated fatty acids unleashes a pro-myelination program that supports myelin homeostasis in the adult spinal cord. These results are crucial for the design of rehabilitative programs to enhance CNS function.

Disclosures: I.A. Scarisbrick: None. **H. Yoon:** None. **A. Kleven:** None. **P. Starski:** None. **A. Paulsen:** None. **J. Wu:** None. **L. Kleppe:** None. **Z. Ying:** None. **F. Gomez-Pinilla:** None.

Poster

213. Oligodendrocytes: Myelination and Remyelination

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 213.02/B98

Topic: B.11. Glial Mechanisms

Support: JSPS KAKENHI Grant Number 25893291

Title: Lactate, a possible metabolite for remyelination by oligodendrocyte progenitor cells

Authors: *Y. ICHIHARA, T. DOI, Y. RYU, M. NAGAO, Y. SAWADA, T. OGATA;
Natl. Rehabil. Ctr. For Persons With Disabilities, Tokorozawa, Saitama, Japan

Abstract: Oligodendrocyte progenitor cells (OPCs) go through remarkable morphological changes to become mature oligodendrocytes, providing thick myelin sheaths to multiple axons. This process is thought to need extraordinary metabolic demands. Lactate has been reported to be used in primary oligodendrocytes as metabolites and rescue hypomyelination induced by low glucose in organotypic slice cultures. Although glycogen in astrocytes is regarded as the source of lactate in neural tissue, lactate usage derived from glycogen in oligodendrocytes *in vivo* is unclear. Therefore we tested the effect of 1,4-dideoxy-1,4-imino-d-arabinitol (DAB), inhibitor of glycogen phosphorylase, glycogen catabolic enzyme, in mice reversible demyelination model, named cuprizone model. A diet containing 0.2% cuprizone was given to mice to induce demyelination particularly in corpus callosum. After six weeks, when the diet was changed to normal chow, we started the administration of either DAB or saline (control) to the mice intracerebroventricularly for two weeks. From histological analysis, we found that the number of Glutathione S-transferase (GST)-π positive cells, indicating mature oligodendrocytes, was significantly decreased in corpus callosum of DAB injected mice compared to saline injected mice. To further examine the possibility whether lactate derived from glycogen affects proliferation or maturation of OPCs, we cultured primary OPCs and tested the effect of lactate. In high glucose condition (36.6 mM), myelin basic protein (MBP), mature oligodendrocyte marker, mRNA level was increased by addition of lactate (10 mM). The ratio of MBP positive cells was also increased by lacate. But BrdU positive cell ratio, proliferating cell marker, was not changed by lactate addition in high glucose condition. On the other hand, 10 mM lactate rescued decreased BrdU positive cell ratio in low glucose condition (0.4 mM). These results suggest that oligodendrocytes use metabolites derived from glycogen for remyelination *in vivo* and lactate might be used for proliferation and maturation in oligodendrocytes.

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Poster

213. Oligodendrocytes: Myelination and Remyelination

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 213.03/B99

Topic: B.11. Glial Mechanisms

Support: National Natural Science Foundation of China for Young Scholars

Natural Science Foundation of Bijing (7153175)

Title: Cathepsin D deficiency delays CNS myelination by inhibiting Proteolipid Protein trafficking from late endosome / lysosome to plasma membrane

Authors: *D. GUO[1], Y. LIU[2], L. XIAO[1], C. HE[3], S. DUAN[2];
[1]Navy Gen. Hosp., Beijing, China; [2]institute of neuroscience, zhejiang university, hangzhou, China; [3]department of neurobiology, second military medical university, shanghai, China

Abstract: Cathepsin D (CathD) deficiencies are fatal neurological diseases that in human infants, in sheep and in mice are characterized by extreme loss of neurons and myelin. However, how loss of CathD leads to the defect in myelination is still unclear. In the present study, we found surprisingly a selective dysmyelination in CNS but normal myelination in PNS of CathD -/- mice. Different from other proteins in myelin (MBP, MAG, et al), PLP is the most abundant protein present in CNS myelin of higher vertebrates, and only found in CNS myelin. PLP plays an important role in the formation and/or maintenance of multilayer myelin. Thus, we further examined intensity of the Fluoromyelin Green and PLP staining in corpus callosum from CathD -/- mice at P7, P14 and P24, we found that there was less extent increase in the level of Fluoromyelin Green staining and PLP expression with the growth of age, and compared with that in CathD+/+ mice, the extent of increasion in PLP expression was significantly delayed, and interestingly,PLP accumulated around nucleus. We also showed that the number of DAPI+ cells, oligodendrocyte lineage cells (olig2+) and postmitotic oligodendrocytes (CC1+) in corpus callosum from CathD -/- mice was significantly lower than from CathD -/- mice between P14 and P24, but there was no difference at P7, and the number of OPCs (NG2+) was normal, suggesting that CathD deficiency only lead to the delay of myelination and oligodendrocyte maturation. To further investigate the mechanisms of CathD deficiency in dysmyelination and defect of oligodendrocyte maturation, we used rat OPCs cultures with CathD antagonist, CathD-/- mouse OPC cultures, we found that CathD, PLP, and VAMP-7 could bind with each other, and CathD deficiency could induce the defect in the trafficking of PLP from LEs/Ls to the plasma membrane of oligodendrocyte, and more PLP gathered in LEs/Ls around nucleus. In addition, VAMP-7 was involved in the process, and delayed the MBP+ oligodendrocytes maturation and myelination in CNS. Together, our findings indicate that CathD may have important significance in the oligodendrocyte maturation and myelination of CNS mediated by protein-protein interation but not by proteolysis.

Disclosures: **D. Guo:** None. **Y. liu:** None. **L. xiao:** None. **C. he:** None. **S. duan:** None.

Poster

213. Oligodendrocytes: Myelination and Remyelination

Location: Hall A

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Topic: B.11. Glial Mechanisms

Support: NIH Grant 5R01NS080153-02

Title: Oligodendroglial expression of the inward-rectifying potassium channel Kir4.1 is not required for oligodendrocyte generation, survival, or myelination

Authors: *V. A. LARSON[1], A. AGARWAL[1], J. E. RASH[2], D. E. BERGLES[1];
[1]Johns Hopkins Univ., Baltimore, MD; [2]Dept. of Biomed. Sci., Colorado State Univ., Fort Collins, CO

Abstract: Oligodendrocytes are generated throughout life from a proliferative population of oligodendrocyte progenitor cells (OPCs). Both OPCs and oligodendrocytes have a highly negative resting potential due to their high resting potassium conductance. The inward rectifier potassium channel Kir4.1 is broadly expressed by CNS glia and is believed to be important in setting the resting membrane potential of these cells. Complete and glia-specific Kir4.1 knockout animals display a profound neurological phenotype that includes severe white matter pathology. This finding has led to the hypothesis that Kir4.1 is critical for the survival of oligodendroglia. However, cell type-specific manipulations of this channel have not been performed, and thus it is not known whether the defects observed in the knockout mice are due to cell-autonomous mechanisms in oligodendroglia, or to interactions among oligodendroglia and astrocytes. Here, we examined the role of Kir4.1 in oligodendroglia using conditional knockout (cKO) mouse lines in which Kir4.1 is selectively removed from OPCs or mature oligodendrocytes. PDGFRα-CreERxRosa-YFPxKir4.1f/f mice were given 4-hydroxytamoxifen at postnatal day 21, corresponding to the peak period of myelination in the brain, and analyzed at postnatal day 35. Whole-cell patch clamp recordings in the cortex, hippocampus, and corpus callosum showed that Kir4.1 cKO OPCs had significantly increased membrane resistance and less negative resting membrane potential than control OPCs. Despite these changes, BrdU incorporation studies and genetic fate tracing showed no change in the proliferation or differentiation of OPCs *in vivo* during this critical time period. We also examined the CNP-CrexKir4.1f/f and MOG-iCrexKir4.1f/f lines, in which Kir4.1 is selectively deleted from mature oligodendrocytes. We found no change in the number of oligodendrocytes, and no deficit in the formation of myelin or structure of myelin in these animals. These results demonstrate that Kir4.1 is not required for OPC proliferation, oligodendrogenesis, oligodendrocyte survival, or myelination. The hypomyelination phenotype observed in Kir4.1 knockout mice may therefore occur secondary to alterations in astrocytes and impaired astrocyte-oligodendrocyte communication.

Disclosures: **V.A. Larson:** None. **A. Agarwal:** None. **J.E. Rash:** None. **D.E. Bergles:** None.

Poster

213. Oligodendrocytes: Myelination and Remyelination

Location: Hall A

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Program#/Poster#: 213.05/B101

Topic: B.11. Glial Mechanisms

Support: National Multiple Sclerosis Society RG5274A1/T

WSU Special Projects Grant

Title: Dynamic and local remodeling of axon caliber during initial myelin ensheathment

Authors: A. J. TREICHEL, M. M. MARTELL, A. J. KAISER, A. G. TRUDEL, B. B. DUXBURY, *J. H. HINES;
Biol. Dept., Winona State Univ., Winona, MN

Abstract: A key feature of vertebrate neural development is the ensheathment of select axons with myelin. At the onset of myelination, oligodendrocytes extend exploratory membranous processes that sample numerous axons in anticipation of initial axon wrapping. How initial axon wrapping is restricted to specific axons is an important gap in our knowledge. In previous studies, we learned that activity-dependent secretion from axons biases axon selection by stabilizing nascent sheaths formed on select axons. To test whether activity also restricts which axons are initially wrapped, we first identified transgenic reporters labeling various myelin-fated and unmyelinated axon sub-types in the zebrafish spinal cord. Pharmacologic and genetic manipulations of neuronal activity did not cause ectopic myelination of axons that are normally unmyelinated, suggesting that activity-independent mechanisms must specify which axons are initially wrapped. We have begun to test the hypothesis that axon choice is determined by an axon's caliber immediately prior to initial wrapping. Supporting this hypothesis, average diameters of myelin-fated axon-sub-types were larger than unmyelinated axon sub-types. Also consistent with the hypothesis, reticulospinal axons that were wrapped first had larger average diameters than those not initially wrapped. However, a comparison of multiple myelin-fated axon types showed that the order of initial wrapping did not follow the order predicted by axon calibers. Rather than overall axon caliber, our findings support the notion that local and transient changes in axon caliber direct the initiation of axon wrapping. Time-lapse confocal microscopy revealed that axon morphology was highly dynamic both before and during initial wrapping. Short axon segments underwent brief and localized radial growth that was associated with interactions with oligodendrocyte membrane processes. Axon diameters at sites of initial wrapping were greater than adjacent unmyelinated segments, suggesting that dynamic and local changes in axon diameter may precede and instruct initial wrapping. Ongoing experiments are aimed to determine whether local axon enlargements precede initial wrapping, and whether changes in axon caliber act instructively during initial wrapping. Collectively, these data show that average axon caliber does not explain the order of axon selection, but raise the possibility that axon-glia interactions stimulate local radial growth of axons to direct initial wrapping, acting to specify select axons for myelination.

Disclosures: **A.J. Treichel:** None. **M.M. Martell:** None. **A.J. Kaiser:** None. **A.G. Trudel:** None. **B.B. Duxbury:** None. **J.H. Hines:** None.

Poster

213. Oligodendrocytes: Myelination and Remyelination

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 213.06/B102

Topic: C.09. Demyelinating Disorders

Support: MS Hope for a Cure

Title: Promotion of remyelination by Vitamin B12 in model with global demyelination, and the cell/s responsible

Authors: ***I. D. DUNCAN**[1], A. B. RADCLIFF[1], A. S. FIELD[3], G. MCLELLAN[2], J. N. VER HOEVE[4];
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Abstract: The current understanding of remyelination is that the oligodendrocyte progenitor cell (OPC) is the primary or only cell capable of giving rise to remyelinating oligodendrocytes (OLs). While the data that supports this statement are strong, however it is both of biological interest and potentially of disease importance to know whether adult OLs might be recruitable in certain circumstances. We are using a large animal model of global demyelination-remyelination to explore this question. Feeding cats a diet irradiated at over 30 Kgrays results in delayed-onset progressive neurologic disease that resolves after return to non-irradiated food (Duncan *et al.* PNAS 2009, 106, 6832). Neurologic dysfunction is caused by generalized demyelination and ongoing remyelination of the CNS, most severe in the optic nerve and spinal cord. Extensive myelin vacuolation leads to myelin breakdown with no cell death of OLs apparent, suggesting that myelin is the primary target. Recovery results from total remyelination of these sites. The disorder has marked similarity to sub-acute combined degeneration in humans, which is caused by a deficiency in Vitamin B12. Early treatment of affected animals with B12 results in recovery while cessation of treatment resulted in relapse. Delay in treatment resulted in neurologic worsening and axon loss in the dorsal columns. Imaging of the brain and spinal cord by MRI, and physiologic evaluation of conduction in the optic nerve (visual evoked potentials) have more recently been performed. MRI showed T2-hyperintensity and reduced magnetization transfer (MT) ratio, primarily in the dorsal columns as well as in optic nerves and cerebral white matter. Recovery was accompanied by increased MT ratio and in one case disappearance of a focal T2-hyperintense lesion in the cerebral peduncle. Photopic flash VEPs and ERGs were recorded simultaneously. The flash VEP declined in amplitude and increased in latency while on diet and showed partial recovery after returning to a normal diet or after cats were given B12

supplementation. ERGs were not altered by the diet, implying the VEP changes are due to alteration in optic nerve and central visual pathway function. While our model does not demonstrate the biochemical hallmarks of B12 deficiency, it is responsive to B12 treatment associated with the promotion of remyelination. Certain aspects of the cellular milieu in areas of demyelination-remyelination suggests that adult OLs may be partaking in the remyelination process and we are exploring this further.

Disclosures: I.D. Duncan: None. **A.B. Radcliff:** None. **A.S. Field:** None. **G. McLellan:** None. **J.N. Ver Hoeve:** None.

Poster

213. Oligodendrocytes: Myelination and Remyelination

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 213.07/B103

Topic: C.09. Demyelinating Disorders

Support: National Multiple Sclerosis Society (NMSS)

National Institutes of Health (NIH)

Title: Therapeutic use of microRNA mimics to promote remyelination

Authors: *A. L. MOYANO[1], A. HEBERT[1], H. LIPTON[2], E. R. BONGARZONE[1];
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Abstract: MicroRNAs (miRs) are small (~22 nt) non-coding RNA molecules controlling gene expression by posttranscriptional regulation. miRs regulate the proliferation, survival and differentiation of oligodendrocytes (OLs). miRs also have functional roles in the pathogenesis of demyelinating diseases such as Multiple Sclerosis (MS). Therefore, there is an intrinsic potential for the therapeutic use of miR-related biology, especially considering that the regenerative capacity of the CNS (remyelination) in demyelinating diseases is generally impaired. The goal of this study is to examine the potential use of artificial miRs to promote remyelination by stimulating the number of functional OL precursor cells. We used an experimental model of demyelination, Theiler's Murine Encephalomyelitis Virus (TMEV), in susceptible SJL mice (*in vivo*) and its susceptible neuroglial progenitor cultures (*in vitro*). We found that miR-17, -19b, and -138 (involved in oligodendrogenesis) were decreased in TMEV-infected cells and TMEV-infected mice. These results were correlated with an increase in caspase-1 mediated death of OL progenitor cells. To test our hypothesis that complementation with miR mimics protects OLs, miR-17 and miR-19a were transfected in TMEV-infected cells. Our results showed that complementation of glial cultures with miR-17 exerted a protective effect on OL progenitor cells. Our results showed that levels of OL-related miRs were significantly decreased during demyelination and these molecules have the potential to exert therapeutic effects by *in vivo*

complementation. This study has an intrinsic clinical value for improving therapies in the treatment of myelin diseases such as MS and highlights the importance of miRs biology in myelinating cells.

Disclosures: **A.L. Moyano:** None. **A. Hebert:** None. **H. Lipton:** None. **E.R. Bongarzone:** None.

Poster

213. Oligodendrocytes: Myelination and Remyelination

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 213.08/B104

Topic: C.09. Demyelinating Disorders

Title: Neuroanatomically distinct vulnerability and response to remyelinating factors in the mouse forebrain of the cuprizone-induced demyelination model: new insights into the progression patterns of demyelination and responses to remyelinating therapy

Authors: ***E. PAVLOPOULOS**, Y. WANG, C. CUI, E. TROY, T. J. PARRY, A. O. CAGGIANO, R. W. COLBURN;
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Abstract: Remyelination is a central focus of a growing number of therapeutic candidates for the treatment of demyelinating diseases, such as multiple sclerosis (MS). However, little is known about whether demyelination or remyelination and their mechanisms are differentially affected by location within the brain, and whether this might lead to a differential response to remyelination therapies. To address these questions, we used the cuprizone mouse model of induced demyelination and spontaneous remyelination and examined the corpus callosum, critical for motor function and a range of cognitive processes, and the hippocampus, the center of learning and memory. Both of these regions and their associated functions are markedly affected in MS and demyelinating diseases. Seven-week old mice were fed with 0.3% cuprizone-containing food for 6 weeks, and their brains were examined immediately after the end of the cuprizone diet as well as 4 weeks later (recovery period), and compared with those of age-matched control mice, fed only regular food. Another group of mice underwent a similar cuprizone diet but they were treated with thyroid hormone T3, a known facilitator of remyelination, beginning at cuprizone cessation. We examined the brains of these mice after 4 weeks of recovery and compared them with those of placebo control animals, as well as mice fed with regular food. We found that cuprizone induced substantial and comparable demyelination in both the corpus callosum and the hippocampus, as measured by 1) myelin detection using the Black Gold and Fluoro-Myelin tracers, and 2) fluorescence-based immunohistochemical examination of myelin proteins, such as the Myelin Basic Protein (MBP) and the Myelin Associated Glycoprotein (MAG). All of these markers were significantly reduced at the end of

the cuprizone diet. Four weeks later, black gold-stained myelin, fluoro-myelin and MBP in the corpus callosum remained unaltered. MAG, however, had returned to control levels, indicating initiation of remyelination. This process was faster in the hippocampus, as, in addition to MAG, MBP was also restored. T3 facilitated remyelination in the corpus callosum, as evidenced by reinstatement of MBP and fluoromyelin after 4 weeks of recovery. The effect of T3 was greater in the hippocampus, as there was also recovery of black-gold stained myelin. These results support the idea that the brain differentially responds to demyelinating insults and facilitators of remyelination depending on the brain area, providing potential new insights into the pathophysiology of demyelinating diseases and the importance of considering regional sensitivity to therapeutic interventions.

Disclosures: E. Pavlopoulos: A. Employment/Salary (full or part-time):; Acorda Therapeutics (Full time). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Acorda Therapeutics (stock holder). **Y. Wang:** A. Employment/Salary (full or part-time):; Acorda Therapeutics (Full time temporary). **C. Cui:** A. Employment/Salary (full or part-time):; Acorda Therapeutics (Full Time). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Acorda Therapeutics (stock holder). **E. Troy:** A. Employment/Salary (full or part-time):; Acorda Therapeutics (Full Time). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Acorda Therapeutics (stock holder). **T.J. Parry:** A. Employment/Salary (full or part-time):; Acorda Therapeutics (Full Time). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Acorda Therapeutics (stock holder). **A.O. Caggiano:** A. Employment/Salary (full or part-time):; Acorda Therapeutics (Full Time). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Acorda Therapeutics (stock holder). **R.W. Colburn:** A. Employment/Salary (full or part-time):; Acorda Therapeutics (Full Time). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Acorda Therapeutics (stock holder).

Poster

213. Oligodendrocytes: Myelination and Remyelination

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 213.09/B105

Topic: C.09. Demyelinating Disorders

Title: Physical exercise promotes remyelination and alters the composition of the lesion extracellular matrix

Authors: *S. K. JENSEN, M. B. KEOUGH, C. BRIDEAU, V. YONG;
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Abstract: Physical exercise is considered a critical component of a healthy lifestyle and is known to have efficacy in treating/controlling many systemic disorders. In the central nervous system, physical exercise is known to promote wellbeing in a number of neurological conditions, including the demyelinating disorder Multiple Sclerosis. Notably, a strong positive correlation between physical activity and white matter integrity (as determined by diffusion tensor imaging) in patients with MS has been described. However, whether physical exercise is capable of promoting repair processes, particularly remyelination, has not been directly established. In the current study, we investigated the ability for physical exercise to promote remyelination. We induced a focal demyelinating lesion via injection of lysolecithin into the ventrolateral white matter of the murine spinal cord. Mice were singly housed and given free access to an electronically monitored running wheel, which were locked in control animals, at the day of injury until sacrifice at either 7 or 14 days post lesion (representing peak demyelination and early remyelination, respectively). Immunohistochemical analysis of day 14 lesions revealed a 47.6% increase in lesion myelin basic protein expression and a 35.9% increase in CC1+ mature oligodendrocytes as a result of access to a running wheel. When examined at the semithin level, we observe a 2.74 fold increase myelinated axons in exercising animals when compared to sedentary controls. Interestingly, lesion versican and chondroitin sulfate A (CSA) expression is altered in the exercise group, and may result in microenvironment more permissive to remyelination in exercising animal. Our previous data has implicated lesion-accumulated chondroitin sulfate proteoglycan (CSPGs) as inhibitors of oligodendrocyte migration, maturation and remyelination (Lau et al., Ann Neurol 72:419, 2012; Nature Rev Neurosci 14:722, 2013). These results indicate that physical exercise can improve white matter regeneration and may be an important tool for the treatment of demyelinating disorders, such as multiple sclerosis.

Disclosures: S.K. Jensen: None. **M.B. Keough:** None. **C. Brideau:** None. **V. Yong:** None.

Poster

213. Oligodendrocytes: Myelination and Remyelination

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Program#/Poster#: 213.10/B106

Topic: C.09. Demyelinating Disorders

Support: NICHD U54 Grant 5U54HD029990

Title: NESTORONE treatment after chronic cuprizone intoxication stimulates the production of transcription factors involved in remyelination in the corpus callosum

Authors: *M. EL-ETR[1], M. RAME[1], A. M. GHOUMARI[1], M. SCHUMACHER[1], R. SITRUK-WARE[2];
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Abstract: We have previously demonstrated that Progesterone and Nestorone®, a synthetic 19-norprogesterone derivative, were able to favor callosal myelin repair, when administered for 3 weeks after a 12-week cuprizone-induced chronic demyelination (El-Etr M et al, GLIA 2015;63:104-117). We also observed that Progesterone exerts its effect via the brain Progesterone receptors (PR), since it was less efficient in heterozygous mice lacking one allele of the PR and had no effect on remyelination in PR-knockout mice. Here we show that after only one week of steroid treatment, Nestorone is already able to enhance the callosal production of some mRNAs coding for transcription factors known to be involved in the proliferation and early differentiation of Oligodendrocyte Progenitor Cells (OPC). Indeed, Myelin transcription factor 1 (Myt1) mRNA expression increases equally after a 1-week treatment with 8 µg/d Nestorone alone, or combined with Estradiol 1µg/d. Myt1 is a zinc-finger DNA binding protein which regulates OPC differentiation and binds to the promoter region of Proteolipid Protein PLP, the most important myelin gene in the CNS (Nielsen JA et al, Mol Cell Neurosci 2004, 25:111-123). While cuprizone alone decreases their mRNA levels, steroid treatments also stimulate the production of Sox17 mRNA, and to the largest extent of Olig2 mRNA. These results are consistent with the increase in NG2 mRNA, a marker of OPCs, after a 1-week treatment and with the increase of mature oligodendrocytes density observed using immunohistological techniques at the end of the 3-week treatment with Nestorone ± Estradiol. However, there is no effect of Nestorone alone or combined with Estradiol on the cuprizone-induced changes of mRNA levels of two other transcription factors which might play a role in OPC differentiation, Sox10 and Ascl1. We are currently investigating the protein levels of the responding transcription factors in order to quantify these responses.

Disclosures: **M. El-Etr:** A. Employment/Salary (full or part-time):; INSERM U 1195. **M. Rame:** A. Employment/Salary (full or part-time):; INSERM U1195. **A.M. Ghoumari:** A. Employment/Salary (full or part-time):; INSERM U1195/Universite Paris 11. **M. Schumacher:** A. Employment/Salary (full or part-time):; INSERM U1195. **R. Sitruk-ware:** A. Employment/Salary (full or part-time):; Population Council.

Poster

213. Oligodendrocytes: Myelination and Remyelination

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 213.11/B107

Topic: C.09. Demyelinating Disorders

Title: The neuronal mitochondrial metabolite NAA supports myelination

Authors: ***N. K. SINGHAL**[1], H. HUANG[2], S. LI[1], R. CLEMENTS[1], J. GADD[1], A. DANIELS[1], E. E. KOOIJMAN[1], F. GUO[3], D. PLEASURE[3], L. SHRIVER[2], E. FREEMAN[1], J. MCDONOUGH[1];
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Abstract: Mitochondrial changes including decreased expression of nuclear encoded electron transport chain subunit genes, inhibition of electron transport chain activity, and decreased levels of the neuronal mitochondrial metabolite N-acetylaspartate (NAA) have been implicated in multiple sclerosis (MS) neuropathology. NAA is synthesized in neuronal mitochondria by the enzyme N-acetyltransferase (NAT8L) and broken down in oligodendrocytes by aspartoacylase (ASPA) into acetate and aspartate. We have hypothesized that NAA links the metabolic activity of axons and oligodendrocytes to support myelination. In the present study we have performed shot-gun lipidomic analysis by mass spectrometry and high performance thin layer chromatography (HPTLC) to identify changes in myelin lipid composition that are correlated with decreased NAA levels in postmortem MS and control brains and in NAT8L knockout (NAT8L-KO) mice. We found that the myelin lipid sphingomyelin was depleted in MS normal appearing white matter (-1.4 fold) compared to controls and was correlated with NAA levels measured by HPLC in adjacent gray matter in the same tissue block. Sphingomyelin and sulfatides were also decreased in myelin lipids isolated from NAT8L-KO brains by 7 and 1.6 fold respectively compared to controls. Metabolomic analysis of primary cultures of oligodendrocytes treated with NAA revealed changes in tricarboxylic acid (TCA) cycle intermediates and increased α-ketoglutarate. Levels of α-ketoglutarate have been shown to regulate histone demethylases. Consistent with this, we found that NAA treatment also resulted in decreased levels of histone H3 trimethylation on lysine 4 (H3K4me3) in the nucleus of oligodendrocytes. H3K4me3 marks promoters of actively transcribed genes and reductions in H3K4me3 result in slowed metabolism and growth, and increased differentiation. These data suggest that dysfunction of neuronal mitochondria and reduced NAA in MS may contribute to defects in oligodendrocyte metabolism and myelin lipid composition.

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Poster

213. Oligodendrocytes: Myelination and Remyelination

Location: Hall A

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Program#/Poster#: 213.12/B108

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant R01 NS052741

NMSS RG4958, PP2009

CA1060A11

The Mayo Clinic Center for Regenerative Medicine

The Mayo Clinic Center for Multiple Sclerosis

Title: Regulatory role of the thrombin receptor in spinal cord myelination

Authors: *H. YOON, M. RADULOVIC, K. L. DRUCKER, J. WU, I. A. SCARISBRICK; Physical Med. & Rehabilitation, Rehabil. Med. Res. Ctr., Mayo Clin., Rochester, MN

Abstract: Myelination in the CNS is achieved through a delicate balance of extrinsic and intrinsic signaling mechanisms with aberrations in the perinatal period resulting in white matter injury and profound sensorimotor and cognitive disabilities. Leakage of blood-derived serine proteases such as thrombin into the CNS is a common feature of infectious, traumatic, hypoxic and hemorrhagic injuries occurring perinatally. In addition to its roles in thrombostasis, elevations in thrombin have been identified as a powerful neurotoxic agent and possible new target for neuroprotection. Thrombin mediates its cellular effects by activation of a G-protein coupled receptor referred to as Protease Activated Receptor 1 (PAR1). Here we use PAR1 knockout mice to evaluate the role of PAR1 in the process of murine spinal cord myelination at a cellular, molecular and ultrastructural level. PAR1 exhibits peak expression levels in the spinal cord at term, including expression by platelet derived growth factor receptor oligodendrocyte progenitor cells (OPCs) and newly generated CC-1+ oligodendroglia. A critical role for PAR1 in the process of myelination is suggested by findings demonstrating that PAR1 gene deficient mice exhibit an earlier onset of spinal cord myelination, including substantially more Olig2-positive oligodendrocytes, more myelinated axons and higher proteolipid protein (PLP) levels at birth. *In vitro*, the highest levels of PAR1 were observed in OPCs, being reduced with differentiation. In parallel, the expression of PLP and myelin basic protein (MBP), in addition to Olig2, were all significantly higher in cultures of PAR1-/- oligodendroglia. Moreover, application of a small molecule inhibitor of PAR1 (SCH79797) to OPCs *in vitro*, resulted in higher levels of expression of both PLP and MBP upon differentiation. Enhancements in myelination associated with PAR1 deficient mice were also observed in adults, including higher levels of MBP and significantly thicker myelin sheaths across large, medium and small diameter axons. Increases in spinal cord myelination in PAR1-/- mice were coupled to developmental increases in the pro-myelination signaling intermediates, extracellular-signal-regulated kinase 1/2 and AKT. Nocturnal ambulation and rearing activity were also elevated in PAR1-/- mice. These studies identify the thrombin receptor as a powerful extracellular regulatory switch that could be readily targeted to improve myelin production in the face of white matter injury and disease. Supported by NIH R01 NS052741 and NMSS RG4958, PP2009 and CA1060A11 and the Mayo Clinic Center for Regenerative Medicine.

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Poster

213. Oligodendrocytes: Myelination and Remyelination

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Topic: A.01. Neurogenesis and Gliogenesis

Support: Bradley Merrill Patten Graduate Fellowship

Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Title: Low density lipoprotein receptor-related protein 1 (LRP1) is a positive regulator of cns myelin development and repair

Authors: ***J.-P. LIN**[1], R. GIGER[1], Y. MIRONOVA[1], P. SHRAGER[2];
[1]Univ. of Michigan, Ann Arbor, MI; [2]Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: LRP1 is a large cell surface protein best known for its role in receptor-mediated endocytosis. In the nervous system, LRP1 is broadly expressed and is required in Schwann cells for proper myelination in the peripheral nervous system. Whether LRP1 participates in CNS myelin development or repair following white matter injury, however, has not yet been determined. In our study, deletion of *Lrp1* in the oligodendrocyte (OL) lineage results in a hypomyelination phenotype. At postnatal-day (P) 10, 21 and 56, optic nerve myelination is greatly reduced compared to wildtype littermate controls. Electrophysiological studies revealed that the propagation of compound action potentials (CAPs) in acutely isolated P21 optic nerves is compromised. Compared to controls, conduction velocity and amplitude of CAPs is reduced in *Lrp1*$^{flox/flox}$*; Olig2-Cre* nerves, and the fraction of abnormally formed nodes of Ranvier is increased. Biochemical studies of membrane extracted from control and *Lrp1*$^{flox/flox}$*; Olig2-Cre* brain tissue revealed reduced levels of proteolipid protein (PLP). Compared to control optic nerves, a decrease in the number of mature OLs was observed in *Lrp1*$^{flox/flox}$*; Olig2-Cre* mice *in vivo*. Primary oligodendrocyte progenitor cells (OPCs) isolated from P6 brains showed normal viability and proliferation. However, in the absence of *Lrp1*, differentiation into mature OLs was significantly attenuated. At P56 inducible deletion of *Lrp1* (*Lrp1*$^{flox/flox}$*; CMV-ER Cre*) does not lead to any overt defects in CNS white matter. However, our data suggests in the adult brain LRP1 is required for white matter repair. Stereotaxic injection of lysophosphatidyl choline (LPC) into the corpus callosum leads to focal axon demyelination. At 21 days after LPC injection, remyelination in the lesion core is significantly more robust in control mice than in *Lrp1* inducible knockout mice. Collectively, these studies show that *Lrp1* functions cell-autonomously

in the OL-lineage during myelin development, and demonstrate that LRP1 is a positive regulator of myelin repair in the adult CNS.

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Poster

213. Oligodendrocytes: Myelination and Remyelination

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Topic: A.01. Neurogenesis and Gliogenesis

Support: CIHR Grant MOP93697

Title: Regulation of central nervous system myelination by the ATRX chromatin remodeling protein

Authors: M. W. EDWARDS[1], *N. G. BERUBE[2];
[1]Biochem., Univ. of Western Ontario, London, ON, Canada; [2]Univ. Western Ontario, London, ON, Canada

Abstract: The myelin sheath is a specialized plasma membrane that encompasses axons of neurons, and is necessary for efficient firing of action potentials, along with providing protection and trophic support to the axonal cell body. In the central nervous system, myelin arises via differentiation of oligodendrocyte precursor cells (OPCs) into oligodendrocytes (OLs). The latter then undergo an additional maturation step into mature OLs, which then extend membraneous fibres and enwrap axons creating the myelin sheath. This is a tightly regulated process, however the mechanisms involved have yet to be fully elucidated. Alpha thalassaemia mental retardation, X-linked (ATR-X) syndrome is a developmental disorder caused by hypomorphic mutations in the ATRX gene, and is characterized by severe cognitive deficits, seizures, microcephaly, loss of white matter, as well as myelin sheath abnormalities. ATRX is an ATP-dependent translocase involved in organizing chromatin structure to influence transcriptional regulation and genomic stability. Microarray analysis from our group revealed that loss of ATRX from the mouse forebrain results in decreased expression of several genes implicated in myelination; therefore, the objective of this study is to determine the mechanism by which ATRX regulates myelin production in the brain. We identified that conditional inactivation of Atrx in the mouse forebrain results in decreased expression of critical components of the myelin sheath at the transcriptional and proteomic level, indicating a decrease in myelination. The observed hypomyelination phenotype was not due to a defect in OPC production, proliferative capacity, or the ability to differentiate into OLs. Moreover, OL-specific deletion of ATRX did not perturb myelination. Together, these results suggest that the myelination defect does not result from abnormalities in the oligodendrocyte lineage and implies that ATRX influences OL maturation in

a cell non-autonomous manner. We are currently testing the hypothesis that neuronal-specific activities of ATRX influence the myelination process using conditional mutant mice and neuron/oligodendrocyte myelinating co-cultures. Clarifying the role of ATRX in myelination presents a novel mechanism that regulates myelin sheath formation, and findings stemming from this study will undoubtedly benefit future treatments of myelin disorders such as ATR-X syndrome.

Disclosures: M.W. Edwards: None. **N.G. Berube:** None.

Poster

213. Oligodendrocytes: Myelination and Remyelination

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Topic: B.11. Glial Mechanisms

Support: National MS Society Center Grant (CA 1064-A-4)

MS Society Postdoctoral Grant

Target ALS

Title: Myelin remodeling in the adult brain

Authors: *E. G. HUGHES, J. L. ORTHMANN-MURPHY, D. E. BERGLES;
Dept. of Neurosci., Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Oligodendrocytes generate myelin sheaths that accelerate action potential propagation and provide metabolic support to axons in the CNS. Although most oligodendrocytes are generated in early postnatal life, the development of myelin continues into adulthood, and recent studies indicate that the characteristics of myelin (number and length of myelin internodes, thickness of myelin sheaths, and myelin protein expression) can be modified by experience. Moreover, axons in the cortical gray matter exhibit discontinuous myelination, and blocking the formation of new oligodendrocytes impairs motor learning, raising the possibility that myelin is continually adjusted to modify the information processing capabilities of cortical circuits. However, these studies have all been performed during early adulthood, when developmental myelination is still ongoing. It is unclear whether the ability to remodel myelin is retained with age. To explore how myelination changes with age, we performed histological analysis and *in vivo* two-photon time-lapse imaging in the somatosensory cortex of transgenic mice in which EGFP is expressed specifically in mature oligodendrocytes (MOBP-EGFP mice). Immunohistological analysis revealed that EGFP is expressed by nearly all myelinating oligodendrocytes in the CNS of MOBP-EGFP mice, and the expression of EGFP was high enough to visualize oligodendrocyte cell somata and myelin sheaths in living tissue. Our studies

indicate that the period of oligodendrogenesis in the cortex is very prolonged, with more than half of the oligodendrocytes produced after 3 months of age. However, isolated myelin internodes were still observed in the upper layers of cortex in animals 12 months of age, indicating that discontinuous myelination is not a transient developmental phenomenon. To determine whether the ability to remodel myelin is retained with age, we used *in vivo* two-photon time-lapse imaging to repeatedly image individual myelin internodes for up to 1.5 months in mice 11-14 months old. Although most oligodendrocyte myelin internodes remained stable over this period, approximately 6% of internodes were dynamically remodeled, either increasing or decreasing in length, and some internodes were completely removed. Together, these results indicate that the myelination state of axons in the adult CNS is not continuous and that some internodes can be remodeled. This ongoing reorganization of existing myelin in the adult CNS may help to alter the pattern of myelination with life experience and perhaps contribute to the plasticity of adult cortical circuits.

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Poster

213. Oligodendrocytes: Myelination and Remyelination

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Topic: C.09. Demyelinating Disorders

Support: CRC SFB-128 (B6, Meuth/Budde/Pape).

Title: Functional consequences of cortical de- and remyelination

Authors: ***M. CERINA**[1,2], V. NARAYANAN[2], K. GÖBEL[2], S. BITTNER[2], T. RUCK[2], A. GORJI[3], N. GHAFFARIAN[3], P. MEUTH[3], A. HERRMANN[2], S. GRAEBENITZ[3], M. STANGEL[4], T. SKRIPULETZ[4], T. DALDRUP[3], T. SEIDENBECHER[3], H. WIENDL[2], E. J. SPECKMANN[3], H. C. PAPE[3], T. BUDDE[3], S. G. MEUTH[2,5];
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Abstract: Multiple sclerosis (MS) is a chronic neurodegenerative disease characterized by the occurrence of many pathophysiological hallmarks like de- and remyelination, axonal and neuronal degeneration and immune cell infiltration and proliferation in the CNS. Important steps forward have been done in understanding consequences of demyelination in the peripheral nerve but little is known about neuronal effects at the central nervous system. Here, by combining *in vivo* and *in vitro* techniques we aim to understand the mechanism underlying de- and

remyelination processes and their consequences in a topographically highly organized neuronal network, namely the auditory thalamocortical system. General demyelination was induced in C57BL6J mice by feeding them with a cuprizone-based diet (0.2%) and then the functionality of the neurons of the primary auditory cortex (AC1) was tested *in vitro* by electrical stimulation and the number of elicited action potentials and changes in the resting membrane potential were taken as read-out. Moreover, the neuronal population properties were tested *ex vivo* by means of field potential and voltage-sensitive dye recordings and, *in vivo* by single unit activity analysis in freely behaving animals exposed to various auditory stimuli. Cuprizone treatment significantly impaired cortical functionality by reducing system' excitability and rendering the network unable to respond to any electrical or auditory stimulus. The latency to response was significantly increased suggesting an altered stimulus conductance. Moreover, *in vivo* electrophysiological data demonstrate single-unit activity to be stimulus-independent after cuprizone-treatment, strongly suggesting demyelination to permanently disrupt AC1-tonotopy. Finally, these alterations of cortical structures have been shown to result in a complete loss of stimulus-discrimination abilities in behavioral experiments after auditory pavlovian conditioning. The very same effect is observed during early and late phases of remyelination. Taken together our data show a severe effect of cortical demyelination on neuronal network activity and it suggests a permanent alteration of cortical processing which may be relevant for MS patients which are often diagnosed with early cortical or thalamic lesions and it may play a crucial role as target in therapeutic approaches.

Disclosures: **M. Cerina:** None. **V. Narayanan:** None. **K. Göbel:** None. **S. Bittner:** None. **T. Ruck:** None. **A. Gorji:** None. **N. Ghaffarian:** None. **P. Meuth:** None. **A. Herrmann:** None. **S. Graebenitz:** None. **M. Stangel:** None. **T. Skripuletz:** None. **T. Daldrup:** None. **T. Seidenbecher:** None. **H. Wiendl:** None. **E.J. Speckmann:** None. **H.C. Pape:** None. **T. Budde:** None. **S.G. Meuth:** None.

Poster

213. Oligodendrocytes: Myelination and Remyelination

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 213.17/C1

Topic: B.11. Glial Mechanisms

Support: 5T32NS041218

Title: Promoting oligodendrocyte survival with creatine: implications for multiple sclerosis

Authors: ***K. A. CHAMBERLAIN**, K. S. CHAPEY, J. K. HUANG;
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Abstract: Oligodendrocytes are glial cells of the central nervous system that enable rapid saltatory conduction by ensheathing neuronal axons with a lipid rich membrane known as the myelin sheath. Expansion of myelin membranes during development requires rapid lipid synthesis and is a highly energy consuming process. Indeed, defects in energy production are associated with oligodendrocyte dysfunction in numerous mitochondrial diseases. Interestingly, genetic mutations affecting the organic energy compound creatine, which maintains intracellular energy supplies via rapid regeneration of ATP, present with delayed myelination and severe cognitive disability. Previous work has shown that the final enzyme in the creatine biosynthetic pathway, guanidinoacetate N-methyltransferase (GAMT), is expressed during oligodendrocyte differentiation and remyelination following experimental demyelination in rodents. Therefore, we investigated the effect of creatine on oligodendrocyte lineage cells following lysolecithin-mediated experimental demyelination of mouse spinal cord at 5,10, and 20 days post lesion (dpl). Our preliminary data suggest that creatine supplementation increases the number of mature oligodendrocytes in lesioned spinal cord compared to control. Conversely, creatine deficient transgenic mice lacking GAMT have significantly less mature oligodendrocytes in lesioned spinal cord. Our *in vitro* studies suggest that creatine may modulate oligodendrocyte cell survival, possibly through mitochondria, as mitochondria distribution and density are altered following creatine treatment of purified mouse oligodendrocytes. Investigating the role of creatine in oligodendrocyte lineage cells may have important implications for the development of novel therapeutics for diseases in which oligodendrocytes are lost or dysfunctional, including multiple sclerosis (MS).

Disclosures: K.A. Chamberlain: None. **K.S. Chapey:** None. **J.K. Huang:** None.

Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 214.03/C2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIGMS P5OGM08273

NINDS NS062184

Title: Decoupling the effects of the amyloid precursor protein and plaque on neuronal transport in the mouse brain

Authors: *E. L. BEARER[1], C. S. MEDINA[2], F. L. CHAVES[2], X. W. ZHANG[3], R. E. JACOBS[3];
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Abstract: Amyloid precursor protein (APP) is the parent protein for amyloid plaques, the pathological hallmark of Alzheimer's disease (AD) with an abundance of plaque in the brain. Here, we decouple the effects of the overexpressed protein itself from the subsequent plaque that it produces. We used a transgenic mouse carrying a human APP with both Swe/Ind mutations, with expression driven by a tetracycline-sensitive promoter. Three groups were studied. Group 'A' (no doxy, +plaques, +APP); group 'B' (doxy at 8 days before sacrifice, +plaques, no APP), and group 'C' (doxy prior to conception, and stopped 8 days before sacrifice, no plaques, +APP). We used manganese-enhanced magnetic resonance imaging (MEMRI) to observe differences in axonal transport dynamics between the three groups. Mn^{2+} was injected into CA3 of the hippocampus. Mn^{2+} enters the neuron through voltage-gated calcium channels. Mn^{2+} travels by kinesin-dependent axonal transport to distal projections. MR images were taken at several time points before and after injection. Mn^{2+} gives a hyperintense signal in T_1-weighted MRI, thus tracing transport over time. Histopathology revealed well-developed plaques in Groups A and B, and Western blots showed human APP expressed 3.2-fold over WT in Groups A and C. Our preliminary results show increased transport in A and C, with APP Swe/Ind expression when compared with B, where expression is suppressed. Transport is increased with APP overexpression independent of plaques, but decreased in the presence of plaques alone in this circuit. Cholinergic neurons in the medial septal nucleus were decreased as determined by anti-ChAT staining in Group C ($p=0.0006$ by one-way ANOVA, $n=15$). These effects could be model-specific. Phospho-tau was present in the dystrophic neuritis surrounding the plaques. In conclusion, we observe separable effects between APP and plaque.

Disclosures: E.L. Bearer: None. **C.S. Medina:** None. **F.L. Chaves:** None. **X.W. Zhang:** None. **R.E. Jacobs:** None.

Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 214.04/C3

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Swedish Research Council

Knut and Alice Wallenberg Foundation

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Alzheimerfonden

Frimurarestiftelsen

Emil and Maria Palm Foundation

Title: Cell-specific function of amyloid precursor protein during neural development

Authors: *R. K. BANOTE[1], M. EDLING[1], F. ELIASSEN[2], P. KETTUNEN[1], H. ZETTERBERG[1,3], A. ABRAMSSON[1];
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Abstract: The amyloid hypothesis comprised that the amyloid plaques are the core cause of neurodegeneration in Alzheimer's disease (AD), which produces from amyloid precursor protein (APP) by proteolytic processing. APP is a transmembrane glycoprotein that has been the subject of intense research because of its implication in Alzheimer's disease pathogenesis. However, its physiological function in the development and maintenance of the central nervous system remains elusive. In this study, we address the role of zebrafish APP homologue, Appb, in the formation of specific hindbrain neurons. We demonstrate that knockdown of appb affects the development of Mauthner neurons, hence plays an essential role for the escape response. Loss of Appb results in increased Notch signaling and decreased neural proliferation and differentiation in the hindbrain. Remarkably, the development defect of the Mauthner cell can be rescued by repression of Notch1a signaling in appb morphants. Furthermore, Appb expression is required to maintain proliferation and differentiation of neural progenitors. In conclusion, we show that Appb negatively regulates Notch1a activity, which is essential for Mauthner cell development and neuronal differentiation. Collectively, our study provides evidence in support of zebrafish model for studying the function of amyloid precursor protein in an *in vivo* framework. We believe that this study may transmit significant information for APP function and its role in neurodegenerative diseases.

Disclosures: R.K. Banote: None. **M. Edling:** None. **F. Eliassen:** None. **P. Kettunen:** None. **H. Zetterberg:** None. **A. Abramsson:** None.

Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 214.05/C4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH NS078363

NIH AG025525

NIH T32AG023477

Title: Contactins as regulators of Amyloid Precursor Protein-dependent neuronal guidance

Authors: *P. F. COPENHAVER[1], J. A. ZWEIG[2], T. L. SWANSON[2], J. M. RAMAKER[2];
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Abstract: Although Amyloid Precursor Protein (APP) is best known as the source of beta-amyloid peptides associated with Alzheimer's disease (AD), factors that disrupt the normal functions of APP may also provoke neurodegeneration. Numerous studies have shown that APP can modulate different aspects of neuronal growth and plasticity, while *in vitro* assays have shown that APP can affect cellular behavior via activation of the heterotrimeric G protein Gαo. However, the mechanisms underlying APP-dependent responses in the nervous system are still controversial. Using Manduca and *Drosophila* as model systems, we have shown that the sole insect ortholog of APP (APPL) co-localizes with Gαo in the leading processes and synaptic terminals of developing neurons, and that the two proteins directly interact *in vivo*. Using both cultured Manduca embryos and rat hippocampal neurons, we also found that stimulating APP/APPL activation inhibits different aspects of neuronal motility in a Gαo-dependent manner. Stimulating Gαo also causes a reduction in Gαo-APPL interactions, supporting the model that APP family proteins can function as unconventional Gαo-coupled receptors. Previous studies by other groups identified members of the Contactin family as potential binding partners for APP that can either induce signaling responses or stimulate proteolytic cleavage of the holoprotein. Recently, we found that insect Contactin represents a promising candidate ligand for APPL. Within the developing nervous system, Manduca Contactin (msContactin) is expressed by proliferating glial cells as they ensheath populations of migratory neurons that express APPL. Short-term treatment with msContactin-Fc fusion proteins selectively labeled the migratory neurons, while more prolonged treatment inhibited their motile behavior, similar to the effects of stimulating APPL-Gαo signaling. Knocking down APPL expression with antisense morpholino oligonucleotides demonstrated that these effects are APPL-dependent. Intriguingly, our initial studies suggest that msContactin-Fc stimulation also promotes the cleavage of APPL by endogenous secretases. These results support the model that Contactins can function as authentic ligands for APP/APPL, regulating neuronal motile responses in a Gαo-dependent manner. Given recent evidence that APP-Gαo signaling may be misregulated in AD, we are currently exploring whether altered Contactin-APP interactions are similarly associated with neurodegenerative responses in the brain.

Disclosures: P.F. Copenhaver: None. **J.A. Zweig:** None. **T.L. Swanson:** None. **J.M. Ramaker:** None.

Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 214.06/C5

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Opposite dysregulation of fragile-x mental retardation protein and heteronuclear ribonucleoprotein c protein associates with enhanced app translation in Alzheimer's disease

Authors: *A. BORRECA[1], K. GIRONI[2], G. AMADORO[3], M. AMMASSARI-TEULE[4]; [1]CNR-IBCN, Rome, Italy; [2]Univ. of Rome- Sapienza, Rome, Italy; [3]Inst. of Translational Pharmacol. (IFT) -National Res. Council (CNR), Rome, Italy; [4]Inst. of Cell. Biol. and Neurobio. (IBCN), Consiglio Nazionale delle Ricerche, Rome, Italy

Abstract: Amyloid precursor protein (APP) is overexpressed in familiar and sporadic Alzheimer Disease (AD) patients suggesting that, in addition to abnormalities in APP cleavage, enhanced levels of APP full length might contribute to the pathology. Based on data showing that the two RNA binding proteins (RBPs) Fragile-X Mental Retardation Protein (FMRP) and heteronuclear Ribonucleoprotein C (hnRNP C) exert an opposite control on APP translation, we have analyzed whether expression and translation of these two RBPs vary in relation to changes in APP protein and mRNA levels in the AD brain at 1, 3 and 6 months of age. Here we show that, as expected, human APP is overexpressed in hippocampal total extract from Tg2576 mice at all age points. APP overexpression, however, is not stable over time but reaches its maximal level in 1-month old mutants in association with the stronger (i) reduction of FMRP and (ii) augmentation of hnRNP C. APP levels then decrease progressively as a function of age in close relationship with the gradual normalization of FMRP and hnRNP C levels. Consistent with the mouse data, expression of FMRP and hnRNP C are respectively decreased and increased in hippocampal synaptosomes from sporadic AD patients. Our findings identify two RBP targets that might be manipulated for reducing abnormally elevated levels of APP in the AD brain, with the hypothesis that acting upstream of amyloidogenic processing might contribute to attenuate the amyloid burden.

Disclosures: **A. Borreca:** None. **K. Gironi:** None. **G. Amadoro:** None. **M. Ammassari-Teule:** None.

Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

Location: Hall A

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Program#/Poster#: 214.07/C6

Topic: C.02. Alzheimer's Disease and Other Dementias

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P01-HD080642

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Fundación Alfonso Martín Escudero

Alzheimer's Drug Discovery Foundation

The Ellison Medical Foundation

R01-NS056049

Title: Increased accumulation of APP-C99 in mitochondria-associated ER membranes triggers early events in the pathogenesis of Alzheimer's disease

Authors: *E. AREA[1], M. PERA[1], D. LARREA[1], R. B. CHAN[1], G. DI PAOLO[1], M. F. MEHLER[2], E. A. SCHON[1];
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Abstract: The pathogenesis of Alzheimer disease (AD) is associated with aberrant proteolytic processing of the amyloid precursor protein (APP) - first by β-secretase to generate a 99-aa C-terminal fragment (C99) and then by γ-secretase (containing presenilins-1 [PS1] or -2 [PS2]) that cleaves C99 to generate the APP intracellular domain and the β-amyloid (Aβ) that is found in extraneuronal plaques - but the relationship of the former to the latter is unclear. Whereas plaques typically form late in disease progression, early events include aberrant lipid metabolism and mitochondrial dysfunction. We recently showed that PS1, PS2, and γ-secretase activity itself are highly enriched in a subdomain of the endoplasmic reticulum (ER) that communicates with mitochondria, called mitochondria-associated ER membranes (MAM), and that ER-mitochondrial connectivity and MAM function are highly upregulated in presenilin-mutant cells and in cells from AD patients. However, the relationship of perturbed APP processing to MAM-related AD phenotypes has also been unclear. MAM, which is a lipid raft rich in cholesterol and sphingomyelin, is a key regulator of lipid metabolism and of mitochondial dynamics, among other processes. We now show that C99 is highly enriched in the MAM, and that it plays a central role in regulating cellular cholesterol and sphingolipid homeostasis and mitochondrial bioenergetics. Furthermore, in cells from AD patients and in cell and animal models of AD, C99 levels are increased significantly, resulting in a disruption of lipid homeostasis that is triggered by the unregulated uptake of extracellular cholesterol, with downstream consequences that mimic the biochemical features of AD, including the bioenergetic deficits. We propose that MAM-localized C99 is a component of the cell's lipid sensing machinery and that its accumulation is a key and early event in AD pathogenesis that can explain many of the biochemical and morphological features of the disease.

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Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 214.08/C7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Cure Alzheimer Fund

NIH

Title: Atxn1, spinocerebellar ataxia type-1 protein, regulates bace1 expression and beta-amyloid pathology in the cerebrum

Authors: *J. SUH[1], D. M. ROMANO[2], J. D. SCHMAHMANN[3], R. E. TANZI[4];
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Abstract: Abnormal expansion of CAG repeat in the ataxin-1 gene (ATXN1) causes the degeneration of Purkinje cells and spinocerebellar ataxia type 1 (SCA1), a neurodegenerative movement disease. Previously our laboratory has reported ATXN1 is associated with Alzheimer's disease (AD) and knockdown of ATXN1 increases Abeta generation in the cultured mammalian cells. Here, we investigate whether ATXN1 expression affects the processing of amyloid precursor protein (APP) and the pathogenesis of AD. We examined the levels of APP cleavage products and the expression of secretases in the brains of ATXN1 knockout (KO) mice. APP processing and Abeta pathology were further analyzed after crossing the ATXN1 KO mice with APPswe/PS1deltaE9 AD mice. We found ATXN1 KO increases the levels of beta-secretase BACE1, but not alpha-secretase ADAM10 in the cortex and hippocampus. No change of BACE1 expression was detected the cerebellum. The increase of BACE1 in cerebrum is concordant with the shift of APP processing into the beta-secretase cleavage pathway along with an increase in Abeta levels and plaque load in the brains of AD mice. In addition, both the proliferation of neural progenitor cells and the dendritic development of immature neurons, the two markers of adult neurogenesis, were severely impaired in the hippocampus of ATXN1 KO mice. Together, these findings suggest that the loss of ATXN1 function potentiates beta-amyloid pathology through the increase of BACE1 expression and the subsequent beta-secretase cleavage of APP.

Disclosures: J. Suh: None. **D.M. Romano:** None. **J.D. Schmahmann:** None. **R.E. Tanzi:** None.

Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

Location: Hall A

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 5R21AG042804-02

NIH Grant 5R01AG018884-10

NIH Grant P30 AG010133- IADC

Alzheimer's Association IIRG-11-206418

Title: Transcription factor Specificity Protein 1 (Sp1) modulation of the amyloid-β precursor protein (APP) and β-site APP Cleaving Enzyme (BACE1) activity as a drug target in Alzheimer's disease (AD)

Authors: *B. L. BAYON[1], K. NHO[2], N. CHOPRA[3], B. MALONEY[4], D. K. LAHIRI[5]; [1]Dept. of Med. & Mol. Genet., [2]Radiology and Imaging Sci., [3]Neurosci., [4]Psychiatry, [5]Psychiatry and Med. & Mol. Genet., Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: The Latent Early-life Associated Regulation (LEARn) model posits that environmental agents epigenetically disturb gene regulation in a long-term manner, but that the pathology may not manifest until much later in life. The LEARn model's molecular mechanisms include changes in DNA methylation within the promoters of specific genes. Expression levels of some transcription factors (TFs) such as Sp1 are perturbed in this latent fashion. Expression of Sp1 parallels expression levels of disease associated genes in AD. BACE1 is the β-secretase responsible for the rate-limiting cleavage of APP to amyloid-β (Aβ), which can reach the pathological levels seen in AD. Sp1 positively regulates APP and induces BACE1 via their respective promoters. We chose single nucleotide polymorphisms (SNPs) within the Sp1 gene from the AD Neuroimaging Initiative GWAS data, performed an association analysis with an AD-specific imaging biomarker (entorhinal cortex thickness), and identified a significant SNP (rs11170553) associated with entorhinal cortex thickness. rs11170553 was also associated with cerebral amyloid deposition. We tested Sp1-mediated regulation of APP with Mithramycin A (MTM), a selective inhibitor of Sp1, and Tolfenamic acid (TA), an inducer of Sp1 degradation, and with siRNAs in mammalian cell lines (rat neuronal PC12 and human glioblastoma U373), a primary human fetal neuron culture (HFN) and mixed cultures derived from human fetal neurospheres (NSPc). Treatment of PC12 reveals minimal changes in confluence, cytotoxicity, neurite length, and neurite outgrowth after Sp1 knockdown via siRNA or treatment with Sp1 modulating drugs. Morphology and cell death tracking studies from U373 reveal increasing cytotoxicity in MTM concentrations above 10 μM after 36 hours. Treatment of HFN with TA did not affect cell viability with doses up to 5 μM. Western blotting shows a significant decrease in expression of BACE1 after MTM treatment in NSPc and U373. Treatment with TA does not significantly decrease APP or BACE1 in NSPc or U373. APP siRNA knocks down expression of APP in both of these cell types. APP expression is not altered by treatment with TA in NSPc, perhaps due to disparate mechanisms of these Sp1-inhibiting drugs. MTM reduces APP and BACE1 expression. Neither treatment with Sp1-inhibiting drugs nor transfection with Sp1 siRNA affects cell viability of primary neurons nor differentiated NSPc. Compounds that can

modify Sp1 binding to sites on the BACE1 and APP promoters could provide a means to limit the production of Aβ peptide and may slow the symptoms of AD. These results show that appropriate modulation of a specific TF could potentially be a novel drug target for AD.

Disclosures: **B.L. Bayon:** None. **K. Nho:** None. **N. Chopra:** None. **B. Maloney:** None. **D.K. Lahiri:** None.

Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 5R21AG042804-02

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NIH Grant P30 AG010133- IADC

Alzheimer's Association IIRG-11-206418

Title: Understanding the neurobiology of Alzheimer's disease (AD) by correlating specific AD-associated miRNAs and the MMSE cognitive scale

Authors: *D. K. LAHIRI[1], B. MALONEY[1], J. M. LONG[1], N. CHOPRA[1], K. SAMBAMURTI[2], B. L. BAYON[1];
[1]Dept. of Psychiatry, Indiana Univ. Sch. of Med., Indianapolis, IN; [2]Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: Alzheimer's disease (AD) is characterized by excess amyloid-β (Aβ) peptide in neuritic plaques. The rate-limiting step in Aβ production is proteolytic cleavage of amyloid- β precursor protein (APP) by β-secretase (BACE1). Dysregulation of such proteins contributes to Aβ deposition. Both APP and BACE1 are regulated by specific microRNAs (miRNAs). E. g., miR-101, miR153, and miR-346 regulate APP, and miR-339-5p lowers BACE1 levels (Long et al-*JBC*, 2012; 2014). The Mini Mental State Examination (MMSE) is a common clinical measure of cognitive decline. We examined relationships between miRNA regulation of AD-associated genes and the MMSE cognitive scale. Levels of APP, BACE 1 expression, and miRNA in brain specimens (BA9, frontal cortex) from AD and age-matched control patients (Dr. Peter Nelson, University of Kentucky) were measured as previously described (Long, et al-JBC 2014). Quantitative RT-PCR was performed on isolated RNA samples and normalized to three control RU-RNAs. The resulting data were modeled as follows: "MMSE ~ (−*APOE*ε4 + miR101+ miR153 + miR346)^2"; where "^2" indicates all two-way interactions, and "−*APOE*ε4" indicates lack of any *APOE*ε4 allele. Our results showed a significant ($p \leq 0.05$)

association between MMSE and miR101 and between MMSE and −*APOE*ε4. A negative relationship between MMSE and miR153 was observed as well as interaction of *APOE* status with each individual miRNA. Interestingly, interactions miR101× miR346 and miR153×miR346 were also significant. Cook's D identified one extreme influential point (D = 7.31). Deleting this point maintained the same relationships except for the association with *APOE* status. *APOE* status interactions were maintained. In summary, we compared levels of miRNAs with MMSE and identified significant relationships between miRNA levels and MMSE scores. Our findings suggest a potential role for specific miRNA activities and cognitive decline measured by MMSE.

Disclosures: D.K. Lahiri: None. **B. Maloney:** None. **J.M. Long:** None. **N. Chopra:** None. **K. Sambamurti:** None. **B.L. Bayon:** None.

Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01AG025952

NIH R01AG033016

NIH R56 NS092497

Title: Loss of BACE1 polarization in GGA3 null hippocampal neurons

Authors: *S. LOMOIO, G. TESCO;
Neurosci., Tufts Univ. Sch. of Med., Boston, MA

Abstract: We have previously reported that GGA3, a clathrin adaptor protein highly expressed in the brain, plays a pivotal role in the degradation and stabilization of BACE1, the rate limiting enzyme in the production of Alzheimer's disease (AD)-associated Aβ protein. It has been described that BACE1 localizes at the presynaptic terminals and accumulates in dystrophic neurites around Aβ plaques in AD. Other studies suggested that high BACE1 levels in AD brains might be due to alterations of its degradation as a result of abnormal cellular trafficking. Our previous findings have shown that the depletion of GGA3 results in increased BACE1 level and activity *in vitro* and *in vivo* owing to accumulation of BACE1 in early endosomes and that the levels of BACE1 and Aβ are increased in GGA3 null hippocampal neurons. More importantly we have previously demonstrated that GGA3 levels are decreased and inversely correlated with BACE1 in post-mortem AD temporal cortex. In spite of compelling studies showing that GGA3 regulates BACE1 trafficking, the role of GGA3 in BACE1 neuronal polarized sorting has not being studied before. Moreover, while increasing evidence is accumulating for a role of the AP

complexes in neuronal sorting, the function of GGA3 in neurons remains poorly understood. Exploring the subcellular localization of GGA3 and BACE1 in cultured hippocampal neurons, we found that BACE1 and GGA3 highly colocalized and are targeted both to the somatodendritic and axonal compartment with a preference for dendritic targeting. As far as we know this is the first study demonstrating the presence of the clathrin adaptor protein GGA3 into the axon. Moreover, live imaging experiments have shown that both BACE1 and GGA3 undergo a bidirectional trafficking along the axon. In fact, while GGA3 trafficking appears slow and saltatory, BACE1 moves with a rapid and constant pace both anterogradely and retrogradely. More interestingly we observed a loss of BACE1 polarization leading to accumulation and increase levels of BACE1 into the axons of GGA3 null hippocampal neurons. To further address the role of GGA3 in BACE1 axonal sorting we performed co-transfection experiments of the two proteins. The results clearly demonstrated that GGA3 reintroduction in GGA3 null hippocampal neurons is sufficient to rescue the polarized distribution of BACE1 to the wild type conditions. Together, these results suggest that GGA3 is able to control and modulate the axonal targeting and the polarized sorting of BACE1. Thus, the depletion of GGA3 observed in AD brains is a leading candidate mechanism underlying BACE1 accumulation in peri-plaque dystrophic axons observed in AD brains.

Disclosures: S. Lomoio: None. **G. Tesco:** None.

Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

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NCRR 1S10 RR031680-01

NIH 1S10OD010398-01

Title: The role of elevated calcium and microtubule destabilization in BACE1 and Aβ accumulation around amyloid plaques in Alzheimer’s disease

Authors: *K. R. SADLEIR[1], A. SOMASUNDARAM[2], V. BUGGIA-PREVOT[3], P. KANDALEPAS[1], G. THINAKARAN[3], M. PRAKRIYA[2], R. VASSAR[1];
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Abstract: β-Secretase, β-site APP Cleaving Enzyme1 (BACE1) initiates the production of the β-amyloid (Aβ) peptide involved in Alzheimer's disease (AD). Genetic reduction of BACE1 levels inhibits Aβ generation and amyloid plaque formation. BACE1 protein and activity are elevated in the brains of Alzheimer's patients and mouse models of AD, accumulating in presynaptic neuronal dystrophies around plaques. These accumulations of BACE1 and APP, lead to increased Aβ production. Immunostaining with antibodies specific to neoepitopes of APP and Aβ created by BACE1 cleavage show accumulation of sAPPβ, β-CTF and Aβ species in dystrophic axons along with BACE1, indicating active processing of APP to Aβ in these areas. Blocking the BACE1 increase could be therapeutically useful for decreasing Aβ generation and in slowing or preventing AD without reducing baseline levels of BACE1, which may cause mechanism-based side effects due to deficient processing of numerous BACE1 substrates. Currently, the mechanism of the BACE1 increase is not clear. We observe that in the brains of 5XFAD mice and AD patients, tubulin is mostly absent in BACE1-containing dystrophic axons around plaques, and remaining tubulin accumulates into spheroidal aggregates. We hypothesize that impaired retrograde transport of BACE1 and other proteins leads to accumulation in swollen dystrophic axons,. We see tubulin beading, varicosity formation, and neurite degeneration in primary neurons exposed to Aβ42, and loss of microtubules in dystrophic axons by EM. Also, some components of the kinesin and dynein-dynactin axon transport complexes are decreased and disorganized in dystrophic axons, while others are enriched. Additionally, Aβ42 disrupts anterograde and retrograde axon transport in primary neurons *in vitro*. High intracellular calcium can depolymerize microtubules and disrupt microtubule-based transport. Elevated resting calcium is observed in dystrophic neurites and in neurons exposed to Aβ42. Calcium channel blockers inhibit the calcium influx observed in Aβ42-treated neurons, implicating a calcium channel as the source of increased resting calcium. We hypothesize that Aβ plaques cause increased resting calcium, disrupted microtubule networks, and impaired BACE1 retrograde transport from dystrophic axons for degradation. We will focus on identifying the calcium channel(s) responsible for increased resting calcium, and using pharmacological methods to inhibit calcium increase and/or microtubule destabilization. Through these approaches we hope to decrease BACE1 accumulation in dystrophic axons around plaques, which we predict will slow Aβ42 generation and amyloid pathology.

Disclosures: K.R. Sadleir: None. **A. Somasundaram:** None. **V. Buggia-Prevot:** None. **P. Kandalepas:** None. **G. Thinakaran:** None. **M. Prakriya:** None. **R. Vassar:** None.

Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 214.13/C12

Topic: C.02. Alzheimer's Disease and Other Dementias

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Alzheimer's Association

Title: Presenilin-1 knockin mice reveal loss-of-function mechanism for familial Alzheimer's disease

Authors: *D. XIA[1], H. WATANABE[1], B. WU[1], S. LEE[1], Y. LI[3], E. TSVETKOV[3], V. Y. BOLSHAKOV[3], J. SHEN[1], R. J. KELLEHER, III[2];
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Abstract: Presenilins play essential roles in memory formation, synaptic function, and neuronal survival. Mutations in the Presenilin-1 (PSEN1) gene are the major cause of familial Alzheimer's disease (FAD). How PSEN1 mutations cause FAD is unclear, and pathogenic mechanisms based on gain or loss of function have been proposed. Here, we generated Psen1 knockin (KI) mice carrying the FAD mutation L435F or C410Y. Remarkably, KI mice homozygous for either mutation recapitulate the phenotypes of Psen1-/- mice. Neither mutation altered Psen1 mRNA expression, but both abolished γ-secretase activity. Heterozygosity for the KI mutation decreased production of Aβ40 and Aβ42, increased the Aβ42/Aβ40 ratio, and exacerbated Aβ deposition. Furthermore, the L435F mutation impairs hippocampal synaptic plasticity and memory and causes age-dependent neurodegeneration in the aging cerebral cortex. Collectively, our findings reveal that FAD mutations can cause complete loss of Presenilin-1 function *in vivo*, suggesting that clinical PSEN mutations produce FAD through a loss-of-function mechanism. We are continuing the analysis of these mutant mice and the new results will be presented.

Disclosures: D. Xia: None. **H. Watanabe:** None. **B. Wu:** None. **S. Lee:** None. **Y. Li:** None. **E. Tsvetkov:** None. **V.Y. Bolshakov:** None. **J. Shen:** None. **R.J. Kelleher:** None.

Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Identification of γ-secretase substrate-binding sites suggests altered substrate positioning as a core mechanism of pathogenic Aβ generation

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Abstract: The failures of Alzheimer´s disease trials with γ-secretase inhibitors to lower Aβ have raised discussions as to whether the enzyme remains a viable drug target. Since lack of substrate selectivity for the Aβ precursor C99 is one likely cause for the failures, a deep understanding of how the enzyme recruits substrates, which has been strikingly understudied, is needed. To address this long-standing unresolved question, we set out to identify the key residues of C99 interacting with the protease complex and to unambiguously identify its substrate-binding subunit(s). By using a tRNA suppressor approach, we site-specifically incorporated the photocrosslinkable amino acid analog para-benzoyl-phenylalanine at every single residue of the Aβ domain of C99 up to the intracellular juxtamembrane domain. Following UV-mediated photoactivation, key substrate residues binding to γ-secretase, such as Val44, were identified as well as the target subunits including the presenilin 1 N-terminal fragment as major substrate-binding site. Strikingly, clinical presenilin 1 mutants displayed altered substrate-binding patterns suggesting that altered substrate positioning is one molecular cause that underlies the pathogenic generation of longer Aβ peptides. By providing a detailed C99 substrate-enzyme interaction map, our photoaffinity-labeling data fill a major gap of basic knowledge of γ-secretase that might also be key for the development of improved γ-secretase-targeting drugs.

Disclosures: A. Fukumori: None. **H. Steiner:** None.

Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R33AG049864

NIH RO1MH101148

Title: Single-cell detection of Aß and sAPPα secreted from human iPSC-derived neurons and glia

Authors: *M.-C. LIAO[1], T. YOUNG-PEARSE[1], J. C. LOVE[2];
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Abstract: Secreted factors play a central role in normal and pathological processes in every tissue in the body. The brain is composed of a highly complex milieu of different cell types, and few methods exist that can identify which individual cells in a complex mixture are secreting specific analytes. By identifying which cells are responsible, we can better understand neural physiology and pathophysiology, more readily identify the underlying pathways responsible for analyte production, and develop and test novel therapeutic strategies that target the cell types of relevance. Accumulation of amyloid beta (Aß) in senile plaques is a pathological hallmark of Alzheimer's disease. Aß is generated from sequential processing of Amyloid Precursor Protein (APP) by ß-secretase followed by γ-secretase. APP also can be cleaved by α-secretase to generate sAPPα, which precludes Aβ generation. We present here the method of microengraving to detect Aβ and sAPPα secreted from single hiPSC-derived neural cells. Through these single cells studies, we have found: 1) unexpectedly heterogeneous responses to secretase inhibitors in putatively "homogeneous" stable clonal cell lines; 2) a previously unappreciated subpopulation of cells that secrete high levels of Aβ in the absence of detectable sAPPα; 3) that cell state can affect the relationship between α- and β-secretase cleavage of APP; 4) that during the time of neural differentiation, the number of cells secreting detectable levels of Aβ and sAPPα increases; and intriguingly 5) that cells expressing forebrain GABAergic neuronal markers are overrepresented in subpopulations of cells that secrete high levels of Aβ and sAPPα, although a variety of cell types can secrete high levels of each. Finally, while there is a widespread belief that neurons are the major source of Aβ in the CNS, the results presented here show that astrocytes are competent to secrete high levels of Aβ and may therefore be a significant contributor to pathology in Alzheimer's disease. Taken together, these results using APP as a model protein describe a novel methodology to examine and quantify analyte secretion from hiPSC-derived neural cells at a single cell level, and they demonstrate the utility of such a system with studies directly relevant to unsettled questions regarding AD pathobiology.

Disclosures: **M. Liao:** None. **T. Young-Pearse:** None. **J.C. Love:** None.

Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

Location: Hall A

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Japan Society for the Ptomotion of Science KAKENHI Grants 22580339

Japan Society for the Ptomotion of Science KAKENHI Grants 25450428

Title: Glycelaldehyde-3-phosphate dehydrogenase (GAPDH) aggregates contribute to the pathogenesis of Alzheimer's disease via the acceleration of Aβ amyloidogenesis

Authors: *M. ITAKURA, H. NAKAJIMA, T. KUBO, Y. SEMI, S. KUME, S. HIGASHIDA, A. KANESHIGE, A. KITA, K. SATO, Y.-T. AZUMA, T. INUI, T. TAKEUCHI;
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Abstract: Glycelaldehyde-3-phosphate dehydrogenase (GAPDH) has been identified as a critical enzyme in glycolysis. In addition to this classical role, GAPDH possesses diverse functions including cell death. It is well known that GAPDH is highly sensitive to reactive oxygen species and oxidatively modified GAPDH forms disulfide-bond related aggregation, leading to cell death. Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is characterized by memory loss and cognitive deficit. Although the exact cause of AD remains to be elucidated, numerous studies have demonstrated the involvement of Amyloid-β peptide (Aβ) in the pathogenesis of AD; Aβ amyloidogenesis is known to be responsible for neuronal dysfunction and cell death. Recently, several reports suggest the relationship between GAPDH and AD, but the underlying mechanism has not been revealed. In the present study, our observation using 3xTg AD mice clarified the co-localization of GAPDH aggregates with Aβ aggregates both in senile plaques and inside neurons in the hippocampal CA3 region, which makes us hypothesize that GAPDH aggregates are involved in Aβ amyoidogenesis. *In vitro* assay using purified GAPDH and Aβ40 revealed that GAPDH aggregates enhanced Aβ40 amyloidogenesis assessed quantitatively and morphologically by Thioflavin-T fluorescence intensity, Congo red birefringence, far-ultraviolet CD spectrum and atomic force microscopy. Further investigation at the cellular level showed that GAPDH aggregates augmented Aβ40-induced mitochondrial dysfunction, causing enhanced cell death. Moreover, the test using intracerebroventricularly Aβ40-injected mice demonstrated that Aβ40-induced neuronal cell death in the hippocampal CA3 region was potentiated by the co-incubation with GAPDH aggregates via the increased cytochrome-c release and nuclear translocation of apoptosis inducing factor. These data indicate that aggregated GAPDH augments Aβ amyloidogenesis and increases neuronal cell death both *in vitro* and *in vivo*, which suggests that GAPDH play an important role for AD pathogenesis.

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Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

Location: Hall A

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Bright Focus Foundation

Cure Alzheimer's Fund

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Title: Partial purification of amyloid-beta oligomers from human Alzheimer's disease brain lysates

Authors: *D. L. BRODY, H. JIANG, T. J. ESPARZA;
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Abstract: Senile plaques constitute a key pathological hallmark of Alzheimer's disease (AD), yet the extent of plaque deposition only moderately correlates with the progression of dementia. In contrast, various forms of soluble amyloid-beta (Aβ) oligomers have demonstrated a stronger correlation with dementia status and exhibit significantly greater toxicity than isolated fibrils, plaques or peptide monomers. We used our plate-based Aβ oligomer assay (Esparza et al, Annals of Neurology, 2013) to quantitatively guide extraction and partial purification of Aβ oligomers from frozen cortical AD brain tissue. Tissue was dounce homogenized in phosphate-buffered saline containing 0.5% CHAPS. Oligomeric Aβ was separated from monomers by 35% ammonium sulfate precipitation followed by size exclusion chromatography. To prevent non-specific loss, tubes and pipet tips were pre-coated with 0.0075% albumin. Immunoprecipitation was performed with combined N-terminal and mid-domain specific Aβ monoclonal antibody-conjugated sepharose beads. After 20 saline washes, immunoprecipitated material was eluted with pH 11 ammonium hydroxide. CHAPS extraction increased yield of Aβ oligomers to approximately 10 ng per gram of frozen cortical tissue. CHAPS extraction and did not result in artifactual oligomerization based on control experiments involving spiking 2 ng/ml AD-brain derived monomeric Aβ into non-demented control brain tissue prior to homogenization. In contrast, triton X-100 and SDS extractions resulted in substantial artifactual Aβ oligomerization. Ammonium sulfate precipitation also did not result in artifactual oligomerization and did not change the predominant size of oligomers: >500 kDa based on Superdex 200 size exclusion chromatography in physiological saline running buffer. In contrast, size exclusion chromatography using ammonium acetate pH 8.5 running buffer produced a heterogenous array of smaller oligomers, suggesting dissociation into sub-assemblies. Ammonium sulfate precipitation produced approximately 10-fold purification, and immunoprecipitation resulted in an additional 1000-fold purification. We have achieved approximately 10,000 fold purification of native Aβ oligomers from human Alzheimer's disease cortex with <30% loss of starting material. Our immediate goal is to test the hypothesis that post-translational modifications and co-associated proteins contribute to the development and accumulation of synaptotoxic Aβ assemblies.

Disclosures: D.L. Brody: None. **H. Jiang:** None. **T.J. Esparza:** None.

Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

Location: Hall A

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Program#/Poster#: 214.20/C17

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: CLAC alters the plaque morphology and dynamics of amyloid-β peptides in the brain

Authors: *T. HASHIMOTO, Y. NAKA, D. FUJII, M. KASHIWAGI, T. WAKABAYASHI, T. IWATSUBO;
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Abstract: Massive deposition of amyloid-β peptides (Aβ) as senile plaques (SPs) characterizes the pathology of the brains of patients with Alzheimer's disease (AD), although the role of SPs in the pathophysiology of AD remains unclear. A variety of SPs in different morphology are observed in AD brains, whereas specific components of SP that determine their morphology are unknown. We examined the role of collagenous Alzheimer amyloid plaque component (CLAC), a non-Aβ proteinaceous component of SP amyloid identified in AD brains, in the formation of SPs and dynamics of Aβ in the brains of AD model mice. To this end, we generated transgenic (tg) mice overexpressing CLAC precursor (CLAC-P) in neurons and crossed them with APP tg mice. Overexpression of CLAC in the brains of APP tg mice caused a remarkable reduction in diffuse-type Aβ plaques and increased well-demarcated and middle-sized Aβ plaques, which were occasionally laden with thioflavin S-positive amyloid cores. Biochemically, the levels of insoluble Aβ in the brains of APP/CLAC-P double tg mice were comparable to those in APP tg mice. These data implicate CLAC in the remodeling of Aβ plaque morphology into maturated form. To further investigate the role of SPs in the dynamics and metabolism of Aβ in brains, we measured the levels of Aβ42 in the interstitial fluid (ISF) of hippocampus by *in vivo* microdialysis using a 1,000 kDa molecular weight cut-off probe. Interestingly, we found a negative correlation between the Aβ plaque burden and the ISF Aβ42 levels in 18-month-old APP tg mice, which may suggest the role of Aβ plaques as a pool of soluble extracellular Aβ in brains. In the APP/CLAC-P double tg mice, however, the levels of hippocampal ISF Aβ42 was significantly lower than those in APP single tg mice, and the negative correlation between Aβ burden and ISF Aβ42 was attenuated. These results altogether suggest that association of CLAC may play an important role in the maturation of Aβ plaques, resulting in an alteration in the dynamics and metabolism of Aβ in AD brains

Disclosures: T. Hashimoto: None. **Y. Naka:** None. **D. Fujii:** None. **M. Kashiwagi:** None. **T. Wakabayashi:** None. **T. Iwatsubo:** None.

Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NINDS grant R01 NS073512

Title: Endothelin-converting enzyme-2 is expressed by somatostatin GABAergic interneurons and modulates intraneuronal Aβ

Authors: *J. PACHECO-QUINTO, E. ECKMAN;
Biomed. Res. Inst. of New Jersey, Cedar Knolls, NJ

Abstract: One of the key features of Alzheimer's disease (AD) is the accumulation of the neuronally-produced amyloid β peptide (Aβ) in the form of aggregates in the brain. These Aβ aggregates, specifically in their soluble states, can cause synaptic toxicity and neuronal loss. Enzymatic degradation is crucial to maintain Aβ homeostasis and endothelin-converting enzyme-2 (ECE-2) is one of the known physiologically important Aβ degrading enzymes. We have previously shown that ECE-2 knockout mice have a significant 1.3 fold elevation in endogenous Aβ. However, ECE-2 has an acidic pH optimum, cannot modulate extracellular Aβ and only degrades Aβ along the endosomal/lysosomal pathway, suggesting that in ECE-2 knockout mice Aβ accumulates intracellularly. To understand better the role that ECE-2 plays in Aβ catabolism, we characterized which cell populations express ECE-2 in CNS and the consequence of ECE-2 inhibition on Aβ accumulation and aggregation. Experiments were performed on brain tissue from wild-type and TgCRND8 APP transgenic mice, as well as temporal cortex from AD patients and non-demented control patients. By combining fluorescence immunohistochemistry with multiplex fluorescence *in situ* hybridization we observed that ECE-2 mRNA was abundantly expressed by somatostatin positive GABAergic interneurons in the soma and along axons. In addition, ECE-2 was found active in synaptosomal preparations, suggesting that it degrades either internalized Aβ in presynapses or Aβ produced along the retrograde transport pathway. We did not observe ECE-2 expression in activated astrocytes or microglia surrounding amyloid plaques, in either APP transgenic mice or in patients with AD. In SH-SY5Y cells overexpressing human wild type APP and in primary neuronal cultures from TgCRND8 mice, ECE-2 inhibition led to increased intracellular Aβ accumulation and the intracellular formation of Aβ aggregates.Our data suggest that somatostatin positive GABAergic interneurons contribute to Aβ homeostasis by degrading Aβ along the retrograde transport pathway. Failure in ECE-2 activity could lead to the formation of Aβ aggregates and represent one of the first steps in the amyloidogenic process.

Disclosures: J. Pacheco-Quinto: None. **E. Eckman:** None.

Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant RO1 GM0980033

Alzheimer's Association (IIRG 08-91256)

Title: Aβ(1-42) fibril structure illuminates self-recognition and replication of amyloid in Alzheimer's disease

Authors: *Y. ISHII[1], Y. XIAO[2];
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Abstract: Increasing evidence suggests that formation and propagation of misfolded aggregates of 42-residue amyloid β (Aβ(1-42)), rather than the more abundant Aβ(1-40), provokes the Alzheimer's cascade. To date, structural details of misfolded Aβ(1-42) have remained elusive. Here we present the atomic model of Aβ(1-42) amyloid fibril based on solid-state NMR (SSNMR) data. It displays triple parallel-β-sheet segments that are different from reported structures of Aβ(1-40) fibrils. Remarkably, Aβ(1-40) is not compatible with the triple-β motif, as seeding with Aβ(1-42) fibrils does not promote conversion of monomeric Aβ(1-40) into fibrils via cross-replication. SSNMR experiments suggest that the Ala42 carboxyl terminus, absent in Aβ(1-40), forms a salt-bridge with Lys28 as a self-recognition molecular switch that excludes Aβ(1-40). The results provide insight into Aβ(1-42)-selective self-replicating amyloid propagation machinery in early-stage Alzheimer's disease. Other topics such as interactions with a pathogenic mutant of Aβ will be also discussed.

Disclosures: Y. Ishii: None. **Y. Xiao:** None.

Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

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Title: Hyperinsulinemia modulates extracellular amyloid-beta *in vitro*

Authors: *M. STANLEY[1], S. L. MACAULEY[2], E. E. CAESAR[2], G. ROBINSON[2], D. M. HOLTZMAN[2];
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Abstract: Type 2 diabetes (T2D) is a risk factor for developing late-onset Alzheimer's disease (AD), the most common form of dementia. T2D is characterized by high blood insulin (hyperinsulinemia), high blood glucose (hyperglycemia), and insulin resistance. Hyperinsulinemia, specifically, has been associated with an increased risk for developing AD and amyloid plaques. Levels of extracellular amyloid-beta (Aβ) in the brain influences Aβ deposition in a concentration-dependent manner and *in vitro* experiments suggest that high insulin can lead to higher concentrations of extracellular Aβ. However, the effects of high insulin on extracellular Aβ *in vivo* are currently unknown. To determine if insulin can modulate brain levels of interstitial fluid (ISF) Aβ in awake, freely-behaving PS1dE9/APPswe mice, insulin was delivered directly into the hippocampus through a microdialysis probe at different concentrations. The response in males was more variable than females, but overall there was a significant increase in ISF Aβ in response to 40nM insulin. T2D is characterized by peripheral hyperinsulinemia and since it is known that blood insulin crosses the blood brain barrier through a saturable transport mechanism, we also determined the effects of peripherally high insulin on ISF Aβ. To manipulate blood insulin levels in awake, freely-behaving PS1dE9/APPswe mice, we performed acute hyperinsulinemic-euglycemic clamps while simultaneously collecting ISF from the hippocampus using microdialysis. We found that moderate hyperinsulinemia (4mU/kg/min) increased ISF Aβ in both young mice without amyloid plaques and old mice with significant amyloid pathology of both genders. We also found that extreme hyperinsulinemia (20mU/kg/min) also modulates ISF Aβ to a similar extent. Our results suggest that increases in peripheral and central insulin can increase the concentration of ISF Aβ both before and after amyloid plaque deposition, suggesting that insulin levels may affect the onset or progression of AD by affecting Aβ aggregation *in vivo*. Funding: P01 NS080675, NSF DGE-1143954(MS), NIH F32 NS080320(SLM)

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Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

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SFB645

KFo177

Neuroallianz

Title: Site-specific effect of amyloid-beta phosphorylation on oligomerization and deposition

Authors: *S. H. KUMAR[1], O. WIRTHS[3], N. REZAEI-GHALEH[4], K. STÜBER[5], P. KOCH[5], S. THEIL[2], M. ZWECKSTETTER[6], T. A. BAYER[3], O. BRÜSTLE[5], D. R. THAL[7], J. WALTER[2]; [1]Dept. of Neurol., [2]Univ. of Bonn, Bonn, Germany; [3]Dept. of Psychiatry and Psychotherapy, Div. of Mol. Psychiatry, Univ. Med. Ctr. (UMG), Göttingen, Germany; [4]Dept. for NMR-based Structural Biol., German Ctr. for Neurodegenerative Dis. (DZNE), Göttingen, Germany; [5]LIFE & BRAIN Ctr., Inst. of Reconstructive Neurobio., Bonn, Germany; [6]Dept. for NMR-based Structural Biol., Max Planck Inst. for Biophysical Chem., Göttingen, Germany; [7]Lab. of Neuropathology, Inst. of Pathology, Univ. of Ulm, Ulm, Germany

Abstract: Background Alzheimer's disease (AD) is the most common form of dementia in the aging population and affects millions of people worldwide. Aggregation and toxicity of the amyloid β-peptide (Aβ) are considered as critical events in the initiation and progression of AD. Several familial AD (FAD) mutations that cause early onset AD are found within the Aβ and result in alterations of peptide conformation and promote oligomerization. However, such mutations are very rare and molecular mechanisms that induce the formation or stabilization of oligomers of the wild-type Aβ remain unclear. Methods We applied cell biological, biochemical, biophysical and neuropathological methods to characterize the effect of Aβ phosphorylation in cell culture models as well as in brains of human AD cases and transgenic mice. Results We demonstrate that Aβ undergoes phosphorylation at different sites and identified potential protein kinases. Phosphorylated Aβ shows increased propensity to form oligomeric and fibrillar aggregates and adopt β-sheet conformation. By using highly specific phosphorylation site specific antibodies, we demonstrate abundant presence of phosphorylated Aβ (pAβ) in transgenic mouse models of AD and human brain. Phosphorylated Aβ shows different spatio-temporal distribution as compared to non-phosphorylated Aβ (npAβ). Importantly, pAβ oligomers exert increased toxicity in human neurons. Conclusions Our studies reveal the effect of site-specific

phosphorylation on Aβ conformation, oligomerization and toxicity. We hypothesize that phosphorylation of Aβ seems to act as a conformational switch and modulate aggregation and toxicity of Aβ peptides. Phosphorylated Aβ species are present in human AD brains and transgenic mouse models. The detection of these variants could be further explored as targets for AD therapy and prevention as well as markers for AD pathogenesis.

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Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

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Illinois Society for the Prevention of Blindness

Research to Prevent Blindness

Title: Nominal amyloid-beta (Aβ) levels, and reduction of Aβ by exogenous neprilysin, in mouse eye tissues

Authors: *R. PARTHASARATHY[1,2], M. K. CHOW[3], Z. DERAFSHI[2], M. P. FAUTSCH[4], J. R. HETLING[2], D. W. RODGERS[3], L. B. HERSH[3], D. R. PEPPERBERG[1,2];
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Abstract: Amyloid-beta (Aβ), an aggregation-prone peptide, is a suspected culprit in Alzheimer’s disease. Recent studies have raised the hypothesis that excessive Aβ build-up in eye tissues may contribute to retinal degenerative diseases such as age-related macular degeneration. Neprilysin (NEP) is a native membrane-anchored endopeptidase that cleaves Aβ into inactive products, and this activity is retained by a recombinant soluble protein (sNEP) that includes

NEP's catalytic domain. We have examined whether sNEP delivered intravitreally to the mouse eye can decrease ocular levels of Aβ40 and Aβ42 (40 and 42 amino acids in length, respectively). Anesthetized 10-month old wild-type C57BL/6J mice and 2-3-month old 5XFAD mice received intravitreal injections (2 μL) of phosphate-buffered saline (PBS) containing 10 μg sNEP. Twenty-four hours after injection, animals were euthanized and the eyes dissected to separately yield (i) combined lens and vitreous, (ii) neural retina, and (iii) combined retinal pigment epithelium and choroid. PBS extracts of homogenized samples were analyzed for Aβ and protein to yield protein-normalized Aβ40 and Aβ42 concentrations. Samples of types (i-iii) from untreated eyes of C57BL/6J and 5XFAD mice exhibited readily measurable Aβ concentrations. For C57BL/6J mice (n=12), Aβ40 concentrations in (i-iii) did not differ significantly from one another ($p>0.3$), and the sum of levels in (i-iii) (43.8 ± 6.9 pmol/g protein) did not differ significantly from Aβ40 levels determined in experiments that involved the recovery of combined samples (i-iii) followed by homogenization, PBS extraction and analysis of the combined eye tissues ($p>0.05$). Similar results were obtained for 5XFAD mice (n=8), where no significant differences were noted between separate-tissue (i-iii) concentrations for either Aβ40 or Aβ42 ($p>0.09$), and where, for both Aβ40 and Aβ42, summed concentrations in (i-iii) did not differ significantly from those determined in combined-eye-tissue experiments ($p = 0.57$ and 0.21, respectively). Treatment with sNEP produced significant Aβ40 reductions in compartments (i-iii) in C57BL/6J mice. In 5XFAD mice, sNEP treatment significantly reduced Aβ40 in all three compartments, and these reductions exceeded those for Aβ42 in each compartment. The results indicate that intravitreal sNEP treatment reduces Aβ levels in multiple compartments of the mouse eye *in vivo*. The data encourage further investigation of the effect of sNEP-mediated Aβ degradation *in vivo* on eye tissue structure and function, with potential for therapeutic application.

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Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 214.26/C23

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Association Grant NIRG-12-242386

Title: Altered vascular morphology and tight junction proteins in presence of oligomeric amyloid-beta in the hippocampus

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[1]Neurosci., Mount Sinai Sch. of Med., New York, NY; [2]Neurosci., [3]Neurol. & Pediatrics, Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Vascular amyloid-beta deposits and cerebral vascular pathology are observed in Alzheimer's disease and in cerebral amyloid angiopathy. The amyloid-beta peptide can exist in soluble oligomeric or fibrillar forms. While oligomeric amyloid-beta is hypothesized to initiate neurotoxicity, it is unclear which of these amyloid-beta species is responsible for vascular changes in disease. We investigated whether soluble oligomeric amyloid-beta alone can trigger cerebral vascular pathology using an experimental model, the Thy-1/hAPP^{E693Q} mouse. These mice express the human amyloid precursor protein with the E693Q Dutch mutation, produce the soluble species of the peptide and do not have visible fibrillar deposits. To identify changes in vascular morphology in Thy-1/hAPP^{E693Q} mice as compared to wild-type (WT) controls, we used collagen IV immunohistochemistry and quantitative stereology. We also quantified the expression of tight junction proteins in the vascular endothelial cells using Western blot, followed by densitometry. Stereological analyses revealed no changes in vascular length density in the hippocampal CA1/2 and CA3 areas of Thy-1/hAPP^{E693Q} mice as compared to WT. However, analysis of vascular morphology revealed that Thy-1/hAPP^{E693Q} mice had more pathological vessels as compared to WT controls. Specifically, we observed increased number of string vessels, with a trend towards increased twisted vessels in the Thy-1/hAPP^{E693Q} mice. Ultrastructural analysis of vessels also revealed changes in the vessel diameters of Thy-1/hAPP^{E693Q} mice. We then investigated the expression of tight junction proteins in various brain regions and found altered expression of the tight junction protein zonula occludens-1 in the hippocampus of Thy-1/hAPP^{E693Q} mice as compared to controls. Taken together, our results indicate that oligomeric amyloid-beta alone, in the absence of fibrillar and amyloid plaque pathology, causes cerebral vascular pathology, reminiscent of those observed in patients with Alzheimer's disease and cerebral amyloid angiopathy.

Disclosures: **M. Varghese:** None. **T. Warda:** None. **A. Mandengue Sosso:** None. **A. Sowa:** None. **D. Dickstein:** None. **M. Ehrlich:** None. **D.L. Dickstein:** None.

Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 214.27/C24

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG046200

Title: Amyloid degradation and Alzheimer's disease

Authors: *K. SAMBAMURTI[1], R. J. BARANELLO[2], N. H. GREIG[3], J. PACHEKO-QUINTO[4], E. ECKMAN[4], D. K. LAHIRI[5], V. PADMARAJU[2];
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Abstract: Accumulation of the β-amyloid (Aβ) protein in the brain as senile plaques is a signature lesion of Alzheimer's disease (AD). The mechanism of Aβ accumulation that fosters its deposition is a subject of intense study and has been considered as the basis for most drug development efforts in the field. However, we still have a poor understanding of the degradation pathways that are responsible for maintaining Aβ homeostasis in normal people. We have previously determined that partial γ-secretase inhibition works through an Aβ degradation bypass mechanism to Aβ levels. In neuronal cells, intracellular (ic)Aβ40 levels are very low and nearly undetectable. Treatment with low doses (25 μM) of a γ-secretase inhibitor -DAPT - increases secreted (s)Aβ40 secreted in the medium substantially, but does not seem to affect icAβ40. High doses of DAPT that completely inhibits sAβ, increases intracellular Aβ40 significantly. Phosphoramidon (PA), an inhibitor of some metalloproteases such as endothelin-converting enzyme (ECE) substantially increase icAβ40 to about 1 ng/mg protein. Inhibiting major protease classes by treatment with E64 and Leupeptin to block serine proteases and cysteine proteases or pepstatin A to inhibit acid proteases did not increase icAβ40 or sAβ40 levels. Treatment with 4-(2-Aminoethyl) benzenesulfonyl fluoride (AEBSF) completely inhibits sAβ40 and icAβ40. However, it only reduces PA-stimulated Aβ40 by 40%. We have used extracts of a popular neuronal cell line - SH-SY5Y - to characterize the Aβ degrading pathways *in vitro*. The studies show that two pools of proteases that are active at acid and neutral pH rapidly degrade Aβ in these lysates. Inhibitor sensitivity profile suggests that metalloproteinase(s) are major contributors to the turnover process at neutral pH, but multiple redundant acid proteases appear to be responsible for its degradation in acid pH. A combination of protease inhibitors fails to substantially block the loss of Aβ at acid pH, suggesting that other uncharacterized activities play a key role under these conditions. The studies suggest that a large redundant process normally prevents amyloid accumulation and the process that overwhelms this safety net needs to be studied in detail to understand the mechanisms of amyloidosis in AD. We are currently examining the miRNA profiles for putative AB-degrading enzymes, including Neprilysin.

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Poster

214. Amyloid Precursor Protein Processing and Abeta Toxicity

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 214.28/C25

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Swedish research council

MultiPark

Title: Role of ESCRT dependent mutivesicular bodies in intracellular beta-amyloid accumulation and exosomal secretion in Alzheimer's disease

Authors: *K. WILLÉN, G. K. GOURAS;
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Abstract: Objectives Alzheimer's-linked beta-amyloid (Aβ) accumulates in endosomal vesicles near synapses with the onset of synaptic dysfunction in Alzheimer's disease (AD). Previous evidence suggested that sorting via the multivesicular body (MVB) pathway is impaired by endosomal Aβ accumulation in cultured AD-transgenic primary neurons. Our aim was to investigate the trafficking and processing of APP and its cleavage products in the endocytic pathway with a focus on the role of ESCRT (endosomal sorting complex required for transport)-mediated sorting. Methods Different steps in the endocytic pathway, including MVB formation, lysosome acidification, autophagy, and exocytosis, were modulated in neuroblastoma cells and primary mouse neurons. APP and its metabolites including Aβ , as well as synaptic proteins, were analysed using WB and immunofluorescence. Differences in the endocytic pathway between wt and AD-transgenic primary neurons (AD-neurons) and neurons treated with synthetic Aβ were also investigated. Results Inhibition of VPS4A, a key component in the ESCRT machinery and the only energy-consuming enzyme that promotes disassembly and recycling of ESCRT-III oligomers, leads to elevated intracellular levels and reduced secretion of Aβ in N2a cells and primary neurons. Inhibition of VPS4A also affects the release of exosomes. Dysfunctional MVBs increase markers of autophagy and chemical stimulation of autophagy partially rescues the dominant negative VPS4A induced rise of intracellular Aβ. Conclusions AD-neurons were shown to have impaired MVB sorting as well as progressive intraneuronal Aβ accumulation and reduced Aβ secretion with time in culture. We now show that inhibition of VPS4A, a critical component of ESCRTs involved in MVB formation, mimics these cellular changes in AD-neurons. Further, our results provide novel insights into the role of MVBs in autophagy and lysosomal degradation, both of which are altered in AD.

Disclosures: K. Willén: None. **G.K. Gouras:** None.

Poster

215. Alzheimer's Disease: Amyloid Precursor Protein Function and Processing

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 215.01/C26

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Function and regulation of APP and Alcadeinα cargoes transported by kinesin-1 in neuron

Authors: *Y. SHIRAKI, K. CHIBA, S. HATA, T. SUZUKI;
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Abstract: Kinesin-1 is the major anterograde motor to transport vesicles in axon. The kinesin-1 is a heterotetramer with two heavy chains (KHC) and two light chains (KLC). Various types of cargos and organelle can be associated with kinesin-1 through the binding to distinct adapter proteins or cargo receptor proteins. Alzheimer's β-amyloid precursor protein (APP) and Alcadeinα (Alcα)/Calsyntenin-1 are major cargo receptor in neuron (EMBO J. [2007] 26, 1475). KLC of kinesin-1 binds APP largely by mediation with JIP1b and Alcα directly (Traffic [2012] 13, 834; Mol. Biol, Cell [2014] 25, 3569). However, cargos transported by APP or Alcα are still controversial. In this study, we showed that APP and Alcα vesicles are largely independent for axonal transport using live cell imaging, and found that the respective cargos transport distinct types of components by proteome analysis. We previously reported that a complex formation of APP with Alcα mediated by X11L neural adaptor protein suppressed both APP and Alcα protein transport to cell surface and overexpression of Alcα intracellular domain fragment (Alcα ICD), which can be generated physiologically by γ-secretase cleavage of Alcα, released APP from the complex to increase APP vesicle transport (J. Biol. Chem. [2015] 290, 987). Taken together with this observation, present results propose a novel insight that APP and Alcα transport a distinct type of vesicular cargo, but the transport of both cargos is regulated ingeniously by an associated mechanism.

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Poster

215. Alzheimer's Disease: Amyloid Precursor Protein Function and Processing

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 215.02/C27

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: DePaul-Rosalind Franklin Pilot Grant

NIH Grant OD010662

Title: Elucidation of the amyloid precursor protein intracellular domain interactome

Authors: *E. M. NORSTROM[1], M. KASPARIAN[1], A. MILLER[1], K. PHILIBERT[2], X. SHAO[2], R. MARR[3], M. GLUCKSMAN[2];
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Abstract: Alzheimer's disease is pathologically characterized by the accumulation of beta-Amyloid (Aβ) plaques in brain, the result of metabolic processing of the Amyloid Precursor Protein (APP). While a definitive function for APP in healthy brains has remained elusive, the series of metabolic events leading to Aβ pathology has been well characterized. APP is a type I integral membrane protein, metabolized by alternative pathways that release either the disease-associated beta-amyloid peptide or the non-pathological p3 peptide. Both pathways ultimately result in the release of a C-terminal fragment into the cytosol - the APP Intracellular Domain (AICD). AICD has been implicated in nuclear signaling, particularly in the regulation of gene expression related to APP processing. This theorized pattern of self-regulation by an intracellular fragment is seen in other metabolic pathways, and AICD protein interactions could be determining factors of the metabolic fate (amyloidogenic or non-) of APP. In this way, AICD and its network of interacting proteins have become an inroad to study, and hopefully translationally influence, APP. The biological role of AICD, if any, remains controversial due to its short half-life and modest effects in *in vitro* gene expression studies. Thus, there is a need to discover potential AICD interacting proteins and to clarify potential functions for this metabolic fragment. Towards this goal, identification of the AICD interactome by affinity purification of a tagged AICD followed by mass spectrometry (MS) and bioinformatic analysis was performed. Affinity tagged AICD fragment were stably expressed in cultured cells. Affinity purification was performed under native conditions on lysates cleared of nuclei to yield extranuclear interaction partners of AICD. We performed both a complete interactome screen as well as targeted proteomics to confirm interactors of interest. Among the targets identified are proteins implicated in nuclear signaling, APP regulation, processing enzymes, and modulation of AICD levels. The post discovery phase will include co-localization studies, transcriptional analysis and *in vivo* analysis with vetted animal models to better comprehend the role of AICD's role in Pathogenesis of Alzheimer's Disease. Supported by DePaul-RFUMS Pilot Grant Program (RAM, MJG, EMN) and NIH OD010662 (MJG)

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Poster

215. Alzheimer's Disease: Amyloid Precursor Protein Function and Processing

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 215.03/C28

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R36AG048247

Title: Translational control of the amyloid precursor protein by a guanine quadruplex

Authors: *E. M. CRENSHAW[1], B. LEUNG[2], C. K. KWOK[3], P. C. BEVILACQUA[4], M. R. AKINS[1], A. J. SAUNDERS[1];
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Abstract: The single stranded nature of RNA enables this molecule to adopt different structural conformations. Guanine rich RNAs can fold into a guanine quadruplex structure through Hoogsteen hydrogen bonding. Guanine quadruplexes are further stabilized by monovalent cations such as potassium or sodium. The involvement of guanine quadruplexes in RNA has shown to be of importance because it can affect translation for a given protein. Recently, we have identified a guanine quadruplex in the 3 prime untranslated region (3' UTR) of the Amyloid Precursor Protein (APP). APP plays a major role in Alzheimer's disease. Neuropathologically, Alzheimer's disease is defined by the accumulation of the β-amyloid (Aβ) peptide. Aβ is produced by the sequential, proteolytic cleavage of the amyloid precursor protein by the β- and γ-secretases. While most Alzheimer's disease cases occur after the age of 65 years (late-onset), there are a small number of cases that occur before the age of 60 years (early-onset). Alzheimer's disease can be caused by genetic changes that result in increased production of the Amyloid Precursor Protein. The increase in APP expression can lead to increased Aβ production and accumulation as seen in Individuals with Down's syndrome (Trisomy 21) who invariably develop AD. This information strongly suggest that dysregulation of APP expression could play a role in the pathogenesis of AD. Therefore it is imperative to identify mechanisms underlying the regulation of APP expression. Given that guianine quadruplexes within the 3' UTR of mRNAs have been shown to play a role in repressing translation, we decided to test what effects this secondary structure would have on regulating the Amyloid Precursor Protein. We first confirmed the presence of the 3 prime untranslated region guanine quadruplex within the APP mRNA by using Circular Dichroism in which we compared the wild type and mutant guanine quadruplex sequence. To address the involvement of the APP 3'UTR guanine quadruplex on translation, we used luciferase reporter constructs and APP reporter constructs to detect changes in mRNA levels (for transcription) or protein levels (for translation). Our results demonstrate that the guanine quadruplex in the 3'UTR of APP negatively regulates APP translation. Moreover, we show that disrupting the guanine quadruplex lead to higher APP protein levels as well as an increase in Aβ levels.

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Poster

215. Alzheimer's Disease: Amyloid Precursor Protein Function and Processing

Location: Hall A

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Program#/Poster#: 215.04/C29

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01NS057295

Drexel University

Commonwealth of Pennsylvania

Title: Cathepsin L mediates the degradation of novel APP fragments

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Abstract: Alzheimer's disease (AD) is characterized by the deposition of amyloid β (Aβ), a peptide generated from proteolytic processing of its precursor, amyloid precursor protein (APP). Canonical APP proteolysis occurs via α-/ β- and γ-secretases. APP is also actively degraded by protein degradation systems. By pharmacologically inhibiting protein degradation with ALLN, we observed an accumulation of several novel APP C-terminal fragments (CTFs). The two major novel CTFs migrated around 15 and 25 kD, and can be observed across multiple cell types. The process was independent of cytotoxicity or protein synthesis. We further determine that the accumulation of the novel CTFs is not mediated by proteasome or calpain inhibition, but by cathepsin L inhibition. Moreover, these novel CTFs are not generated by increased amount of BACE. Here, we name the CTF of 25 kD as η-CTF (eta-CTF). Our data suggests that under physiological conditions, a subset of APP undergoes alternative processing and the intermediate products, the 15 kD- and the η-CTFs get rapidly degraded/ processed via the protein degradation machinery, specifically, cathepsin L.

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Poster

215. Alzheimer's Disease: Amyloid Precursor Protein Function and Processing

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 215.05/C30

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The Shoemaker Award for Neurodegenerative Research

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Title: Modeling CD74 directed APP trafficking for Alzheimer's disease

Authors: *T. KIYOTA[1], C. M. MORRISON[1], Y. LU[1], W. DONG[1,3], B. DYAVARSHETTY[1], H. E. GENDELMAN[1,2];
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[3]Integrated Traditional Chinese and Western Med., Fujian Univ. of Traditional Chinese Med., Fujian, China

Abstract: Control of amyloid-β (Aβ) precursor protein (APP)/Aβ trafficking represents an exciting therapeutic directive in harnessing β-amyloidosis. If successful it could positively affect the tempo of Alzheimer's disease (AD). The current work is based on our prior observations that recombinant adeno-associated virus (AAV)-mediated gene delivery of CD74, the major histocompatibility complex class II-associated invariant chain, reduces Aβ production in the mouse hippocampus and improves learning performance when measured by Morris water maze tasks. To elucidate how CD74 effects APP/Aβ modification, we investigated its trafficking pathways. Stereotaxic injection of the AAV-tet-response element (TRE)-green fluorescent protein (GFP) or CD74 into the hippocampi of AD mice [TgCRND8 strain x CamKII (calmodulin-dependent protein kinase II derived promoter)-tet-controlled transactivator (tTA, binding to TRE to regulate gene expression) double-transgenic mice] demonstrated hippocampal expression of GFP and CD74. Neuronal production of CD74 reduced intraneuronal Aβ accumulation when compared to GFP-controls. Immunoprecipitation tests demonstrated that CD74 binds to neuronal APP in neural progenitor cell (NPC)-derived neurons co-infected with adenovirus expressing human APP Swedish mutant (APPsw) and AAV-CD74. Endolysosomal trafficking of APP was shown from NPC-derived neurons co-infected with AAV-tTA, AAV-TRE-APPsw and AAV-GFP or CD74. Here immunofluorescence studies for Rab5 and 7 and Lamp1 showed that APPsw was co-localized with Rab5 and Lamp1, but not Rab7 in CD74 expressing neurons. These results support the idea that CD74 reduces Aβ production by endolysosomal trafficking and promotes APP degradation. This previously undisclosed pathway opens a new therapeutic window for AD.

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Poster

215. Alzheimer's Disease: Amyloid Precursor Protein Function and Processing

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH 1R01AG042819

Title: A novel role for amyloid precursor protein in the endocrine pancreas

Authors: *J. KULAS, K. L. PUIG, C. K. COMBS;
Basic Sci., Univ. of North Dakota, Grand Forks, ND

Abstract: Amyloid Precursor Protein (APP) has been widely studied due to its role in the formation of Aβ plaques in Alzheimer's disease (AD) patients. Despite rigorous examination the endogenous function of this protein has remained elusive. Epidemiological data has indicated a link between AD and type II diabetes, with recent evidence of insulin resistance occurring in the hippocampi of AD patients. Our lab has focused on documenting the expression and function of APP in peripheral tissues. We have observed APP expression within human and murine pancreatic Islets of Langerhans, the site of peripheral insulin production, with abundant overexpression in the pancreatic islets of APP/PS1 transgenic mice. In order to gain a mechanistic understanding of APP within this tissue, we have compared pancreatic function and protein expression across a variety of transgenic mice, including C57BL/6 wild type, APP -/- and APP/PS1 animals. Our data suggests a novel role for APP in the regulation of both BACE2 and Insulin Degrading Enzyme (IDE) expression as revealed by western blotting. Additionally, our assessments of isolated murine pancreatic islets indicate APP as a regulator of both islet glucose uptake and insulin secretion. Together, these data suggest a previously unknown function for APP within the endocrine pancreas.

Disclosures: J. Kulas: None. **K.L. Puig:** None. **C.K. Combs:** None.

Poster

215. Alzheimer's Disease: Amyloid Precursor Protein Function and Processing

Location: Hall A

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Health and Medical Research Fund

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CUHK direct grant scheme

Title: The role of FE65 phosphorylation in regulating Alzheimer's disease amyloid precursor protein processing

Authors: *K.-F. LAU, W. LI, J. C. NGO, H. E. CHAN, W. V. CHOW;
The Chinese Univ. of Hong Kong, Shatin, NT, Hong Kong

Abstract: Alzheimer's disease (AD) is a fatal neurodegenerative disease affecting 36 million people worldwide. Genetic and biochemical research indicated that the excessive generation of amyloid-β peptide (Aβ) from Amyloid Precursor Protein (APP), is essential to AD pathology. A number of APP-interacting proteins have been implicated in modulation of APP processing, including FE65. FE65 is a neuronal adaptor protein with multiple protein-protein interaction domains. It has been shown that FE65 interacts with APP to alter APP processing and Aβ generation. Evidence indicates that FE65 is a phospho-protein with a number of phosphorylation sites. However, the role of FE65 phosphorylation in APP metabolism is still not fully understood. In our work, we found a number of phosphorylated residues in FE65 that could modulate APP processing. Moreover, the kinases for the sites were identified. We therefore provide evidence that FE65 phosphorylation plays important role in the regulation of FE65-mediated APP processing.

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Poster

215. Alzheimer's Disease: Amyloid Precursor Protein Function and Processing

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant OD008761

AFAR Joint Grant on Alzheimer's Disease

Title: Homeostatic coupling between surface trafficking and cleavage of amyloid precursor protein

Authors: *C. DELBOVE[1], C. E. STROTHMAN[2], K. E. KITKO[2], R. M. LAZARENKO[1], Q. ZHANG[1];
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Abstract: Amyloid precursor protein (APP) is a ubiquitously expressed neuronal membrane protein with multiple routes of proteinase processing involving different secretases in different lipid membrane compartments. Among those routes, only intracellular cleavages of APP by β- and γ-secretases contribute to the production of amyloid beta peptides (Aβ). Due to its pathological association with Alzheimer's disease (AD), the expression, trafficking, and processing of APP have drawn tremendous attention and effort in the field of neurodegeneration.

Recent studies have revealed that APP trafficking and processing are influenced by synaptic activity. Despite tremendous interest and research effort, a few major questions about APP remain unanswered: (1) the distribution of APP in different lipid compartments at neuronal synapses, (2) the role of synaptic transmission in APP trafficking between the surface membrane and intracellular compartments, and (3) the regulation of APP allocation to different cleavage pathways. These issues are not only important for the understanding of Aβ generation but also precede any therapeutic strategies targeting APP cleavage. To directly address these questions in live neurons, we devised a multicolor fluorescent probe as a ratiometric reporter for APP trafficking and processing. Using immunocytochemistry and quantitative measurements, we confirmed that this probe is a faithful proxy for endogenous APP. In combination with an optically separable reporter for synaptic activity, we evaluated the activity-driven APP shuttling between surface and internal membrane compartments. Pharmacological inhibition of selected secretases revealed an unexpected homeostatic coupling between different APP cleavage processes and APP trafficking. Homeostatic mechanisms maintain a constant level of APP on the synaptic surface and α- and/or β-secretase inhibitors cause an intracellular APP accumulation. Unexpectedly, this homeostasis heavily depends on γ-secretase activity. As those secretases become major drug targets for AD treatment, tools to quantify APP allocation, trafficking, and cleavage in live neurons are indispensible. More importantly, a mechanistic understanding of APP distribution and trafficking among different subcellular locations, between major secretase pathways and under the influence of neuronal activity is essential for designing effective therapeutic strategies to prevent Aβ accumulation and neurodegeneration.

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Poster

215. Alzheimer's Disease: Amyloid Precursor Protein Function and Processing

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Arkansas Biosciences Institute

NIGMS IDeA award P30GM110702

Title: Amyloid precursor protein affects energy utilization parameters manifest at the cellular and organismal levels

Authors: *R. D. HENDRIX[1], A. K. ODLE[1], S. ROSE[2], R. E. FRYE[2], G. V. CHILDS[1], S. W. BARGER[3,4];
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Abstract: Diverse and extensive data sets indicate disturbances in energy metabolism in Alzheimer's disease (AD), including mitochondrial deficits and a comorbidity with type-2 diabetes. The amyloid precursor protein (APP) is genetically and biochemically connected to AD, and we have begun to assess its role in glucose regulation and other aspects of energy utilization through the analysis of APP-knockout mice. We examined glucose maintenance via glucose- and insulin-tolerance tests, and we explored other aspects of metabolism through the use of a Comprehensive Laboratory Animal Monitoring System (CLAMS). We also assessed cell-autonomous respiration via Seahorse assays of cultured astrocytes. Wild-type mice fed a western (high-fat, high-sucrose) diet developed an impaired ability to respond to glucose and insulin challenges, and they exhibited hyperinsulinemia; their APP-KO littermates were resistant to these effects. These results appear to involve both reduced food consumption and elevated metabolism in the APP-KO mice. Moreover, respiratory changes were apparent at the cellular level, as APP-KO astrocytes showed elevated oxygen consumption rates. These findings indicate fundamental roles for APP in cellular metabolism that are translated to global physiological effects. This illuminates yet another caveat for disease models that involve overexpression of APP.

Disclosures: R.D. Hendrix: None. **A.K. Odle:** None. **S. Rose:** None. **R.E. Frye:** None. **G.V. Childs:** None. **S.W. Barger:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SWB receives royalties from Sigma-Aldrich Chem. Co. related to the sales of secreted APP.

Poster

215. Alzheimer's Disease: Amyloid Precursor Protein Function and Processing

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 215.10/C35

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSERC-RGPIN-2015-04645

Pfizer-FRQS 31288

Title: Alternative processing of APP through the rhomboid protease RHBDL4

Authors: *S. PASCHKOWSKY[1], M. HAMZÉ[1], F. OESTEREICH[2], B. MICHALSKI[3], M. FAHNESTOCK[3], L. M. MUNTER[1];
[1]Pharmacol., [2]Integrated Program in Neurosci., McGill Univ., Montreal, QC, Canada; [3]McMaster Univ., Hamilton, ON, Canada

Abstract: Alzheimer's disease (AD) is the most common neurodegenerative disease, with 44 million affected patients in 2014 and numbers increasing. So far, no FDA-approved treatment is able to halt or prevent disease progression. With molecular changes occurring as early as 25 years prior to manifestation of clinical symptoms, preventive treatment strategies seem very promising. One major hallmark of AD pathology is the toxic amyloid-beta (Aβ) peptide, which is generated from the larger amyloid precursor protein (APP) in two sequential cleavage events. Reducing Aβ production is one of the main goals of AD therapeutics. Rhomboid proteases are a highly conserved class of intramembrane serine proteases, which cleave their substrates within the transmembrane and ectodomain regions. The human rhomboid-related protein 4 (RHBDL4) resides in the endoplasmic reticulum (ER) and is involved in essential cell processes such as ER-associated degradation (ERAD) of proteins and apoptosis. We found that RHBDL4 efficiently cleaves APP when expressed in HEK 293T cells. Cleavage of APP by RHBDL4 occurs in the ectodomain, leading to the generation of stable, 75 kDa N-terminal fragments and multiple 15-25 kDa C-terminal fragments, as determined by Western blot analysis. All fragments were detected in the cell lysate, while the N-terminal fragment was additionally observed in the supernatant. Co-immunoprecipitation of APP and RHBDL4 indicated an interaction between both proteins. Furthermore, an inactive form of RHBDL4 caused accumulation of the immature, ER-residing form of APP, but not mature APP in cell lysates. In addition, inhibition of other known APP-processing enzymes such α-,β- and γ-secretase did not affect production of RHBDL4-specific APP fragments, indicating that fragment generation is independent of other proteases. Most importantly, RHBDL4-mediated degradation of APP led to a drastic reduction in Aβ peptide levels, presumably by preventing APP from reaching the cell surface. Finally, analysis of human brain tissue from AD cases and age-matched controls revealed an increase in RHBDL4 mRNA and protein. This may be a protective, compensatory response to accumulating Aβ. Thus, our results suggest a new metabolic pathway for APP, which removes it from amyloidogenic processing and therefore reduces Aβ levels. We conclude that RHBDL4-mediated APP processing is a new potential mechanism to reduce Aβ peptide levels that could be exploited as novel drug target for AD.

Disclosures: S. Paschkowsky: None. **M. Hamzé:** None. **F. Oestereich:** None. **B. Michalski:** None. **M. Fahnestock:** None. **L.M. Munter:** None.

Poster

215. Alzheimer's Disease: Amyloid Precursor Protein Function and Processing

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 215.11/C36

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Belgian F.N.R.S

S.A.O./F.R.A. Foundation for Research on Alzheimer's disease

Interuniversity Attraction Pole Programme-Belgian Sate-Belgian Science Policy (IAP-P7/16 and IAP-P7/13

Title: APP regulates the Glial cell line-Derived Neurotrophic Factor (GDNF) gene expression driving functional neuromuscular junctions formation

Authors: *S. STANGA[1,2], N. ZANOU[3,2], E. AUDOUARD[3,2], B. TASIAUX[3,2], S. CONTINO[3,2], F. CLOTMAN[3,2], P. GAILLY[3,2], I. DEWACHTER[3,2], J.-N. OCTAVE[3,2], P. KIENLEN-CAMPARD[3,2];
[1]Univ. Catholique De Louvain (UCL), Bruxelles, Belgium; [2]Inst. of Neurosci. (IoNS), Bruxelles, Belgium; [3]Univ. catholique de Louvain (UCL), Bruxelles, Belgium

Abstract: Objectives: Beside its crucial role in Alzheimer's disease (AD) pathogenesis, different functions have been attributed to the amyloid precursor protein (APP) but its pivotal role is difficult to depict. Here, we show that APP regulates the transcription of the Glial cell line-Derived Neurotrophic Factor (GDNF). Our work aims at investigating the role of APP-dependent GDNF expression in neuromuscular junctions. Methods: Following transcriptome analysis, qRT-PCR, ELISA and reporter gene assays were performed on APP knock-out mouse embryonic fibroblasts (APP -/- MEFs) stably re-expressing APP695 and APP751 isoforms. APP and GDNF expression have been monitored throughout skeletal muscle differentiation (C2C12 cells) upon APP silencing (small interfering RNA) and/or GDNF plasmid expression. We performed grip strength tests, mechanic measurements on isolated muscles and immunohistochemistry (IHC) of neuromuscular junctions (NMJs) on APP -/- mice. We set up a nerve-muscle co-culture model to generate NMJs *in vitro* and to analyze the effects of APP silencing or GDNF expression on NMJs formation and neuronal maturation by immunocytochemistry (ICC). Results: GDNF mRNA and protein levels together with GDNF transcriptional activity are down-regulated in APP -/- MEFs and restored specifically by APP751 isoform. GDNF and APP levels increase during muscular differentiation. Their overexpression or silencing favors or affects the process, respectively. APP-dependent regulation of GDNF expression affects muscles strength, muscular trophy and both neuronal and muscular differentiation fundamental for neuromuscular junctions (NMJs) maturation *in vivo*. Silencing of muscular APP induces a significant decrease in secreted GDNF levels, in the total number of NMJs, in the density of acetylcholine vesicles at the presynaptic site and in neuronal maturation. These defects are rescued by GDNF expression in muscle cells. Conclusions: Our findings highlight for the first time that APP-dependent GDNF expression drive the process of neuronal and muscular differentiation contributing to a better understanding of APP gene regulatory network and physiological functions.

Disclosures: **S. Stanga:** None. **N. Zanou:** None. **E. Audouard:** None. **B. Tasiaux:** None. **S. Contino:** None. **F. Clotman:** None. **P. Gailly:** None. **I. Dewachter:** None. **J. Octave:** None. **P. Kienlen-Campard:** None.

Poster

215. Alzheimer's Disease: Amyloid Precursor Protein Function and Processing

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 215.12/C37

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Swedish Alzheimer foundation

The Stohne foundation

The foundation Lars Hiertas Minne

Foundation for Gamla Tjänarinnor

Title: Changes in APP processing in response to inhibition of endocytosis

Authors: ***L. T. AGHOLME**[1], T. SATIR[1], P. BERGSTRÖM[1], E. PORTELIUS[1], K. BLENNOW[1], H. ZETTERBERG[1,2];
[1]Neurosci. and Physiol., Univ. of Gothenburg, Gothenburg, Sweden; [2]Dept. of Mol. Neurosci., Inst. of Neurology, Univ. Col. London, London, United Kingdom

Abstract: Generation of β-amyloid (Aβ), by cleavage of APP in endosomes has been postulated as a central event in AD pathogenesis. Both β- and γ-secretase have been localized to endosomes, and the acidic environment favors β-secretase activity. On the other hand, cleavage of APP by α-secretase is thought to take place at the plasma membrane. Accumulation of enlarged endosomes is one pathological finding in Alzheimer's disease (AD), indicating that disturbances in the endocytic pathway could contribute to disease progression in AD. We have earlier identified a novel pathway of APP cleavage, generating short Aβ fragments by β- followed by α-secretase cleavage. However, the localization of this type of cleavage is still unknown. The aim of this study was to investigate how inhibition of endocytosis (in general and of recycling endosomes) affects APP cleavage and generation of different Aβ peptides in a human, iPSc derived, cortical cell model. Human IPS cells were differentiated into cortical neurons [1]. Endocytosis was inhibited using dynasore, as well as siRNA knockdown of specific proteins in the endocytosis machinery. Transferrin uptake was analyzed to confirm inhibition of dynamin dependent endocytosis. After 24 hours of endocytosis inhibition, cell culture media and cells were collected. Cell culture media was analyzed for Aβ 38/40/42 and sAPPα/β using the Meso Scale Discovery system. Aβ peptides were analyzed using immunoprecipitation followed by mass spectrometry (IP-MS). IP-MS analysis of Aβ fragments ranging from 14 to 42 amino

acids (aa) showed a change in the ratio of secreted peptides when cells were treated with dynasore. Shorter fragments (ending at aa 14 to 20), were generally increased whereas longer fragments (ending at aa 34 to 40) tended to be decreased upon inhibition of endocytosis. Cleavage pattern, secretion and intracellular accumulation of Aβ were also analyzed upon Rab11B siRNA treatment, successfully inhibiting expression of Rab11B protein. The cleavage patterns of Aβ peptides, analyzed with IP-MS, seem to be affected by endocytosis inhibition. These results indicate two different cellular localizations for APP cleavage into Aβ, and this could have implications for the understanding on APP processing in healthy neurons, as well as in AD. 1. Shi, Y., P. Kirwan, and F.J. Livesey, Directed differentiation of human pluripotent stem cells to cerebral cortex neurons and neural networks. Nat. Protoc., 2012. 7(10): p. 1836-46.

Disclosures: L.T. Agholme: None. **T. Satir:** None. **P. Bergström:** None. **E. Portelius:** None. **K. Blennow:** None. **H. Zetterberg:** None.

Poster

215. Alzheimer's Disease: Amyloid Precursor Protein Function and Processing

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 215.13/C38

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Memory deficits in rats using a model of Alzheimer’s disease with an overexpression of APP21 and the presenilin 1 transgene

Authors: D. KLAKOTSKAIA[1], R. A. RICHARDSON[1], T. A. LARSON[1], E. WOODALL[1], K. PATEL[1], M. K. BURRUS[1], K. M. CLARK[1], C. AGCA[2], *T. SCHACHTMAN[3], Y. AGCA[2]; [1]Psychological Sci., [2]Vet. Pathology, [3]Univ. of Missouri, Columbia, MO

Abstract: The accumulation of amyloid plaques in the brain due to aggregate forms of amyloid beta (Aβ) peptides is known to lead to Alzheimer’s disease. Previous studies have found that the cleavage of beta amyloid precursor protein (APP) is the mechanism by which Aβ peptides are released. In this study, spatial memory performance in the Barnes maze was assessed in two strains of transgenic rats that overexpress human beta amyloid precursor protein (APP) in comparison to Fischer controls. All animals received eight 5-min acquisition trials over the course of four days, after which 3-day retention was evaluated, and subsequently followed by three days of reversal training trials. The number of nose-poke errors made and latency to enter the target hole were recorded. No significant group or sex differences were found during acquisition or reversal training, but there were significant group differences during the retention interval test. It was found that APP21 rats with an additional presenilin 1 transgene (APP21-PS1) made significantly more errors than both singly transgenic APP21 rats and control rats. Doubly transgenic (APP21-PS1) rats also took longer to enter the target hole than control rats, as

measured by their significantly longer latency scores. These results suggest a larger spatial memory deficit for APP21-PS1 rats than the singly transgenic APP21 rats.

Disclosures: **D. Klakotskaia:** None. **R.A. Richardson:** None. **T.A. Larson:** None. **E. Woodall:** None. **K. Patel:** None. **M.K. Burrus:** None. **K.M. Clark:** None. **C. Agca:** None. **T. Schachtman:** None. **Y. Agca:** None.

Poster

215. Alzheimer's Disease: Amyloid Precursor Protein Function and Processing

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 215.14/C39

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH 5RO1AG042819-03

Title: Characterization of amyloid precursor protein-dependent brain changes in a murine model of high fat diet-dependent obesity

Authors: ***K. L. PUIG**, S. A. BROSE, M. Y. GOLOVKO, C. K. COMBS;
Basic Sci., Univ. of North Dakota, Grand Forks, ND

Abstract: Mid-life obesity has been implicated as a significant risk factor for AD. This suggests that the relationship between AD and obesity may be more than correlative and involve a common pathophysiology. It is well known that mutations in the gene coding for amyloid precursor protein, APP, are responsible for autosomal dominant forms of AD and our prior work has demonstrated a role for APP in regulating monocyte/microglial activation. Therefore, we tested whether APP is involved in inflammatory changes that occur in brain tissue during high fat diet-induced obesity. Six week old male wild type (C57BL/6) and $APP^{-/-}$ mice were maintained on either a control or high fat diet for 22 weeks. As we and others have reported, neuronal APP and reactive microglia immunoreactivity did appear to have slight increases in the temporal cortex region of high fat diet fed wild type mice. $APP^{-/-}$ brains exhibited reduced microglial immunoreactivity on a control diet but noticeably increased staining in high fat diet fed mouse brains. High fat diet fed brains had significantly increased protein levels of phosphorylated tau, CFABP, and APOE in wild type but not $APP^{-/-}$ cortex. $APP^{-/-}$ mice on high fat diet also showed reduced CD36, TLR2, TLR4, LRP, CD68, Iba-1, Cox-2, GFAP, CFABP, and APOE protein levels compared to wild type mice on high fat diet. On either diet, $APP^{-/-}$ mice had significantly less PGE_2, PGD_2, thromboxane B_2 and 6-*keto*$PGF_{1\alpha}$ compared to their diet-matched wild type mice. $APP^{-/-}$ neurons demonstrated attenuated 16:0 uptake ability whereas $APP^{-/-}$ astrocytes demonstrated attenuated 16:0, 20:4n6, and 22:6n3 uptake ability. A cytokine array analysis of temporal cortex lysate revealed a significant increase in eotaxin-2,fractalkine, IL-2, IL-13, IL-17, I-TAC, KC, MIP-1γ, RANTES, and TCA-3 in $APP^{-/-}$ mouse brains. This data

demonstrated a fundamental role for APP in regulating brain immune and lipid-metabolism associated events particularly during high fat diet feeding.

Disclosures: **K.L. Puig:** None. **S.A. Brose:** None. **M.Y. Golovko:** None. **C.K. Combs:** None.

Poster

215. Alzheimer's Disease: Amyloid Precursor Protein Function and Processing

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

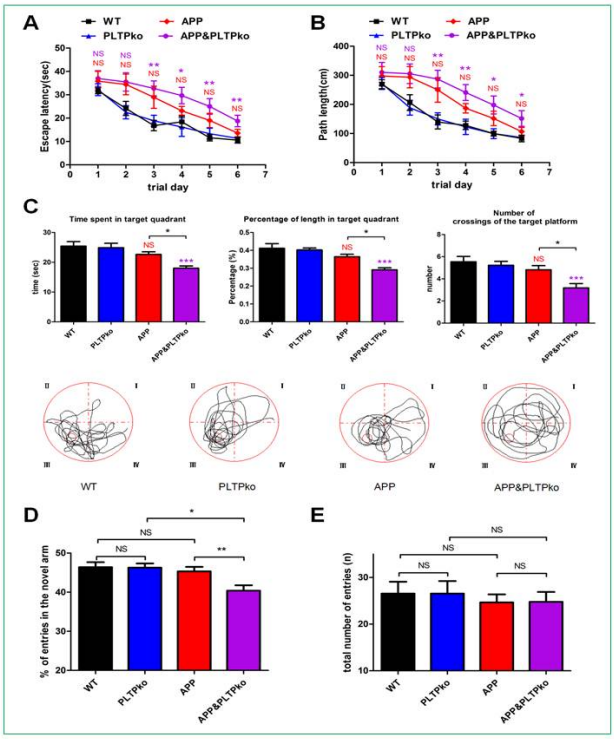
Program#/Poster#: 215.15/C40

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Phospholipid transfer protein (PLTP) deficiency accelerates memory dysfunction through altering amyloid precursor protein (APP) processing in a mouse model of Alzheimer’s disease

Authors: *D. CHUI;
Peking Univ., Beijing, China

Abstract: Disordered lipid metabolism is increasingly recognized in the pathogenesis of several neurodegenerative diseases, such as Alzheimer’s disease (AD). As a widely expressed lipid transfer protein, phospholipid transfer protein (PLTP) participates in the transport of cholesterol and other lipids in the plasma and peripheral tissues. Recently, elevated amyloid β (Aβ) in young and aged PLTP-deficient brains had been reported. However, the role of PLTP in amyloid precursor protein (APP) processing and AD pathology remains elusive. Here we first found that deficiency of PLTP accelerated memory dysfunction in APP/PS1ΔE9 AD model mice at the age of 3 months. Further characterization showed that PLTP deficiency increased soluble Aβ peptides, and intracellular accumulation of Aβ was illustrated, which might be due to disrupted APP turnover and the enhanced amyloidogenic pathway. Besides, reduced brain-derived neurotrophic factor (BDNF) was found in PLTP deficient APP/PS1ΔE9 mice, and BDNF level was negatively correlated with Aβ42 content, instead of Aβ40 content. In addition, autophagic dysfunction was found in the PLTP deficient APP/PS1ΔE9 mice. Our data suggested that PLTP played an important role in APP processing and PLTP deficiency could increase susceptibility to early onset of AD. ACKNOWLEDGMENT This work was supported by the 973 Program, No.2012CB911004; Grant No. 61450004, 61471005, 81241040 and 81171015 Correspondence to: Prof. Dehua Chui, Neuroscience Research Institute & Peking University Third Hospital. E-mail: dchui@bjmu.edu.cn



PLTP deficiency accelerated memory dysfunction in APP/PS1ΔE9 mice but did not affect wild type mice at the age of 3 months.

Disclosures: **D. Chui:** A. Employment/Salary (full or part-time):; Neuroscience Research Institute, HSC, Peking University, Beijing, China.

Poster

215. Alzheimer's Disease: Amyloid Precursor Protein Function and Processing

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 215.16/C41

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NSF GRFP DGE-1143954

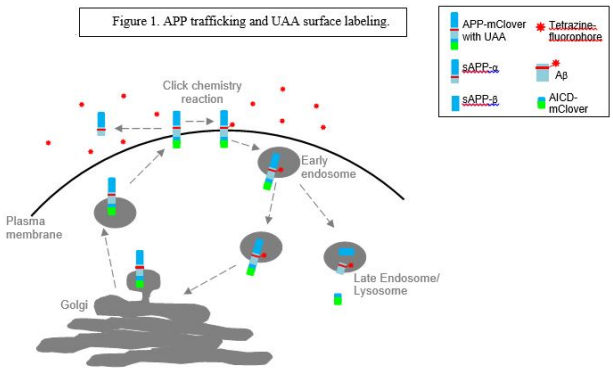
NIH Grant R21NS083529

Title: Using an unnatural amino acid to fluorescently label amyloid beta generated de novo from amyloid precursor protein expressed within cells

Authors: *L. R. CZERNIEWSKI, S. L. CRICK, J.-M. LEE;
Washington Univ. In St. Louis, Saint Louis, MO

Abstract: Amyloid beta (Aβ) plays a fundamental role in Alzheimer's disease pathogenesis, but its production, trafficking, degradation, and eventual aggregation into extracellular plaques is poorly understood. A key reason for our inability to study these processes in cells and *in vivo* is that there is no method for specifically labeling and subsequently monitoring Aβ, endogenously produced from its amyloid precursor protein (APP). Current trafficking studies utilize APP fused with fluorescent proteins at either terminus to study its intracellular trafficking. In this work, we will present a method for labeling the Aβ segment within APP, so that we can track *de novo* Aβ production within a cell using an unnatural amino acid (UAA). We generated an APP construct mutated at a single codon within Aβ to allow for incorporation of an UAA. The seventh amino acid in Aβ was mutated to a stop codon to prevent translation in the absence of an UAA. In the presence of an UAA and its cognate tRNA/tRNA-synthetase pair the UAA is inserted at the stop codon in Aβ outside of the transmembrane domain. This allowed us to use two alternative UAA variants to label Aβ: 1) an intrinsically fluorescent UAA could be used to label Aβ within APP from the start of translation; and 2) a different UAA harboring a strained dienophile could also be incorporated—with a subsequent click chemistry reaction at the cell surface linking fluorescently tagged tetrazine molecules to the UAA incorporated in APP as it is trafficked to the plasma membrane (figure 1). APP was also fused at its C-terminus to the mClover fluorescent protein in order to visualize cleavage events. The incorporation of an unnatural amino acid allows for direct visualization of Aβ through use of an intrinsically fluorescent unnatural amino acid or an unnatural amino amenable to click-chemistry labeling. This strategy of unnatural amino acid incorporation can be used to site-specifically label endogenous Aβ within the amyloid precursor protein.

Figure 1. APP trafficking and UAA surface labeling.



Disclosures: **L.R. Czerniewski:** None. **S.L. Crick:** None. **J. Lee:** None.

Poster

215. Alzheimer's Disease: Amyloid Precursor Protein Function and Processing

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 215.17/C42

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Society of Alberta and the Northwest Territories (ASANT)

Queen Elizabeth II Graduate Scholarship

Title: The effect of oligomeric aβ42 on app processing and aβ40 generation in cultured u373 astrocytes

Authors: *D. I. OURDEV[1], B. V. FOROUTANPAY[2], Y. WANG[2], S. KAR[3];
[2]Psychiatry, [3]Med. and Psychiatry, [1]Univ. of Alberta, Edmonton, AB, Canada

Abstract: Background: Amyloid-β (Aβ) peptides are a family of proteins that are considered to be a principal aspect of Alzheimer's disease (AD), the most common cause of senile dementia affecting elderly. These peptides result from the proteolytic processing of Amyloid precursor protein (APP) by the sequential cleavage mediated via β- and γ-secretases. Evidence suggests that an overproduction and/or a lack of degradation may increase brain Aβ levels which, in turn, contribute to neuronal loss and development of AD. **Objectives:** In this study, we seek to determine what effect Aβ has on APP processing in cultured astrocytes. **Methods:** Using human astrocytoma cell line U373, we investigated the effects induced by $A\beta_{42}$ treatment on the cellular expression of APP and its products, αCTF, βCTF, and $A\beta_{40}$. In conjunction with these experiments, we examined the relative levels and activity of β- and γ-secretases in Aβ-treated astrocytes. **Results:** We report here that $A\beta_{42}$ treatment of astrocytes increased the expression of APP and its cleaved products including $A\beta_{1\text{-}40}$ in a time-dependent manner. **Conclusions:** These results suggest that astrocytes following activation can contribute to the development of AD by enhancing levels and processing of APP leading to increased production/secretion of Aβ-related peptides.

Disclosures: D.I. Ourdev: None. **B.V. Foroutanpay:** None. **Y. Wang:** None. **S. Kar:** None.

Poster

215. Alzheimer's Disease: Amyloid Precursor Protein Function and Processing

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 215.18/C43

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR

Title: Effects of Aβ1-42 on APP processing in cultured N2a cells

Authors: *B. V. FOROUTANPAY[1], T. J. REVETT[2], D. VERGOTE[2], D. WESTAWAY[2], S. KAR[1];
[1]Psychiatry, [2]Ctr. for Prions and Protein Folding Dis., Univ. of Alberta, Edmonton, AB, Canada

Abstract: Alzheimer's disease (AD), the most common cause of senile dementia and a growing global medical concern, is characterized by neuronal loss, tau-positive neurofibrillary tangles and amyloid-β (Aβ) containing neuritic plaques in selected brain regions. Aβ peptides are a family of proteins that are considered to contribute to AD pathogenesis. These peptides are derived from Amyloid Precursor Protein (APP) via sequential processing by β- and γ-secretases. Although much work has been focused on the interactions between Aβ peptide and neurons, the potential role of Aβ in APP processing is relatively unclear. Here we aimed to elucidate the effects of Aβ peptide on APP processing using cultured neuronal N2a cells. Through use of RT-PCR, we have shown that APP transcription is increased after Aβ treatment. Further analysis indicates an increase in APP and APP-CTFs levels in a time- and dose-dependent manner; however, the levels of secretases do not appear to alter following Aβ treatment. Similarly, our ELISA results have shown an increase in Aβ levels both in cell lysates and conditioned media. As a follow up, we will examine activity of β- and γ-secretases, as well as the subcellular localization of the various components involved in the cellular processing of APP and clearance of Aβ. These experiments will provide an insight into the molecular mechanisms by which Aβ can regulate the proteolytic processing of APP that can influence the loss of neurons and subsequent development of AD pathology.

Disclosures: B.V. Foroutanpay: None. **T.J. Revett:** None. **D. Vergote:** None. **D. Westaway:** None. **S. Kar:** None.

Poster

216. Alzheimer's Disease: *In vitro* and *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 216.01/C44

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Estate of Clem Jones AO

Title: Scanning ultrasound opening of the blood-brain-barrier reduces amyloid pathology and improves memory in APP23 transgenic mice

Authors: *G. LEINENGA[1], J. GOETZ[2];
[1]Queensland Brain Inst., Brisbane, Australia; [2]Clem Jones Ctr. for Ageing Dementia Research, Queensland Brain Inst., The Univ. of Queensland, Brisbane, Australia

Abstract: The brain of Alzheimer's disease (AD) patients is characterized by plaques, with the amyloid-β (Aβ) peptide being a major constituent. Here, we aimed to establish whether a transient opening of the blood-brain barrier (BBB) by 'scanning' the brain with focused ultrasound could assist in Aβ clearance. The protocol we developed caused neither edemas nor erythrocyte extravasation, nor upregulation of inflammatory markers associated with tissue

damage. We found, however, that repeated scanning ultrasound (SUS) treatments of the brain of Aβ plaque-forming APP23 mice, without the need for any additional therapeutic agents, significantly reduced Aβ levels and Aβ plaque load. Moreover, memory functions were restored to wild-type levels, as shown with three complementary memory tasks, spontaneous alternation in the Y-maze, novel object recognition and active place avoidance. Spinning disk confocal microscopy, histology and high-resolution 3D-reconstruction revealed an increased activation of microglia, but no increase in microglial numbers. Microglia in SUSed brains were found to fragment and engulf plaques. Moreover, Aβ was internalized into microglial lysosomes. Cleared plaques were observed in 75% of the SUSed mice but never in the sham-treated animals. Given that repeated SUSing and caused no overt damage to brain tissue, our study highlights the potential of this non-pharmacological approach, including as a possible vehicle for drug delivery, given that the BBB remains a major obstacle for the brain uptake of therapeutic agents.

Disclosures: **G. Leinenga:** None. **J. Goetz:** None.

Poster

216. Alzheimer's Disease: *In vitro* and *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 216.02/C45

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Annexin A1 restores Aβ1-42- induced blood brain barrier disruption

Authors: ***J. PARK**, H. CHO, S. BAIK, S.-H. HAN, A. KIM, I. MOOK-JUNG;
Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: The blood brain barrier (BBB) is composed of capillary endothelial cells, which are connected by tight junctions with a high selective permeability. It has an important role in maintaining homeostasis of the brain separated 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). Annexin A1 (ANXA1), an anti-inflammatory messenger, is expressed in brain endothelial cells and there was some reports that it can regulate BBB integrity. However, its role and mechanisms for protecting BBB in AD have not been identified. We found that Aβ1-42 induced BBB disruption was rescued by human recombinant ANXA1 (hrANXA1) in the murine brain endothelial cell line bEnd.3. Also, ANXA1 was reduced in human serum of AD patients. In addition, we attempted to find out what is the mechanism that ANXA1 recovers BBB integrity in AD. These data propose that ANXA1 is a therapeutic agent, protecting against the breakdown of the BBB in AD.

Disclosures: **J. Park:** None. **H. Cho:** None. **S. Baik:** None. **S. Han:** None. **A. Kim:** None. **I. Mook-Jung:** None.

Poster

216. Alzheimer's Disease: *In vitro* and *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 216.03/C46

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Effects of MRK-016 on amyloid-beta induced learning deficits in mice in a contextual conditioning paradigm

Authors: *S. L. HODGES[1], M. J. EIMERBRINK[2], J. D. WILES[2], J. D. WHITE[2], M. J. CHUMLEY[2], G. W. BOEHM[2];
[1]Baylor Univ., Waco, TX; [2]Texas Christian Univ., Fort Worth, TX

Abstract: Alzheimer's disease pathology is associated with cognitive decline, the presence of amyloid-beta (Aβ) plaques, and disruption of gene expression related to BDNF. Previously, our lab has demonstrated that repeated bouts of peripheral inflammation are sufficient to significantly increase central Aβ peptide in the dorsal hippocampus, and that this elevation is congruent with cognitive deficits in learning and memory. In the present study, we examined the protective effects of the inverse benzodiazepine agonist MRK-016 on the cognitive deficits associated with inflammation-induced accumulation of amyloid-beta (Aβ). Animals were injected (i.p.) with either LPS (250μg/kg) or saline, once per day, for seven consecutive days to increase central Aβ. On day eight, 24 hours after the last injection, animals were trained in a contextual fear conditioning paradigm, and received either an i.p. injection of either MRK-016 (2mg/kg) or saline to create 4 treatment groups. The following day, animals were returned to the conditioning chamber for testing to evaluate freezing behavior. Analysis of the behavioral data identified a significant interaction between MRK and LPS treatments, such that animals treated with MRK and LPS exhibited freezing behavior comparable to controls, while animals treated with Saline and LPS froze significantly less than all other conditions. After testing, hippocampi were collected to evaluate levels of central Aβ. In a comparable set of animals, dorsal hippocampus sections were collected both prior to training as well as 4 hours after training to identify the influence MRK-016 and LPS has on BDNF-related gene expression, using qRT-PCR. Between-groups analysis of the qRT-PCR data showed that animals treated with MRK and LPS had unique, and significantly different, expression of BDNF-related genes. Overall, results from this study support the hypothesis that MRK-016 can prevent Aβ-induced cognitive deficits, and alter gene expression related to BDNF activity in the hippocampus.

Disclosures: S.L. Hodges: None. **M.J. Eimerbrink:** None. **J.D. Wiles:** None. **J.D. White:** None. **M.J. Chumley:** None. **G.W. Boehm:** None.

Poster

216. Alzheimer's Disease: *In vitro* and *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 216.04/C47

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Chronic low level beta1-adrenergic receptor activation decreases amyloid beta, modulates the neuroimmune response and improves neurocognitive function in two models of Alzheimer's disease

Authors: *A. K. EVANS, P. M. ARDESTANI, B. YI, M. SHAMLOO;
Neurosurg., Stanford, Stanford, CA

Abstract: Severe degeneration of noradrenergic (NA) neurons has been reported in Alzheimer's Disease (AD) and may contribute to progression of pathology, neuroinflammation and cognitive dysfunction. In addition to a neuronal role in learning and memory, beta adrenergic receptors (ADRB1 and ADRB2) on microglia and astrocytes regulate neuroinflammation and govern compensatory and protective mechanisms for neuronal function and survival. Pharmacological and genetic ablation of NA neurons has been shown to exacerbate amyloid beta deposition, markers of neuroinflammation, and behavioral deficits in mouse models of AD. We have previously implicated acute activation of ADRB1 in restoration of cognitive deficits in mouse models of AD. In a series of studies we aimed to determine effects of chronic low level activation of the ADRB1 receptor on behavioral, pathological, and neuroimmune endpoints in 2 different mouse models of AD. Using 2 different transgenic models in which mice either express 5 mutations related to Familial Alzheimer's Disease, [5XFAD; 3 mutations in the amyloid precursor protein and 2 in presenilin 1] or 2 mutations in amyloid precursor protein (T41B), transgenic and wildtype male mice were chronically dosed with vehicle or with the specific ADRB1 partial agonist, xamoterol (5XFAD, 6 mg/kg daily oral gavage or 3 mg/kg subcutaneous pump; T41B, 0.3-1.0 mg/kg daily subcutaneous injection). Mice were dosed for 3 months and run through a series of behavioral tests during the last 6 weeks of dosing (Activity Chamber, Y-maze, Novel Object Recognition, Morris Water Maze, Elevated Plus Maze, Fear Conditioning). At conclusion (6-9 months of age in respective studies), plasma was collected and brains were perfused with saline and were either fresh frozen or paraformaldehyde fixed for neurobiological analyses. Chronic dosing with the specific ADRB1 partial agonist, xamoterol, improved novel object recognition and spatial learning in 5XFAD mice, and reduced hyperactivity and improved contextual fear conditioning in T41B mice. Chronic dosing with xamoterol decreased amyloid beta in both models and modulated indices of neuroimmune activation in both models (microglia/macrophage immunoreactivity, and immune-related mRNA and protein expression). Chronic dosing with an ADRB1 partial agonist attenuated upregulation of mRNA expression for several markers of neuroimmune activation while having a unique potentiating effect on CD68 mRNA expression in 5XFAD mice. These data support the hypothesis that ADRB1 activation

improves pathology and cognitive function in mouse models of AD and may do so by modulating the neuroimmune response.

Disclosures: **A.K. Evans:** None. **P.M. Ardestani:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent. **B. Yi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent. **M. Shamloo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent.

Poster

216. Alzheimer's Disease: *In vitro* and *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 216.05/C48

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA 5R01AG044404

Title: Azelnidipine attenuates amyloid-β altered cerebral endothelial functions

Authors: ***T. TENG**[1], D. M. RIDGLEY[1], A. TSOY[3], Y. NI[1], G. Y. SUN[2], S. ASKAROVA[4], J. C. M. LEE[1];
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Abstract: Alzheimer’s disease (AD) is an irreversible progressive neurodegenerative disease among older people. Although the cause of AD remains unknown, Amyloid-β (Aβ) is thought to play a crucial role in the AD pathology. Aβ interferes different cell types through different mechanisms. Cerebral endothelial cells (CECs) are integral components of the blood brain barrier (BBB), which is important for maintaining neuron function. The BBB regulates transportation of materials between peripheral blood and brain parenchyma. Aβ has been reported to disrupt calcium homeostasis and increase oxidative stress in CECs resulting in alterations of CEC functions. In this project, azelnidipine (ALP), an anti-hypertension drug, was examined for its ability to attenuate Aβ-altered CEC function with immortalized mouse cerebral endothelial cells (bEnd.3). We found that Aβ oligomers triggered calcium influx in cells within 15 min and lasted for at least 45 mins, and calcium influx was suppressed by ALP treatment. Aβ also induced production of superoxides activation of ERK, cytosolic phospholipase A2 and NF-κB activity, and all these events were suppressed by ALP. Taken together, these results demonstrate that ALP is capable of reducing Aβ-induced calcium influx, and downstream

oxidative and inflammatory pathways in CEC, and may be potentially useful for development of therapeutic treatment for AD.

Disclosures: **T. Teng:** None. **D.M. Ridgley:** None. **A. Tsoy:** None. **Y. Ni:** None. **G.Y. Sun:** None. **S. Askarova:** None. **J.C.M. Lee:** None.

Poster

216. Alzheimer's Disease: *In vitro* and *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 216.06/C49

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Trigonelline is effective in scopolamine induced memory deficits in mice due to its anti-acetylcholinesterase and anti-oxidative properties

Authors: ***A. JUVEKAR**, A. CHOWDHURY, N. GAWALI, P. KOTHAVADE, P. DESHPANDE;
Dept. of Pharmaceut. Sci. and Technol., Inst. of Chem. Technol., Mumbai, India

Abstract: Background: Alzheimer's disease (AD) is a progressive, neurodegenerative disease of the brain, and is the most common form of dementia among the elderly population. It is a chronic disease characterized symptomatically by progressive decline of daily living activities, behavioral disturbances and loss of cognition. Trigonelline, a pyridine alkaloid is commonly found in coffee beans and Trigonella foenum-graecum L. (fenugreek) seeds. Recent reports have emerged regarding the beneficial effects of trigonelline in β- amyloid induced memory impairment in rats. Also, Trigonelline demonstrated *in vitro* neurite outgrowth in rats and human neuronal cells. However detailed mechanism of the neuroprotection has not been explored yet. Therefore present study was conducted to explore the neuroprotective mechanism of Trigonelline in scopolamine induced amnesia mice model. Methods: Scopolamine (2.5 mg/kg) was used to induce amnesia in male swiss albino mice. Donezepil (1 mg/kg i.p) was used as positive control. Animals were divided into 5 groups (n=6) and Trigonelline (50 and 100 mg/kg) was administered by oral gavage for 2 weeks. Memory performance was evaluated by Morris water maze test and elevated plus maze. Post treatment, the mice were euthanized, brains isolated and extent of oxidative stress (elevated malionaldehyde, nitrite concentration, reduced glutathione, superoxide dismutase and catalase) was estimated. Histopathology studies were carried out to corroborate the neuroprotective effect of Trigonelline. Results: Administration of Trigonelline (50 and 100 mg/kg) resulted in improved cognitive performance in both, Morris water and elevated plus maze models as compared to negative control. Further pretreatment with Trigonelline caused significant attenuation of brain acetyl choline esterase activity and strengthened the oxidative defense. Moreover the neuroprotective effect of Trigonelline was well supported by photomicrographs of the hippocampus of brain where minimal vacuolar

degeneration of neurons were observed as compared to negative control. Conclusions: In conclusion, these results indicate that Trigonelline may exert anti-amnesiac effects which may be mediated, at least in part due to its anti-acetylcholinesterase and anti-oxidant properties.

Disclosures: A. Juvekar: None. **A. Chowdhury:** None. **N. Gawali:** None. **P. Kothavade:** None. **P. Deshpande:** None.

Poster

216. Alzheimer's Disease: *In vitro* and *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 216.07/C50

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA grant R37 AG-10880

Title: Cerebrospinal fluid and plasma ceramides are modified by dietary intervention in adults with mild cognitive impairment and healthy controls

Authors: *B. J. NETH[1], J. L. BAYER-CARTER[2], A. HANSON[3], L. D. BAKER[1], K. S. NAIR[4], S. CRAFT[1];
[1]Wake Forest Sch. of Med., Winston Salem, NC; [2]Veterans Affairs Puget Sound Hlth. Care Syst., [3]Div. of Gerontology and Geriatric Med., Univ. of Washington Sch. of Med., Seattle, WA; [4]Metabolomics Resource Core, Mayo Clin., Rochester, MN

Abstract: Background: Sphingolipid-derived ceramides play an important role in cell signaling and disease pathology. Serum ceramides are higher in insulin resistance, Type 2 Diabetes and other metabolic conditions, and are modulated by diet in rodent studies (Haus et al., 2009; Turpin et al., 2014). Moreover, recent studies have described elevated blood ceramide concentrations in adults with Alzheimer's disease (AD) and other neurologic conditions (Mielke et al., 2012; Filippov et al., 2012). Interestingly, several cerebrospinal fluid (CSF) ceramides are lower in adults with Mild Cognitive Impairment (MCI) and AD and correlate with AD biomarkers (Fonteh et al., 2015). Modification of ceramide levels through diet and other metabolic manipulations may reveal therapeutic targets to alleviate AD pathology. Methods: Data from 49 participants were used in this analysis. Twenty participants (13F, 7M) were cognitively normal (CN) with a mean [SD] age of 69.3 [7.4] and 29 participants (13F, 16M) were adults with amnestic MCI with a mean [SD] age of 67.6 [6.8]. Participants were randomized to one of two equicaloric dietary interventions (High Saturated Fat/Glycemic Index – "High Diet" & Low Saturated Fat/Glycemic Index – "Low Diet") for 4 weeks. All meals were supplied during the study period. Blood (plasma) and CSF samples were collected prior to starting diet (week 0) and ending diet (week 4). CSF and plasma ceramide concentrations were measured with Liquid Chromatography–Mass Spectrometry, and subjected to analysis of covariance adjusting for age,

gender, and APOE-e4 status. Results: In CSF, adults with MCI showed decreased concentrations of C16:0 ($p<0.05$) and C22:0 ($p<0.05$) on the Low relative to the High diet. The ceramide C18:0 trended toward significance ($p=0.08$). Conversely, concentrations of C16:0 ($p<0.05$) for CN were increased on the Low relative to the High diet. In plasma, concentrations of 5 ceramides (C14:0 ($p<0.0001$), C16:0 ($p<0.0001$), C18:0 ($p<0.0001$), C20:0($p<0.0005$), C24:1($p<0.001$)) decreased significantly after consumption of the Low diet relative to the High, with exception to C24:1, which showed an opposite pattern. Conclusions: Our results demonstrate both plasma and CSF ceramides are modified after a 4-week dietary intervention in cognitively normal adults, as well as in adults with amnestic MCI. Further study is needed to fully understand the role of ceramides in AD and whether dietary intervention is an effective therapeutic or preventative approach.
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Disclosures: **B.J. Neth:** None. **J.L. Bayer-Carter:** None. **A. Hanson:** None. **L.D. Baker:** None. **K.S. Nair:** None. **S. Craft:** None.

Poster

216. Alzheimer's Disease: *In vitro* and *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 216.08/C51

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01 AG037481

NIH R01 AG037919

NIH R01 ES024233

Department of Defense W81XWH-13-0384

NIH K01 AG044490

Title: Effects of high fat diet on cognition and neurodegeneration in APOE3 and APOE4 mice

Authors: *V. L. REEVES, A. Y. CARTER, N. F. FITZ, I. LEFTEROV, R. KOLDAMOVA; Envrn. and Occup. Hlth., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Multiple genes, metabolic stimuli, and environmental factors control the variation in susceptibility to Alzheimer’s disease (AD). The inheritance of ε4 allele of *APOE* (APOE4) is the major genetic risk factor for late-onset AD; however, the mechanisms underlying this association remain elusive. Notably, high fat diet (HFD) and related metabolic stimuli are associated with increased risk of AD. Recently, we have demonstrated the detrimental effects of HFD on cognitive performance and cerebral amyloidosis in animal models of AD. Gene-environment interactions are critical for the development and progression of AD. We hypothesize that high fat

diet results in changes in transcriptional activity and expression level of genes important for developing AD, accelerating the course of the disease, or aggravating AD phenotypes. We have utilized the targeted replacement humanized APOE3 and APOE4 mouse models and a HFD to simulate typical Western diet. The mice were compared for behavioral, amyloid deposition, and transcriptional profiles in the brain. Our data demonstrate that HFD aggravate behavioral deficits in an isoform-specific manner with APOE4 and mice fed HFD having worse cognitive performance than APOE3 mice, and mice fed control diet. The results of this study will reveal transcriptional changes instigated by HFD nutritional signals and how these modifications contribute to AD pathology. The ability to associate changes at gene expression level, or transcript enrichment within gene networks and clusters, to pathogenic features of AD will have a significant impact on defining various molecular mechanism of AD pathogenesis. This study further defines how diet and dietary patterns influence epigenetic mechanisms relevant to neurodegeneration.

Disclosures: V.L. Reeves: None. **A.Y. Carter:** None. **N.F. Fitz:** None. **I. Lefterov:** None. **R. Koldamova:** None.

Poster

216. Alzheimer's Disease: *In vitro* and *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 216.09/C52

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NINDS grant T32NS43115

CDC grant R49/CCR811509

Title: Insulin and Insulin-Like Growth Factors (IGFs) may protect against susceptibility to brain atrophy and dementia in Late-Onset Alzheimer’s disease (LOAD) and diabetes

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Colorado State Univ., Fort Collins, CO

Abstract: It is herein proposed that insulin and IGFs are age-dependent, environmentally-responsive growth factors that normally protect against weak susceptibility genes to prevent brain atrophy, synapse loss and dementia in LOAD. Age is a major risk factor for LOAD (comprises 95% of Alzheimer's cases; onset arises after age 65; dementia and brain atrophy with loss of up to 1/3 brain mass). Irrespective of presence or absence of susceptibility genes, subjects are protected against disease onset for 65+ years. Indeed, two decades after onset of amyloid-beta plaques and neurofibrillary tangles (P&T) as well as dementia in LOAD patients, their identical twins can be free of P&T and cognitively normal (Brickell et al., 2007). Environmental factors can clearly prevent or delay disease onset for decades in subjects with identical genetic

susceptibility. Autopsy confirmed LOAD brains have an 81% association with mid-life diabetes or insulin resistance. Obesity, insulin resistance, and diabetes are each correlated with brain atrophy and cognitive disorder in mid-life. Elderly diabetic patients have increased risk of dementia even after correcting for cerebrovascular disease. Neither learning/memory nor glucose utilization are altered following conditional knock-out of the neuronal insulin receptor in mice. LOAD, clinical diabetes, and diabetic rats share brain atrophy, impaired learning/memory, and reduced insulin and IGF levels in brain. IGFs are neurotrophic factors required for synapse formation and learning, IGF levels decline with age, and IGF treatment prevents impaired learning in diabetic rats independently of hyperglycemia (Lupien et al., 2003). The combination of insulin and IGF restoration is discovered to prevent massive brain atrophy, including loss of total brain protein and DNA involving both neurons and glia (Serbedzija et al., 2009). Disease onset may be delayed for decades by a) lifestyle modifications that prevent obesity, insulin resistance, and diabetes, and/or b) pharmacologic interventions that increase both insulin and IGF levels in the CNS, but not systemic glucose levels. The safe doses of insulin and IGF are known, and clinical trials should explore whether the combination of insulin and IGF can prevent brain atrophy and subsequent dementia.

Disclosures: D.N. Ishii: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Colorado State University; Aurogen Inc.. Other; Founder of Aurogen Inc..

Poster

216. Alzheimer's Disease: *In vitro* and *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 216.10/C53

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: 3-iodothyronamine rescues β-Amyloid-dependent inhibition of long-term potentiation in the entorhinal cortex

Authors: *A. ACCORRONI[1], C. CRISCUOLO[2], M. SABATINI[3], R. DONZELLI[3], A. SABA[3], R. ZUCCHI[3], N. ORIGLIA[2];
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Abstract: Entorhinal cortex (EC) is a parahippocampal region involved in learning and memory that is early affected in Alzheimer’s disease (AD). Beta-Amyloid (Aβ) oligomers have been proven to be involved in the inhibition of long-term potentiation (LTP) in the EC. Thyroid hormones (TH) have been demonstrated to regulate cognitive function and there is evidence that TH cerebral metabolism may be altered, with an increased production of the inactive form of TH, in AD patients. Furthermore, the acute administration of 3-iodothyronamine (T1AM), a

derivative of TH, has been shown to stimulate memory acquisition in the mouse. To investigate whether T1AM can restore Aβ-induced impairment of synaptic plasticity, we performed extracellular *in vitro* recordings in EC slices taken from wild type (WT) mice and treated with Aβ oligomers. In addition, we evaluated T1AM restorative effect on LTP in EC slices taken from AD mice expressing human mutations of APP gene (hAPP-J20 line), which show EC-LTP impairment at a very early stage (2months). Field Potentials (FPs) were evoked in layer II after stimulation of the same layer and LTP was elicited by high frequency stimulation (HFS), consisting of three trains of 100 pulses at 100 Hz. In addition to electrophysiological evaluations, T1AM endogenous levels were assayed in the EC by HPLC coupled to tandem mass spectrometry. First, we identified a concentration (5μM) of T1AM that did affect neither basal synaptic transmission nor LTP induction and maintenance in WT EC slices. T1AM was administered for 10 minutes starting 5 minutes before the delivery of HFS. To verify its protective effect, T1AM (5μM) was administered in combination with Aβ oligomers at 200 nM, a concentration that has been previously demonstrated to inhibit EC-LTP. The results indicate that T1AM perfusion completely restores LTP in Aβ-treated WT slices. Moreover, T1AM confirmed its protective efficacy in slices from hAPP-J20 mice. Indeed, LTP was completely rescued in hAPP-J20 slices perfused with T1AM, with respect to transgenic untreated slices and was comparable to LTP recorded in WT control slices. Furthermore, T1AM endogenous concentration was reduced in 2 month old mhAPP EC slices when compared to age-matched WT controls. Our results suggest that T1AM plays a neuroprotective effect, rescuing Aβ-induced neuronal dysfunction, and that its reduced levels may contribute to EC vulnerability in AD. A more thorough understanding of the neuroprotective properties of T1AM and the underlying mechanisms of its effect, may be beneficial for clinical research and might lead to the identification of new pharmacological targets to delay disease progression.

Disclosures: **A. Accorroni:** None. **C. Criscuolo:** None. **M. Sabatini:** None. **R. Donzelli:** None. **A. Saba:** None. **R. Zucchi:** None. **N. Origlia:** None.

Poster

216. Alzheimer's Disease: *In vitro* and *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 216.11/C54

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Protective effects of Anthocyanins against Amyloid beta-induced neurotoxicity *in vitro* and *in vitro*

Authors: *T. KIM, H. BADSHAH, M. KIM;
Gyeongsang Natl. Univ., Jinju, Korea, Republic of

Abstract: Alzheimer's disease (AD) is one of the most common neurodegenerative disorders in recent world, characterized by increased production of amyloid beta in the nervous system with an ultimate effect of apoptotic neurodegeneration. This study was aimed to investigate the neuroprotective effect of black soybean anthocyanins in a neurodegenerative model of amyloid beta 1-42 (Aβ1-42). Aβ1-42 was treated to HT22 cell lines or adult male rats via intra-cerebro-ventricular injection to induce neurotoxicity in these experimental models. Anthocyanins were treated 0.2mg/kg in case of cell lines or 4mg/kg intragastrically to adult rats to protect against Aβ-induced neurodegeneration. Assay for cell viability, mitochondrial membrane potential (Ψm), intracellular free Ca2+ and apoptotic cells (Fluorojade-B and TUNEL) were performed *in vitro* while western blot analysis were performed to the hippocampal proteins of adult rats. Our results showed that Aβ1-42 treatment reduced cell viability, disturbed the Ψm and Ca2+ homeostasis in and out of the cell, and increase neuronal apoptosis. Treatment with anthocyanins for 12 hr retained the cell viability, normalized Ψm and Ca2+ level, and decreased the neuronal cell death. In accordance, anthocyanins reversed Aβ-induced effect on proteins expression of mitochondrial apoptotic pathway (Bax, cytochrome c, caspase-3 and caspase-9) and major Alzheimer's markers i.e. Aβ, APP, P-tau and BACE-1. Overall, our results showed that anthocyanins are potential candidates to treat neurodegenerative disorders like AD.

Disclosures: **T. Kim:** None. **H. Badshah:** None. **M. Kim:** None.

Poster

216. Alzheimer's Disease: *In vitro* and *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 216.12/C55

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RCMI-BRIDGES (NIMHD)

INBRE III (NIGMS)

Title: The neuroprotective action of a hexapeptide core sequence within beta amyloid

Authors: *K. FOREST, J. LAWRENCE, N. ALFULAIJ, R. NICHOLS;
Univ. of Hawaii, Manoa, Honolulu, HI

Abstract: PURPOSE: Alzheimer's disease (AD) is a progressive neurodegenerative disease, characterized by memory loss as well as cognitive decline and language dysfunction. To date, there is no effective cure for the disease. Identifying and characterizing the cellular mechanisms underlying AD will potentially elucidate a target pathway for potential treatment. One possible pathway is the neurotoxicity triggered by the accumulation of beta amyloid (Aβ), a short peptide found in the brains of individuals with Alzheimer's disease. Aβ was originally identified in dense neuritic plaques which are one of two histopathological hallmarks in AD. However, considerable

evidence has shown that in normal healthy brains, soluble, oligomeric Aβ functions as a neuromodulator. Our laboratory has recently shown that at low concentrations (pM-nM) the N-terminal Aβ fragment (N-Aβ) is twice as effective as full-length Aβ as a neuromodulator, stimulating receptor-linked increases in Ca^{2+}, enhancing long-term potentiation (LTP) and enhancing contextual fear conditioning. In addition, we have shown that N-Aβ inhibits the synaptotoxicity triggered by full-length Aβ ($Aβ_{42}$). Preliminarily, we have found that N-Aβ also protects against $Aβ_{42}$-induced neurotoxicity. We have further identified a hexapeptide core sequence within N-Aβ ($Aβ_{core}$), YEVHHQ, encompassing a putative metal binding site, which is equally as effective as N-Aβ in Ca^{2+} signaling. We have therefore postulated that the $Aβ_{core}$ would have neuroprotective potential. DESIGN METHODS: We investigated the extent of neuroprotection of $Aβ_{core}$ and mutants against full-length Aβ toxicity by measuring oxidative stress (as production of reactive oxygen species, ROS), nuclei and DNA fragmentation, and cell death in an *in vitro* $α_4β_2$-nicotinic receptor (nAChR)-transfected neuroblastoma-based model nerve cell culture system. The presence of target nAChRs was previously shown to sensitive nerve cells to Aβ toxicity. In addition, we assessed the neuroprotective action of $Aβ_{core}$ and mutant peptides on synaptic plasticity in rodent hippocampal slice LTP. RESULTS and CONCLUSION: Through mutational analysis, we have identified residues in the $Aβ_{core}$ essential for functional regulation. In addition, co-treatment with the $Aβ_{core}$ was shown to protect against $Aβ_{42}$-induced ROS and cellular toxicity in $α_4β_2$-nAChR-transfected neuroblastoma cells. SIGNIFICANCE: The neuroprotective action of the $Aβ_{core}$ suggests the possibility of using this core sequence as a scaffold for optimization of a potential biologic for protection against Aβ-induced toxicity.

Disclosures: **K. Forest:** None. **J. Lawrence:** None. **N. Alfulaij:** None. **R. Nichols:** None.

Poster

216. Alzheimer's Disease: *In vitro* and *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 216.13/C56

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RCMI-BRIDGES (NIMHD)

INBRE III (NIGMS)

Title: Neuroprotection by a n-terminal fragment of beta amyloid

Authors: ***N. ALFULAIJ**, G. YOSHIKAWA, J. CHENG, K. ARORA, R. NICHOLS; Dept. of Biol., Univ. of Hawaii At Manoa, Honolulu, HI

Abstract: Amyloid-beta (Aβ) is the primary component of plaques associated with Alzheimer's disease (AD). In normal brain, Aβ is present in soluble oligomeric form at low picomolar levels

where it appears to have a neuromodulatory role. We have previously shown that the presence of nicotinic receptors (nAChRs) sensitizes neurons to Aβ neurotoxicity. We recently discovered that a N-terminal Aβ fragment, $A\beta_{1-15,}$ has neuromodulatory activity but is not toxic. We have been able to show that picomolar levels are able to enhance fear memory and increase long-term potentiation (LTP). We postulated therefore that $A\beta_{1-15}$ may have a neuroprotective action against full-length Aβ neurotoxicity. Changes in reactive oxygen species (ROS) and nuclear disintegration are early measures of toxicity and apoptosis. Using a ROS assay as a measure of toxicity, we examined the potential neuroprotective action of $A\beta_{1-15}$ on a hybrid neuroblastoma cell line, NG108-15, expressing α4β2 nAChRs as compared to α7 nAChRs or controls, subjected to treatment with full-length $A\beta_{1-42}$ at 100nM for 3 days. We also examined effects of withdrawing $A\beta_{1-42}$ after varying lengths of treatment to assess the time-dependency of receptor stimulation for toxicity. To confirm this in an *ex vivo* model, we utilized organotypic hippocampal slice cultures subjected to full-length Aβ or combination treatment of $A\beta_{1-42}$ and $A\beta_{1-15}$. We assessed cell viability using a propidium iodine stain and then homogenized the tissue and extracted proteins to examine cell death signaling proteins in each treatment group. Electrophysiology was performed on acute slices to examine the effect of $A\beta_{1-15}$ on the inhibitory effects of $A\beta_{1-42}$ on LTP. While we've previously shown that $A\beta_{1-15}$ enhanced LTP, we now examined how various combinations of $A\beta_{1-42}$ and $A\beta_{1-15}$, or delayed $A\beta_{1-15}$ perfusion after $A\beta_{1-42}$ affects LTP. Following $A\beta_{1-42}$ early withdrawal, ROS production continued, approaching levels found for 3 days of Aβ treatment, indicating that the neuroprotection window is in the early phase of $A\beta_{1-42}$-induced toxicity. With co-incubation, $A\beta_{1-15}$ blocked $A\beta_{1-42}$-induced ROS and nuclear disintegration in cultures expressing α4β2 nAChRs. In contrast, α7 nAChRs may not sensitize the cultures to Aβ neurotoxicity, precluding a neuroprotective action of $A\beta_{1-15}$ via this pathway. Further confirming neuroprotection by $A\beta_{1-15}$, *ex vivo* slice cultures show significantly less cell death in combination-treated slices compared to high levels of cell death when treated with $A\beta_{1-42}$ alone. The action of the $A\beta_{1-15}$ fragment indicates that it may serve a competitive, neuroprotective role via α4β2 nAChRs at the synapse in the context of accumulating Aβ in AD.

Disclosures: **N. Alfulaij:** None. **G. Yoshikawa:** None. **J. Cheng:** None. **K. Arora:** None. **R. Nichols:** None.

Poster

216. Alzheimer's Disease: *In vitro* and *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 216.14/C57

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: "Cooperative Research Program for Agriculture Science & Technology Development (PJ009830022015)" Rural Development Administration, Republic of Korea

Title: Cyanidin-3-glucoside protects against amyloid beta (25-35)-induced neuronal cell death in cultured rat hippocampal neurons

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Abstract: Increasing evidences implicate changes in $[Ca^{2+}]_i$ and oxidative stress as causative factors in amyloid beta (Aβ)-induced neuronal cell death. Cyanidin-3-glucoside (C3G), a component of anthocyanin, has been reported to protect against glutamate-induced neuronal cell death by inhibiting Ca^{2+} and Zn^{2+} signaling. The present study was investigated to determine whether C3G has a protective effect against Aβ-induced neuronal cell death in cultured rat hippocampal cells and pure hippocampal neurons from embryonic day 17 fetal Sprague-Dawley rats using digital imaging methods for Ca^{2+}, Zn^{2+}, MMP and ROS, and MTT assay for cell survival. Pretreatment with C3G (10 μg/ml) for 30 min inhibited Aβ-induced $[Ca^{2+}]_i$ increases in the cultured rat hippocampal neurons**.** C3G significantly inhibited Aβ-induced mitochondrial depolarization. C3G blocked Aβ-induced formation of ROS. C3G also significantly inhibited Aβ-induced $[Zn^{2+}]_i$ increases. Treatment with C3G (10 μg/ml) for 48 h attenuated Aβ-induced neuronal cell death in cultured rat pure hippocampal neurons. Taken together, all these results suggest that cyanidin-3-glucoside inhibits Aβ-induced Ca^{2+} signaling, mitochondrial depolarization, formation of reactive oxygen species and Zn^{2+} signaling in cultured rat hippocampal neurons, which is involved in neuroprotection.

Disclosures: J. Yang: None. **K. Yoon:** None. **S. Yoon:** None.

Poster

216. Alzheimer's Disease: *In vitro* and *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 216.15/C58

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: LMTX reduces tau-pathology in transgenic mice and restores microtubule stabilisation and synaptic transmission

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Abstract: Alzheimer’s disease (AD) is an irreversible neurological disorder characterised by cognitive and motor impairments and the microtubule-associated protein tau is believed to drive the underlying pathology. We recently reported beneficial effects of LMTX, a reduced form of

methylthioninium, on pathology and motor skills in an AD transgenic mouse model overexpressing mutated human tau (P301S/G335D). We have used a proteomics approach to identify signaling networks responsible for the mediation of beneficial actions of LMTX. Brain tissue from untreated and LMTX-treated transgenic mice was subjected to large scale two-dimensional electrophoresis and nano-LC-ESI-MS (orbitrap). We found that oral LMTX administration affects biological processes involved in microtubule stabilisation and synaptic transmission, protein ubiquitination and response to oxidative stress, as well as metabolic processes associated with energy generation. Ingenuity Pathway Analysis revealed tau to be a highly significant upstream regulator of the observed changes. We conclude that LMTX specifically targets tau aggregation and that tau represents a reasonable therapeutic target for the treatment of AD and other tauopathies.

Disclosures: **K. Schwab:** None. **S. Frahm:** None. **V. Melis:** None. **M. Magbagbeolu:** None. **G. Riedel:** None. **C.M. Wischik:** None. **C.R. Harrington:** None. **F. Theuring:** None.

Poster

216. Alzheimer's Disease: *In vitro* and *In vivo* Experimental Therapeutics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 216.16/C59

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR

Title: Effects of U18666A on APP metabolism in cultured N2a cells

Authors: *J. CHUNG[1], A. MOHAMED[2], M. MAULIK[3], G. THINAKARAN[4,5,6], E. POSSE DE CHAVES[2], S. KAR[1,3];
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Abstract: Amyloid β (Aβ) peptides originating from β-amyloid precursor protein (APP) are considered to play a critical role in the development of Alzheimer's disease (AD). Multiple lines of evidence suggest that elevated levels of cholesterol can influence amyloidogenic processing of APP, leading to increased production of Aβ peptides. However, it remains unclear how sequestration of cholesterol within endosomal-lysosomal (EL) system, the major site of Aβ production, can regulate APP metabolism. In this study we investigate how alteration in cholesterol level/distribution following treatment with U18666A, a class II amphiphile that triggers redistribution of cholesterol to the EL system, can influence the levels/processing of APP in cultured N2a cells grown in media containing 0%, 5% or 10% fetal bovine serum (FBS). The N2a cells used in the study includes wild type N2a cells ($N2a_{wt}$), N2a cells transfected with

either wild type human APP ($N2aAPP_{wt}$) or human APP containing Swedish mutation ($N2aAPP_{sw}$). Our results indicate that U18666A treatment in 0% FBS, but not in 5% or 10% FBS, decreases the levels of total and free cholesterol in all categories of N2a cells. Changes in SREBP2 activation reflect this decrease in cholesterol. The levels of APP, APP-CTFs and intracellular Aβ1-40/42 are not markedly altered in $N2a_{wt}$ cells but are differentially increased in $N2aAPP_{wt}$ and $N2aAPP_{sw}$ cells. However, levels of α-secretase ADAM10, β-secretase BACE1 and components of γ-secretase (PS1, Nicastrin, PEN2 and APH1) remain unaltered in all three types of N2a cells following U18666A treatment. We are currently evaluating levels of secreted Aβ and activities of APP processing enzymes in U18666A treated N2a cells. Our results, obtained so far, suggest that redistribution of cholesterol into the EL system differentially alters the levels/processing of APP depending on the cultured conditions and endogenous levels of APP.

Disclosures: J. Chung: None. **A. Mohamed:** None. **M. Maulik:** None. **G. Thinakaran:** None. **E. Posse de Chaves:** None. **S. Kar:** None.

Poster

216. Alzheimer's Disease: *In vitro* and *In vivo* Experimental Therapeutics

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Program#/Poster#: 216.17/C60

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH P50AG05136

NIH R01AG042819

Title: Characterization of novel Src family kinase inhibitors to attenuate microgliosis

Authors: G. D. MANOCHA[1], K. L. PUIG[1], S. A. AUSTIN[2], K. SEYB[3], M. A. GLICKSMAN[3], *C. K. COMBS[1];
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Abstract: Microgliosis is a major hallmark of Alzheimer's disease (AD) brain pathology. Aβ peptide is hypothesized to act as a stimulus for microglia leading to activation of non-receptor tyrosine kinases and subsequent secretion of pro-inflammatory cytokines. Therefore, the signaling pathways mediating microglial activation may be important therapeutic targets of anti-inflammatory therapy for AD. Four novel compounds were chosen after high throughput screening kinase activity assays determined them as potential Lyn kinase inhibitors. Their kinase inhibitory and anti-inflammatory effect on Aβ-stimulated activation was assessed using the murine microglial cell line, BV2. Cells were treated with the compounds determine effects on active, phosphorylated levels of Src family kinases, Src and Lyn, as well as an unrelated Ser/Thr

kinase, ERK. This study identifies a novel small molecule Src family tyrosine kinase inhibitor with anti-inflammatory effects in response to Aβ stimulation of microglia. Further *in vitro*/*in vivo* characterization of LDDN-0003499 as well as structural modification may provide a new tool for attenuating microglial mediated brain inflammatory conditions such as that occurring in AD.

Disclosures: G.D. Manocha: None. **K.L. Puig:** None. **S.A. Austin:** None. **K. Seyb:** None. **M.A. Glicksman:** None. **C.K. Combs:** None.

Poster

217. Therapeutics of Parkinson's Disease: Target Validation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 217.01/C61

Topic: C.03. Parkinson's Disease

Support: CONACyT Grant: 154131

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IN-202914. IMPULSA 03

Title: Unexpected receptors interactions in striatal neurons during a rodent model of Parkinson´s disease

Authors: *E. RENDON-OCHOA, T. HERNANDEZ-FLORES, O. HERNANDEZ-GONZALEZ, M. PEREZ-RAMIREZ, M. PALOMERO-RIVERO, R. DRUKER-COLÍN, E. GALARRAGA, J. BARGAS;
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Abstract: The "two pathways model" of the basal ganglia (BG) proposes that the direct pathway facilitates whereas the indirect pathway inhibits movement execution. Half of striatal projection neurons (SPNs) belong to the direct (dSPNs) and the other half to the indirect (iSPNs) pathway. dSPNs express D1-type dopamine (DA) receptors while iSPNs express D2-type DA receptors. D1-receptors facilitate neuronal discharge while D2-receptors repress neuronal discharge in part by enhancing or reducing, respectively, CaV1 Ca2+ currents. Adenosine A1 receptors are located in both neuron classes, while adenosine A2A receptors are preferential expressed in iSPNs. A1-type receptors in the SPNs reduce CaV2.2 Ca2+ currents, increasing neuronal excitability. A2A-type receptors enhance CaV1 Ca2+ currents in iSPNs, increasing neuronal excitability. However, A2A receptors need the previous occupation of A1 receptors to modulate Ca2+ channels. Here, we asked whether another receptor type could facilitate A2A receptors activation. Using voltage-clamp recordings in acutely dissociated SPNs we found that activation of D2-type receptors also facilitate A2A receptors activation in iSPNs. These interactions (A1-A2A and D2-A2A) are preserved in the 6-OHDA rodent model of Parkinson´s disease (PD).

Currently, L-DOPA and dopamine agonists are used therapeutically. While antagonists of A2A receptors are being tested as adjuvants. However their efficacy has been lower than expected. One reason is that D2-receptors induced activity may be counteracting antagonists actions on A2A -receptors.

Disclosures: **E. Rendon-Ochoa:** None. **T. Hernandez-Flores:** None. **O. Hernandez-Gonzalez:** None. **M. Perez-Ramirez:** None. **M. Palomero-Rivero:** None. **R. Druker-Colín:** None. **E. Galarraga:** None. **J. Bargas:** None.

Poster

217. Therapeutics of Parkinson's Disease: Target Validation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 217.02/C62

Topic: C.03. Parkinson's Disease

Title: An in silico analysis of α-synuclein interactions using seed

Authors: *J. J. MORRISON, III, J. W. RYAN, K. MCLAUCHLAN, A. M. GREEN, L. SCHAPPELL, K. S. INMAN, A. D. LEE, B. BEHROUZ;
Neuroinitiative, Jacksonville, FL

Abstract: Parkinson's Diseases (PD) is a progressive neurodegenerative disorder with debilitating symptoms and unknown etiology. The protein α-synuclein has been at the forefront of PD research for two main reasons: aggregated forms of the protein within Lewy Body inclusions are a hallmark pathology of sporadic PD and gain of function mutations and multiplications in the gene encoding for α-synuclein cause autosomal dominant PD. Since the discovery of α-synuclein's involvement in PD in 1997, over four thousand peer reviewed manuscripts have described numerous interactions with other proteins and involvement in multiple subcellular pathways. Although the complexity of the neuronal system and the heavy interplay between biochemical pathways within the cell make it incredibly difficult to derive meaningful conclusions, recent computational advances open the door to organizing, visualizing and analyzing this data in a comprehensible manner. In these experiments, we used the Simulation Environment for Experimental Design (SEED) to model molecular interactions that affect or are impacted by α-synuclein. These in Silico experiments allow for manipulation of proteins in such a way that can model aspects of disease, for example by accumulation and aggregation of α-synuclein. These models can be used as a platform to test potential therapeutics cheaply and efficiently, reducing negative data and highlighting important molecular pathways that can be further exploited using laboratory research.

Disclosures: **J.J. Morrison:** None. **J.W. Ryan:** None. **K. Mclauchlan:** None. **A.M. Green:** None. **L. Schappell:** None. **K.S. Inman:** None. **A.D. Lee:** None. **B. Behrouz:** None.

Poster

217. Therapeutics of Parkinson's Disease: Target Validation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 217.03/C63

Topic: C.03. Parkinson’s Disease

Title: In silico visualization of the endosomal pathway in Parkinson's disease using SEED

Authors: *J. W. RYAN, J. J. MORRISON, A. M. GREEN, L. SCHAPPELL, K. S. INMAN, A. D. LEE, B. BEHROUZ;
Neuroinitiative, Jacksonville, FL

Abstract: Recent genetic findings have implicated the endosomal pathway as a contributor in the development of Parkinson’s disease. Much is known about the molecular interactions that occur within this and other pathways involved in the pathogenesis of this disease. However, the heavy interplay between endosomal proteins, their neuronal locations, organelle associations, and associated feedback loops make it difficult to predict downstream effects of genetic alterations. Recent computational advancements provide a unique opportunity to create *in silico* models of these known interactions, and to predict downstream events associated with abnormalities within these pathways. Using the Simulation Environment for Experimental Design (SEED), we consolidated, simplified, and visualized available data on known molecular interactions. Using video game technology, we created a 3D environment in which to visualize the interplay between multiple molecules within the endosomal pathway in virtual space, in real time. Further, we manipulated expression of these molecules, including VPS35 to alter downstream molecular interactions within a virtual test tube. These *in silico* models can serve as a platform to quickly and inexpensively obtain detailed insight into the spatio-temporal behaviors of molecules inside the cell.

Disclosures: J.W. Ryan: None. **J.J. Morrison:** None. **A.M. Green:** None. **L. Schappell:** None. **K.S. Inman:** None. **A.D. Lee:** None. **B. Behrouz:** None.

Poster

217. Therapeutics of Parkinson's Disease: Target Validation

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NIH/ORIP Grant P51-OD011132

Title: Structural plasticity of the GABAergic pallidothalamic system in MPTP-treated Parkinsonian monkeys

Authors: *A. J. SULLIVAN[1], A. SHIBATA[1], C. KATONA[1], T. WICHMANN[1,2], Y. SMITH[1,2];
[1]Udall Ctr. of Excellence for Parkinson's Dis. Res., Yerkes Natl. Primate Res. Center, Emory Uni, Atlanta, GA; [2]Dept. of Neurol., Sch. of Med., Atlanta, GA

Abstract: Parkinson's disease is characterized by progressive loss of the dopaminergic neurons of the substantia nigra pars compacta, and manifests clinically with severe motor impairments including slowness of movement, resting tremor, rigidity, loss of balance and gait problems. Although the decreased motor activity seen in PD is thought to result from an abnormally increased inhibitory outflow from the internal globus pallidus (GPi) onto the thalamus, structural and functional data to support such a network change remain scarce. While studies have reported parkinsonism-associated changes in thalamic metabolism, neurochemistry and GABA receptor binding, the findings were inconsistent. Our preliminary light microscopic data from 3 control and 3 MPTP-treated parkinsonian monkeys suggest ~20% increase in vesicular GABA transporter (vGAT) immunoreactivity in the GPi-receiving region of the ventral motor thalamic nuclei in parkinsonian animals. In order to determine the source of this increased immunolabeling, we undertook an ultrastructural analysis of the morphology and prevalence of GABAergic terminals from the GPi in the anterior portion of the ventrolateral nucleus (VLa), known as the main target of sensorimotor pallidal projections in primates. Based on data about structural changes of pallidal terminals in the subthalamic nucleus (Fan et al. J Neurosci 32, 13718. 2012) and other GPi-like GABAergic terminal subtypes in the thalamus (Bodor et al. J Neurosci 28, 3090. 2008), we hypothesize that the synaptic microcircuitry of the GABAergic connections between the GPi and the anterior portion of the VLa undergoes major plastic reorganization in the parkinsonian state so that the GPi is capable of over-inhibiting its thalamic targets through an increased release of GABA. Data obtained so far demonstrate that GPi terminals have a large size, contain numerous mitochondria and form multiple symmetric synapses with large dendrites of VLa neurons. Some also contact vesicle-filled dendrites of GABAergic interneurons. A quantitative analysis is in progress to determine changes in the relative density, pattern of connectivity and number of synapses formed by individual GPi terminals in parkinsonian monkeys. The results of this study will help us to gain a better understanding of the complex network changes that affect the pallidothalamic system in the parkinsonian state, thereby contributing to our knowledge of the development of PD pathophysiology and the refinement of antiparkinsonian therapies.

Disclosures: **A.J. Sullivan:** None. **A. Shibata:** None. **C. Katona:** None. **T. Wichmann:** None. **Y. Smith:** None.

Poster

217. Therapeutics of Parkinson's Disease: Target Validation

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Support: UDALL Center Grant P50NS071669 (NIH/NINDS)

R01NS037948

Yerkes Base Grant OD P51OD011132 (NIH/ORIP)

Title: Glutamatergic denervation of striatal cholinergic interneurons in MPTP-treated Parkinsonian monkeys

Authors: *R. M. VILLALBA[1], S. LEE[1], J.-F. PARE[1], Y. SMITH[1,2];
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Abstract: The thalamus is a major source of glutamatergic innervation to the striatum. The caudal intralaminar nuclei, namely the centre median (CM) and parafascicular (Pf) nuclei, are the main sources of thalamic inputs to the putamen and caudate nucleus, respectively. Previous tract-tracing studies have shown that both striatal projection neurons and cholinergic interneurons are the main targets of CM/Pf inputs in the monkey striatum (Sidibe and Smith, 1999, Neuroscience 89:1189). Recent behavioral data indicate that the CM/Pf plays a critical role in regulating the physiological responses of cholinergic interneurons to reward-related salient sensory stimuli (Matsumoto et al., 2001, J Neurophysiol 85: 960). The CM/Pf undergoes severe degeneration in Parkinson's disease and MPTP-treated monkeys (Henderson et al., 2000, Ann Neurol 47:345; Villalba et al., 2014, Brain Struct Funct 219:381). This neuronal death is associated with a significant loss of vGluT2-immunoreactive thalamostriatal terminals in the putamen of parkinsonian monkeys (Villalba et al., 2013, Soc Neurosci Abstr 240.02). However, the impact of this breakdown of the thalamostriatal system on the thalamic innervation of specific neuronal populations in the striatum remains unknown. To address this issue, we performed a double immunolabeling study for the vesicular glutamate transporter 2 (vGluT2) (specific marker of thalamostriatal terminals) and choline acetyltransferase (ChAT) at the electron microscopic level to determine if striatal cholinergic interneurons undergo thalamic denervation in the caudate nucleus and putamen of MPTP-treated parkinsonian monkeys. Three control and three MPTP-treated adult rhesus monkeys were used in this study. The pre-embedding immunogold method was used to localize vGluT2-positive profiles, while ChAT-containing elements were labeled with immunoperoxidase. Quantitative electron microscopic analysis revealed ~30% reduction in the prevalence of unlabeled terminals forming asymmetric synapses with ChAT-containing dendrites in both the caudate nucleus and putamen of parkinsonian monkeys. In contrast, there

was no significant difference in the proportion of ChAT-positive dendrites contacted by vGluT2-positive terminals between the two groups of animals. In conclusion, these findings demonstrate that striatal cholinergic interneurons undergo glutamatergic synaptic denervation that largely affects non-vGluT2-positive terminals in parkinsonian monkeys. Studies are in progress to determine the exact source of these terminals.

Disclosures: **R.M. Villalba:** None. **S. Lee:** None. **J. Pare:** None. **Y. Smith:** None.

Poster

217. Therapeutics of Parkinson's Disease: Target Validation

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Topic: C.03. Parkinson's Disease

Support: NHMRC

Title: Direct impact of dopaminergic and noradrenergic systems on adult-hippocampal neurogenesis in adult rats and the relevance to dementia in Parkinson's disease

Authors: ***C. ERMINE**[1,2], J. L. WRIGHT[1,2], C. L. PARISH[1,2], L. H. THOMPSON[1,2];
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Abstract: A key pathological feature of Parkinson's disease (PD) is the progressive degeneration of midbrain dopaminergic neurons, causing motor dysfunction. However there are a range of 'non-movement' related features (including cognitive dysfunction, dementia and sleep disorder), which are not alleviated by dopamine replacement therapy. We are currently investigating the hypothesis that reduced hippocampal neurogenesis contributes to cognitive dysfunction in PD. We aim to characterise the effect of the dopaminergic and noradrenergic system on the adult-hippocampal neurogenesis in order to identify potential targets for the treatment cognitive impairments related to neurogenesis. We induced lesions of the different systems in adult rats using stereotaxic injections of toxins: 6-hydroxydopamine (dopaminergic system) and anti-dopa-β-hydroxylase-saporin (noradrenergic system). Four weeks later, the new cells were marked by pulses of bromodeoxyuridine (Brd-U) twice daily for 1 week. The animals were then sacrificed 4 weeks later for tissue collection. A high-performance liquid chromatography has confirmed that both lesions were successful: dopamine level in the striatum dropped to 20% and noradrenaline level in the hippocampus dropped to 8.3%. Surprisingly there was no difference in the number of Brd-U positive cells or in the number of double positive Brd-U/NeuN cells between our groups. The results show that while both noradrenergic and dopaminergic systems are implicated in the onsets of non-motor symptoms, they may not act through the regulation of adult-hippocampal neurogenesis like it was previously thought. Importantly our project has allowed reconsideration

of how neurogenesis is involved in PD and redirected the therapies to better potential targets for treatment.

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Poster

217. Therapeutics of Parkinson's Disease: Target Validation

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Topic: C.03. Parkinson's Disease

Support: NIH Grant T32DA031111

NINDS R01NS082650

Title: Loss of VGLUT3 produces hyperdopaminergia and ameliorates motor dysfunction and L-dopa mediated dyskinesias in a model of Parkinson's disease

Authors: *C. B. DIVITO[1], S.-P. G. WILLIAMS[1], J. A. STANCATI[3], E. C. HOLMSTRAND[1], D. T. CASE[1], L. ZHI[4], C. YUAN[1], N. E. CAGLE[1], T. SUN[1], M. E. RUBIO[2], C. E. SORTWELL[3], T. J. COLLIER[3], D. SULZER[5], R. H. EDWARDS[6], K. STEECE-COLLIER[3], H. ZHANG[4], R. P. SEAL[1];
[1]Neurobio., [2]Otolaryngology, Univ. of Pittsburgh, Pittsburgh, PA; [3]Translational Sci. and Mol. Med., Michagan State Univ., Grand Rapids, MI; [4]Neurosci., Thomas Jefferson Univ., Philadelphia, PA; [5]Neurol. & Psychiatry, Columbia Univ., New York, NY; [6]Neurol., Univ. of California, San Francisco, San Francisco, CA

Abstract: The striatum is essential for many aspects of mammalian behavior including motivation and movement and is dysfunctional in motor disorders such as Parkinson's disease. Previous studies have suggested a key role for the vesicular glutamate transporter (VGLUT) 3 in regulating locomotor activity, a canonical measure of basal ganglia output, through its expression by striatal cholinergic interneurons. Here we show that the hyperlocomotor activity observed in mice lacking VGLUT3 occurs during the waking cycle and is accompanied by increased dopamine synthesis, packaging and release in the striatum. We also show that contrary to the prevailing hypothesis, locomotor activity and striatal dopamine levels are surprisingly unaffected by the loss of the transporter from cholinergic neurons. The mice do however show defects in sensorimotor gating and habituation. Importantly, we find that loss of VGLUT3 prevents the development of motor deficits and markedly diminishes the appearance of L-dopa mediated dyskinesias in a model of Parkinson's disease. VGLUT3 thus profoundly regulates striatal function through multiple mechanisms, opening new avenues for understanding the regulation of

basal ganglia circuitry and potentially finding new treatment options for Parkinson's disease and related disorders.

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Poster

217. Therapeutics of Parkinson's Disease: Target Validation

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Program#/Poster#: 217.08/C68

Topic: C.03. Parkinson's Disease

Title: Combined D1-D3 dopamine receptor stimulation synergistically exacerbates dyskinesia in L-DOPA-primed hemi-Parkinsonian rats

Authors: ***S. M. MEADOWS**, L. GROSS, E. NUSS, N. CHAMBERS, C. BISHOP; Binghamton Univ., Binghamton, NY

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterized by motor deficits that result from the loss of nigro-striatal dopamine (DA) cells. Thus exogenous stimulation of DA receptors with direct and/or indirect DA agonists is standard treatment for early stage PD patients. However, chronic treatment often results in a loss of efficacy and abnormal involuntary movements (AIMs), known as dyskinesia. Recent evidence has shown that D1R and D3R co-localize in the striatum and may augment G-protein independent signaling implicated in dyskinesia. Therefore, the present study tested the behavioral effects of individual and combined D1R and D3R agonists in L-DOPA-primed hemi-parkinsonian rats. Adult male Sprague-dawley rats were given unilateral 6-hydroxydopamine lesions to the medial forebrain bundle. Three weeks later, rats were given daily L-DOPA (6 mg/kg; s.c.) for 2 weeks to establish stable dyskinesia expression. Using a within-subjects, counterbalanced design, dose-response AIMs testing was performed with the D1R agonist SKF38393 (0, 0.3, 1.0, 3.0 mg/kg; s.c.) followed by dose-response AIMs testing with the D3R agonist (+)PD128907 (0, 0.1, 0.3, 1.0 mg/kg; s.c.). Results demonstrated that both D1R and D3R agonists alone dose-dependently induced AIMs. Rats were then administered threshold doses of either SKF38393 (0.3 mg/kg; s.c.), (+)PD128907 (0.1 mg/kg; s.c.), or both after which AIMs were rated. The results showed that D1R-D3R co-activation produces significantly greater dyskinesia than individual stimulation of either receptor alone. Thus, the present study elucidates the pronounced contribution of combined D1R-D3R signaling to dyskinesia, suggesting a potential pharmacotherapeutic target for the attenuation of dyskinesia.

Disclosures: **S.M. Meadows:** None. **L. Gross:** None. **E. Nuss:** None. **N. Chambers:** None. **C. Bishop:** None.

Poster

217. Therapeutics of Parkinson's Disease: Target Validation

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Title: Early repetitive transcranial magnetic stimulation intervention exerts neuroprotective effects and ameliorates motor deficits on Parkinson's disease model of rats

Authors: ***T.-H. HSIEH**[1,2], Y.-Z. HUANG[4], A. ROTENBERG[5], Y.-H. CHIANG[3], J.-J. CHEN[6]; [1]Dept. of Physical Therapy and Grad. Inst. of Rehabil. Sci., Chang Gung Univ., Taoyuan, Taiwan; [2]Grad. Inst. of Neural Regenerative Med., [3]Dept. of Neurosurg., Taipei Med. Univ., Taipei, Taiwan; [4]Dept. of Neurol., Chang Gung Mem. Hosp. and Chang Gung Univ. Col. of Med., Taipei, Taiwan; [5]Dept. of Neurol., Boston Children's Hosp. and Harvard Med. Sch., Boston, MA; [6]Dept. of Biomed. Engin., Natl. Cheng Kung Univ., Tainan, Taiwan

Abstract: Non-invasive repetitive magnetic stimulation (rTMS) technique, including theta burst stimulation (TBS) paradigm, has been proven to be able to effectively modulate motor cortical plasticity which might have potential for the novel treatment of Parkinson's disease (PD). However, the therapeutic benefits in PD are still controversial. The severity of dopamine depletion could be critically involved for the expression of motor plasticity induced by rTMS and might further interfere the improvement of motor performance. Accordingly, we conducted the PD animal model for elucidating the possible therapeutic effects after early and long-term intervention of rTMS. An acute hemiparkinsonian rat model, generated by unilateral injection of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle (MFB), was applied to evaluate therapeutic potential of rTMS in neuroprotective effects and motor behaviors following an intermittent theta burst stimulation (iTBS) protocol. The detailed gait analysis, akinesia, apomorphine-induced turning behavior as well as dopaminergic neurons degeneration level were evaluated up to 4 weeks after daily administration of iTBS over the motor cortex. Under rTMS intervention over the course in acute PD rats, we found that the long-term and daily iTBS significantly reduced and postponed the 6-OHDA induced motor deficits in gait, akinesia and rotational behavior. Immunohistochemically, tyrosine hydroxylase (TH)-positive neurons and fibers in the substantia nigra and striatum were significantly preserved, respectively. Taken

together, these results suggest that early and daily iTBS exerts neuroprotection and reduces the aggravation of PD symptoms in PD rats model. Also, our data further highlight the potential therapeutic effects of rTMS and confirm the existence of a long-term effect of consecutive daily applications of iTBS that might be relevant to the clinical effect and useful for further potential application of human PD subjects.

Disclosures: **T. Hsieh:** None. **Y. Huang:** None. **A. Rotenberg:** None. **Y. Chiang:** None. **J. Chen:** None.

Poster

217. Therapeutics of Parkinson's Disease: Target Validation

Location: Hall A

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Program#/Poster#: 217.10/C70

Topic: C.03. Parkinson's Disease

Support: PRIN 2010-2011 #2010AHHP5H

Title: Pharmacological blockade of mTORC1 or genetic deletion of Rhes inhibit levodopa-induced dyskinesia along with GABA and glutamate release in mice substantia nigra reticulata

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Abstract: Evidence that levodopa-induced dyskinesia (LID) is mediated by activation of mammalian target of rapamycin complex-1 (mTORC1) has been provided (Santini et al, Sci Signal 2: 1-10, 2009). mTORC1 signaling cascade in striatum is modulated by both Rheb (Ras homolog enriched in brain) and Rhes (Ras homolog enriched in striatum). It was recently proved that Rhes gene deletion significantly reduces LID in 6-hydroxydopamine (6-OHDA) hemilesioned mutant mice (Subramaniam et al, Nat Neurosci 15: 191-193, 2012). In order to investigate whether mTORC1 pathway activation is involved in the modulation of direct pathway medium-sized spiny neurons (MSNs), we performed *in vivo* microdialysis in C57BL/6J 6-OHDA-hemilesioned mice subacutely treated with rapamycin and levodopa. The levodopa-induced rise of GABA and glutamate levels in substantia nigra reticulata (SNr), a neurochemical marker of LID (Bido et al, J Neurochem 118: 1043-1055, 2011; Mela et al, Neurobiol Dis 45: 573-582, 2012), was monitored simultaneously with abnormal involuntary movements. Subacute rapamycin attenuated LID development by about 50%, without compromising the therapeutic effect of levodopa. Different from control animals, acute levodopa challenge in rapamycin-treated mice did not evoke the rise of nigral amino acids. To investigate whether lack of Rhes replicated the effect of pharmacological blockade of mTORC1, microdialysis was performed in knockouts (Rhes-/-) 6-OHDA-hemilesioned mice and wild-type controls (Rhes+/+). Rhes-/-

mice developed less severe dyskinesia upon subacute levodopa treatment compared with controls (~40%), without showing changes of levodopa therapeutic effect. Different from Rhes+/+ mice, Rhes-/- mice undergoing microdialysis did not show the increase of nigral GABA and glutamate levels upon acute levodopa challenge. These data suggest that mTOR activity alterations induced either by rapamycin administration or Rhes gene targeting prevent the sensitization of striato-nigral MSNs to levodopa, further pointing to Rhes protein as a promising target in LID therapy.

Disclosures: **A. Brugnoli:** None. **A. Usiello:** None. **M. Morari:** None.

Poster

217. Therapeutics of Parkinson's Disease: Target Validation

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Program#/Poster#: 217.11/C71

Topic: C.03. Parkinson’s Disease

Title: Increased striatal acetylcholine and dopamine efflux is associated with L-DOPA-induced dyskinesia in hemi-Parkinsonian rats

Authors: ***M. CONTI**, N. PALUMBO, J. A. GEORGE, N. CHAMBERS, C. BISHOP; Psychology, Binghamton Univ., Binghamton, NY

Abstract: The dopamine (DA)-acetylcholine (ACh) balance hypothesis describes an antagonistic balance in the striatum where increased DA reduces ACh efflux and vice versa. Parkinson’s disease (PD) is typically characterized by the progressive loss of nigrostriatal DA resulting in elevated striatal ACh and hypokinetic motor symptoms. Replacing depleted DA levels with L-DOPA is the standard PD treatment, but chronic L-DOPA typically leads to the development of abnormal involuntary movements (AIMs) referred to as L-DOPA-induced dyskinesia (LID). However, it is unclear how L-DOPA treatment affects ACh neurotransmission in the PD brain. Thus, the current study sought to examine L-DOPA-induced changes in extracellular striatal DA and ACh following systemic L-DOPA treatment in L-DOPA-primed, hemi-parkinsonian rats. Adult male Sprague-dawley rats received either sham or unilateral 6-hydroxydopamine lesions of the left medial forebrain bundle and microdialysis guide cannulae into the DA-lesioned striatum. Animals were then primed with daily L-DOPA (6 mg/kg + benserazide 15 mg/kg; s.c.) for 2 weeks to establish stable AIMs. Lesion and L-DOPA-mediated striatal DA and ACh efflux was monitored with *in vivo* microdialysis while concurrent AIMs and rotations were quantified in response to L-DOPA. Surprisingly, both DA and ACh efflux positively and temporally correlated with severe LID expression and rotations in DA-lesioned rats. These findings shed new light on DA-ACh interactions in the context of L-DOPA treatment and point to novel therapeutic targeting of the ACh system.

Disclosures: **M. Conti:** None. **N. Palumbo:** None. **J.A. George:** None. **N. Chambers:** None. **C. Bishop:** None.

Poster

217. Therapeutics of Parkinson's Disease: Target Validation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 217.12/C72

Topic: C.03. Parkinson's Disease

Support: Center for Development and Behavioral Neuroscience

Title: Contribution of muscarinic acetylcholine receptors to L-DOPA-induced dyskinesia

Authors: ***N. E. CHAMBERS**[1], M. CONTI[1], C. NAMBA[1], N. PALUMBO[1], C. FELDER[2], D. MCKINZIE[2], C. BISHOP[1];
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Abstract: Parkinson's disease (PD), a neurodegenerative disorder caused by a depletion of dopamine (DA) cells in the substantia nigra, is characterized by motor symptoms that eventually necessitate the use of L-DOPA DA replacement therapy. However, chronic L-DOPA treatment results in L-DOPA-induced dyskinesia (LID), characterized by abnormal involuntary movements (AIMs). The exact cause of LID is unknown, but recent evidence has suggested that an L-DOPA-induced imbalance in striatal DA and acetylcholine (ACh) may contribute. With improved drug discovery and synthesis, muscarinic ACh receptors (mAChRs) have been identified as potential therapeutic targets for attenuating LID. In order to better understand the contribution of ACh and mAChRs to LID, the current study examined the potential for a panel of mAChR agonists and antagonists to modulate LID in unilateral 6-OHDA-lesioned Sprague-Dawley rats. After 3 weeks of recovery from surgery, rats were primed with L-DOPA, and monitored for AIMs to ensure stable expression of LID. Selective and non-selective mAChR agonists and antagonists were administered subcutaneously before L-DOPA in a counterbalanced within-subjects design, after which rats were then tested for AIMs as well as motor performance using the forepaw adjusting steps test. The results of this study suggest that mAChRs can modulate LID and preliminarily implicate the promise of the mAChR 4 subtype for the treatment of LID.

Disclosures: **N.E. Chambers:** None. **M. Conti:** None. **C. Namba:** None. **N. Palumbo:** None. **C. Felder:** None. **D. McKinzie:** None. **C. Bishop:** None.

Poster

217. Therapeutics of Parkinson's Disease: Target Validation

Location: Hall A

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Program#/Poster#: 217.13/C73

Topic: C.03. Parkinson's Disease

Support: Swedish Research Council

European Community FP7

Title: Involvement of phospholipase C in the dopaminergic treatment of Parkinson's disease: effects on dyskinetic behaviors and ERK1/2 signaling activation

Authors: *I. SEBASTIANUTTO, N. MASLAVA, M. A. CENCI;
Exptl. Med. Science; Basal Ganglia Pathophysiology Unit, Lund Univ., Lund, Sweden

Abstract: In animal models of Parkinson's disease (PD) treated with L-DOPA, a large striatal activation of extracellular signal-regulated kinases 1 and 2 (ERK1/2) is associated with the development of dyskinesia. Antagonists of metabotropic glutamate receptor type 5 (mGluR5) reduce the striatal activation of ERK1/2 by L-DOPA and have antidyskinetic effects. In the dopamine (DA)-denervated striatum *ex vivo*, the ERK1/2 activation induced by SKF38393 (a D1/D5 receptor agonist) can be inhibited by antagonizing mGluR5 or its downstream signaling mediator, phospholipase C (PLC) (Fieblinger et al. J. Neurosci. 2014). Based on these results, we set out to examine the effects of PLC inhibition *in vivo*. Rats sustained unilateral 6-OHDA lesions of the nigrostriatal DA pathway followed by chronic treatment with either L-DOPA or SKF38393. Thereafter, animals that had developed dyskinesia received challenge injections of the PLC inhibitor U73122 (10 or 30 mg/kg) or its inactive analogue, U73343, together with the dopaminergic treatment. U73122 dose-dependently reduced the severity of peak-dose dyskinesia without altering the rats´ performance in tests of forelimb use and general motor dexterity. The effects of U73122 were more potent when the dyskinesia-inducing agent was SKF38393. By contrast, U73122 was ineffective on dyskinesias evoked by the D2/D3 agonist, Quinpirole. The dose of U73122 exerting significant antidyskinetic effects blunted the striatal activation of ERK1/2 and the phosphorylation of histone 3 (pH3) induced by either L-DOPA or SKF38393 (Quinpirole did not induce these markers). In keeping with previous findings *ex vivo*, these results demonstrate that the striatal activation of ERK1/2 downstream of supersensitive D1 receptors requires PLC activity. These results will hopefully inform the development of novel antidyskinetic treatments that selectively target an abnormal communication between D1 receptors and PLC-dependent signaling.

Disclosures: I. Sebastianutto: None. **N. Maslava:** None. **M.A. Cenci:** None.

Poster

217. Therapeutics of Parkinson's Disease: Target Validation

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Topic: C.03. Parkinson's Disease

Support: The Michael J. Fox Foundation

Jean Perkins Foundation

Title: A small molecule ligand for TrkB/TrkC neurotrophin receptors improves motor dysfunction and pathology in cellular and mouse MPTP-induced Parkinson's models

Authors: *F. M. LONGO[1], T. YANG[1], K. C. TRAN[1], N. PHELPS[1], C. J. CONDON[1], D. A. SIMMONS[1], S. M. MASSA[2,3];
[1]Dept. of Neurol. and Neurolog. Sci., Stanford Univ. Med. Ctr., Stanford, CA; [2]San Francisco Veterans Affairs Med.Ctr., San Francisco, CA; [3]Neurol., Univ. of California, San Francisco, CA

Abstract: Multiple lines of evidence suggest that the neurotrophin receptors TrkB, which selectively binds brain-derived neurotrophic factor (BDNF), and TrkC, the cognate NT-3 receptor would be particularly effective therapeutic targets for PD. Both receptors are expressed by substantia nigra (SN) dopaminergic (DA) and striatal neurons, their survival depends on BDNF and NT-3, and loss of BDNF may occur in the SN of PD patients. TrkB and/or TrkC mutations cause hypersensitivity of nigrostriatal DA neurons to MPTP and BDNF/TrkB protects against MPTP-induced degeneration. These findings suggest that deficiencies in Trk receptor signaling contribute to PD pathogenesis and that compounds which restore this signaling could be important therapeutic candidates. Our laboratories have developed a small molecule, non-peptide ligand, BC-2, that binds to and activates both TrkB and TrkC, but not TrkA or p75 receptors. We hypothesized that BC-2 would have positive effects on morphological, behavioral and biochemical deficits in cell culture and/or mouse MPTP-induced PD models. In *in vitro* studies, BC-2 at nanomolar concentrations rescued mouse embryonic DA neuron degeneration induced by MPTP and promoted human stem cell differentiation into TH-positive neurons. In normal mice, BC-2 from 10-150 mg/kg (intraperitoneal, IP) achieved significant brain levels, and activated TrkB and TrkC and their downstream targets. 50mg/kg was determined to be an optimal dose for *in vivo* studies. In a neuroprotection study in mice, daily IP BC-2 began 3 days before and continued throughout MPTP injections and behavior testing for 14 days. In a neurorestoration study, BC-2 treatment was begun one day after the last MPTP injection and continued throughout behavioral testing until mice were euthanized at 28 days. In both studies, BC-2 significantly improved motor outcomes, reduced the loss of SN TH-positive neurons and neurites, inhibited decreases in striatal dendritic spine density, and reversed deficits in the levels of phosphorylated Trks and their downstream signaling components. Overall, these results support the idea that concomitant targeting of TrkB and TrkC might offer a novel, pharmacologically feasible small molecule strategy for treatment of PD patients.

Disclosures: **Drs. Longo and Massa** are listed as inventors on patents relating to a compound in this report, which are assigned to the University of North Carolina, University of California, San Francisco and the Dept. of Veterans Affairs. Drs. Longo and Massa are entitled to royalties distributed by the assigned universities per their standard agreements. Dr. Longo is a principal of, and has financial interest in PharmatrophiX, a company focused on the development of small molecule ligands for neurotrophin receptors, which has licensed several of these patents **T. Yang:** None. **K.C. Tran:** None. **N. Phelps:** None. **C.J. Condon:** None. **D.A. Simmons:** None.

Keyword(s): NEUROTROPHIN
SUBTANTIA NIGRA
PARKINSON'S DISEASE

Support: The Michael J. Fox Foundation
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Disclosures: **F.M. Longo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of North Carolina, University of California, San Francisco and the Dept. of Veterans Affairs. **T. Yang:** None. **K.C. Tran:** None. **N. Phelps:** None. **C.J. Condon:** None. **D.A. Simmons:** None. **S.M. Massa:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of North Carolina, University of California, San Francisco and the Dept. of Veterans Affairs..

Poster

217. Therapeutics of Parkinson's Disease: Target Validation

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 217.15/C75

Topic: C.03. Parkinson's Disease

Support: Horngren Family Alzheimer's Research Fund

Jean Perkins Foundation

Title: A small molecule mediates co-activation of TrkB/TrkC neurotrophin receptors, and promotes novel signaling patterns and bioactivities including neurite outgrowth on inhibitory surfaces

Authors: *T. YANG[1], S. M. MASSA[2,3], K. C. TRAN[1], D. A. SIMMONS[1], J. RAJADAS[1], T. JANG[1], S. CARSANARO[1], F. M. LONGO[1];
[1]Neurol. and Neurology. Sci., Stanford Univ., Stanford, CA; [2]San Francisco Veterans Affairs Med.Ctr., San Francisco, CA; [3]Neurol., Univ. of California, San Francisco, CA

Abstract: These authors made equal contributions to this work. Neurotrophin receptors are coupled to numerous signaling cascades that play critical roles in neuronal survival and plasticity. Several non-peptide small molecule ligands have recently been reported that bind to and activate specific tyrosine-receptor kinase (Trk) neurotrophin receptors, stimulate their downstream signaling and cause biologic effects similar to, though not completely overlapping, those of the native protein ligands. Using *in silico* screening coupled with low-throughput neuronal survival screening (Massa et al., JCI. 2010), a new compound has been identified, BC-2, that unlike prior small molecule compounds is capable of: selectively binding to and activating two of the three Trk receptors, TrkB and TrkC; promoting neurite outgrowth superseding maximal levels obtainable with brain derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3) or a combination of the two in cultured mouse hippocampal neurons; and unlike BDNF or NT-3, supporting neurite outgrowth in an inhibitory environment consisting of myelin-associated glycoprotein (MAG) and chondroitin sulfate proteoglycans (CSPGs). In young and aged mice, the compound activates hippocampal and striatal TrkB and TrkC, and their downstream signaling AKT, ERK and PKC, and increases hippocampal dendritic spine density and synaptic markers in aged mice. Thus, BC-2 constitutes a new tool for the study of TrkB and TrkC signaling, and supports the possibility of developing ligands that stimulate unique combinations of Trk receptors and activity patterns applicable to neuronal populations and deficits present in various disease states.

Disclosures: **Drs. Longo and Massa** are listed as inventors on patents relating to a compound in this report, which are assigned to the University of North Carolina, University of California, San Francisco and the Dept. of Veterans Affairs. Drs. Longo and Massa are entitled to royalties distributed by the assigned universities per their standard agreements. Dr. Longo is a principal of, and has financial interest in PharmatrophiX, a company focused on the development of small molecule ligands for neurotrophin receptors, which has licensed several of these patents. **T. Yang:** None. **K.C. Tran:** None. **D.A. Simmons:** None. **J. Rajadas:** None. **T. Jang:** None. **S. Caranaro:** None.

Keyword(s): TROPOMYOSIN-RELATED KINASE B AND C
BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF)
NEUROTROPHIN 3 (NT-3)

Support: Horngren Family Alzheimer's Research Fund
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Disclosures: **T. Yang:** None. **S.M. Massa:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of North Carolina, University of California, San Francisco and the Dept. of Veterans Affairs. **K.C. Tran:** None. **D.A. Simmons:** None. **J. Rajadas:** None. **T. Jang:** None. **S. Carsanaro:** None. **F.M. Longo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); University of North Carolina, University of California, San Francisco and the Dept. of Veterans Affairs.

Poster

217. Therapeutics of Parkinson's Disease: Target Validation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 217.16/C76

Topic: C.03. Parkinson's Disease

Support: Santos Dumont Institute

AASDAP

FINEP

INCEMAQ (Programa INCTs do CNPq/MCT)

FAPERN

CAPES

CNPq

Title: Lasting spontaneous negative motor effect of dopamine synthesis blocker alpha-methyl-para-tyrosine (AMPT) in common marmosets

Authors: *H. S. PEREIRA[1,2], K. COSTA[1], M. F. P. ARAÚJO[1];
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Abstract: The most prominent symptom in many pathological states, such as Parkinson disease, is motor disability. Most animals models of motor system impairments either induce chronic irreversible deficits or highly acute deficits lasting just a few hours. Alpha-methyl-para-tyrosine (AMPT) injections have been previously used in chronic animal models to enhance the motor impairments for a few hours. In the present study we evaluated the effects of AMPT injections in four normal marmosets. The common marmoset (Callithrix jacchus) is a New world primate used extensively in biomedical and behavioral research which have many brain structures similarities with humans allowing for better translational inferences. Four marmosets were injected subcutaneously with AMPT (2 doses of 240 mg/kg, dissolved in physiological saline, with a 3 hours interval between them). The animals daily spontaneous locomotion 3 days before and 6 days after AMPT injections were measured by an actimeter (Actiwatch Mini©, CamNtech Ltd) placed inside a bag attached to the torso of each animal. Motor disabilities following AMPT were also evaluated in 2 animals using the Manual Parkinson Disease Scoring (MPDS) developed by Santana (adapted from Fahn's UPDRS). The MPDS adapted scale consists of 16 categories scored from zero to three, which corresponds to absence of altered state to more intense symptomatology, respectively. Some categories involve symptoms that were evaluated for each body part individually (i.e., limbs, trunk, head). Hence, the maximum total score of the scale is 48 points. The motor examination was performed daily in the animal's home cage. The daily spontaneous locomotion decreased during days 1-3 after AMPT injection in all 4 animals. In addition, the MDPS score increased during 3 days after AMPT injection in both marmosets evaluated. These results suggest that subcutaneous injection of AMPT can be used as an acute model to study motor disabilities in commom marmosets.

Disclosures: **H.S. Pereira:** A. Employment/Salary (full or part-time):; State University of Rio Grande do Norte. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Edmond and Lily Safra International Institute of Neuroscience. **K. Costa:** None. **M.F.P. Araújo:** None.

Poster

217. Therapeutics of Parkinson's Disease: Target Validation

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Topic: C.03. Parkinson's Disease

Support: Drexel University Innovation Fund

Drexel-coulter translational research fund

Title: Characterization of D3 receptor agonists with atypical signaling and receptor trafficking properties

Authors: *S. KORTAGERE[1], W. XU[2];
[1]Microbiology and Immunol., [2]Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Dopamine D3 receptor (D3R) has been evidenced to play a critical role in the etiology of both the Parkinson's disease (PD) and levodopa (L-dopa)-induced dyskinesia (LID). A number of dopaminergic agents from partial agonists to antagonists have been tested either as L-dopa replacement agents or as adjuvants with L-dopa therapy with limited success. Our previous results showed that compound SK609, a novel selective D3R agonist with atypical properties such as biased-ERK signaling, did not induce desensitization of D3R *in vitro*. SK609, significantly improved motor impairments associated with PD-like symptoms in a 6-OHDA induced hemiparkinson rat model of PD. Chronic treatment of SK609 did not induce abnormal involuntary movements (AIMs) but significantly reduced AIMs induced by L-dopa when used adjuvantly with L-dopa. In our quest to identify novel analogs of SK609 that possess similar atypical properties but with improved affinity for D3R, we have identified SK608, a selective D3R agonist. SK608, like its parent compound does not induce desensitization of D3R stably expressed in CHO cells and dose- and time-dependently induces internalization of D3R. These results are in complete contrast with the signaling properties of other known D3R agonists such as Dopamine and PD128907, which significantly induce desensitization but not internalization of D3R. D3R are only known to undergo pharmacological sequestration in response to these known D3R agonists. Our preliminary results suggest that the internalization induced by SK608 contributes to the re-sensitization of D3R signaling and receptor trafficking which may explain its novel therapeutic efficacy observed in alleviating the symptoms of PD and LID in rodent model.

Disclosures: **S. Kortagere:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Polycore Therapeutics LLC. **W. Xu:** None.

Poster

217. Therapeutics of Parkinson's Disease: Target Validation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: C.03. Parkinson's Disease

Support: NRF Grant 2013-008773

Title: Pharmacological rescue of defective mitophagy in PINK1 deficiency

Authors: J. KIM, *S. YANG, J. HAN, M. KANG, J. H. SON;
Ewha Womans Univ., Seoul, Korea, Republic of

Abstract: At present, no effective therapeutic measures are available to alleviate mitochondrial deficits in Parkinson's disease (PD), despite the implication of mitochondrial dysfunction as one of the major pathological mechanisms underlying the dopaminergic neuronal loss. Mitophagy is the selective autophagy of mitochondria, which is a critical quality control mechanism involved in removal of damaged or excess mitochondria. The failure in mitophagy is implicated in the mitochondrial dysfunction observed in familial PD that is caused by PINK1 or Parkin gene mutations. According to the PINK1-Parkin signaling model, mitophagy is promoted by the mitochondrial translocation of Parkin, an essential PINK1-dependent step. We have identified that low levels of nitric oxide (NO), produced by phospho-nNOS on damaged mitochondria, was sufficient to induce the mitochondrial translocation of Parkin even in PINK1 deficiency (Han J. et al. J. Biol. Chem. (2015)) Especially, optimum levels of NO treatment enabled PINK1-null cells to regain the mitochondrial translocation of Parkin, which appeared to be significantly suppressed by both NAAN, an nNOS inhibitor, and nNOS-null mutation in wild type cells. Moreover, nNOS-null mutation resulted in the similar mitochondrial OXPHOS enzyme deficits as PINK1-null mutation. A functional significance of nNOS activation in mitophagy was further confirmed by demonstrating the increased interaction of the full-length PINK1 with nNOS on mitochondria, which was accompanied by mitochondrial accumulation of phospho-nNOS during mitophagy. Of interest, L347P PINK1 mutant, a causal monogenic mutation of familial PD, failed to interact with nNOS and to mediate subsequent induction of mitophagy. Furthermore, NO-induced mitophagy help recover from the mitochondrial OXPHOS deficits in PINK1-null cells. Therefore, we first demonstrate a novel functional role for NO in induction of Parkin translocation and mitophagy, suggesting the feasibility of pharmacotherapy for defective mitophagy in PINK1 deficiency.

Disclosures: J. Kim: None. **S. Yang:** None. **J. Han:** None. **M. Kang:** None. **J.H. Son:** None.

Poster

217. Therapeutics of Parkinson's Disease: Target Validation

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Program#/Poster#: 217.19/C79

Topic: C.03. Parkinson's Disease

Support: NRF Grant 2013-008773

Title: Oxi-alpha and Oxi-beta as potential therapeutic targets in autophagic dysregulation caused by oxidative stress in a dopaminergic neuron model

Authors: *J.-S. KIM, Y. JANG, J. H. SON;
Ewha Womans Univ., Seoul-City, Korea, Republic of

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder which is characterized by specific cell death of dopaminergic (DA) neurons in the substantia nigra. Both altered autophagy and oxidative stress (OS) are considered as major pathogenic factors contributing to DA neurodegeneration, but it is largely unknown how autophagy is disrupted by OS. We have characterized the autophagy regulator gene family including Oxi-alpha and Oxi-beta (Choi et al., J. Neurochem. 2010). Oxi-alpha and Oxi-beta exhibited the opposing actions towards mTOR phosphorylation, one of the critical regulatory steps of autophagy and translation. Oxi-alpha was down-regulated by OS, and overexpression of Oxi-alpha increased mTOR phosphorylation via unknown mechanism, subsequently suppressing the accumulation of autophagosome. In contrast, Oxi-beta was up-regulated during OS-induced dopaminergic cell death. Oxi-beta inhibited mTOR phosphorylation and subsequently induced autophagy. We have identified that Oxi-β acted to stabilize tuberous sclerosis complex 2 (TSC2) through competitively interacting with FBXW5, E3-ubiquitin ligase (Ha et al., J. Neurochem. 2014), resulting in decreased mTOR phosphorylation and increased autophagy under OS. Our results suggest that, under pathogenic OS condition, the altered expression of Oxi-α and Oxi-β may act synergistically to increase autophagy in dopaminergic neurons, and they might be potential therapeutic targets in dysregulated autophagy under OS condition observed in PD.

Disclosures: J. Kim: None. **Y. Jang:** None. **J.H. Son:** None.

Poster

217. Therapeutics of Parkinson's Disease: Target Validation

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 217.20/C80

Topic: C.03. Parkinson's Disease

Title: Photobiomodulation in aged nonhuman primates

Authors: *J. H. KORDOWER[1], S. MULLER[2], K. MAREK[4], G. TAMAGNAN[4], R. HARKER[3], B. HILLER[3], K. OH[3], B. LOVISA[5], A. PITZSCHKE[5], G. WAGNIERES[5];
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Abstract: The purpose of this study was to develop a medical device capable of altering the progression of Parkinson's disease through photobiomodulation (PBM) using near-infrared light in the substantia nigra pars compacta (SNpc) region of aged Rhesus monkeys. Four aged (≥19 yrs) rhesus monkeys were unilaterally implanted with a hollow catheter into the substantia nigra,

through which an optical fiber was later introduced to illuminate the target region. Pre- and post-operative MRIs were taken to confirm positioning of the catheter. Monkeys received illumination for 100 seconds with near-infrared light at 808 nm once every other day for four weeks with irradiances ranging between 1 and 100 mW/cm^2 to determine dose-response. *In vivo* PE2I (DAT) SPECT scan results were variable across animals and dose levels. Relative to the unimplanted and unilluminated side, reductions in the dopaminergic nigrostriatal system on the implanted/illuminated side were seen with tyrosine hydroxylase (TH)-immunoreactive stereological cell counts, (Monkey 1: -29.73%, Monkey 2: -29.92%, Monkey 3: -40.25%, Monkey 4: 52.67%), TH optical density in the putamen (Monkey 1: +1.71%, Monkey 2: -42.44%, Monkey 3: +9.46%, Monkey 4: -29.60%) and caudate nucleus (Monkey 1: 18.78%, Monkey 2: -34.79%, Monkey 3: + 4.85%, Monkey 4: -27.33%), and striatal dopamine via HPLC (Monkey 1: -41.72%, Monkey 2: -51.66%, Monkey 3: +1.26%, Monkey 4: 67.24%). This pilot data suggests that surgical implantation of the catheter plus near-infrared illumination of the SNpc can, in certain conditions, induce a degeneration of nigrostriatal neurons in aged nonhuman primates. Caution should be employed prior to clinically using this approach until parameters can be established that induce a positive influence on this system, as is the case in separate studies performed in rodent models.

Disclosures: **J.H. Kordower:** 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.; ARETFACT. **S. Muller:** None. **K. Marek:** None. **G. Tamagnan:** None. **R. Harker:** None. **B. Hiller:** None. **K. Oh:** None. **B. Lovisa:** None. **A. Pitzschke:** None. **G. Wagnieres:** None.

Poster

217. Therapeutics of Parkinson's Disease: Target Validation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 217.21/C81

Topic: C.03. Parkinson’s Disease

Support: National Natural Science Foundation of China No.81201861

Title: Effects of mesenchymal stem cell differentation to DA induced by linear micro and nano-topology for Parkinson`s disease

Authors: ***L. QI**, X. ZHANG;
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Abstract: Precursor cell differentiation of Dopamine (DA) neural is the most feasible way for Parkinson`s disease (PD) treatment. Mesenchymal Stem Cells (MSC) have potentiality into DA neuron differentiation which was influenced by many factors. Physics factor was focused on the

MSC proliferation and differentiation. We used processing technique of micro and nano-meter topology to get 0.7um and 2um substrates with linear pattern (LMP) for MSC culturing. We found that LMP depressed adult neural stem cells proliferation when compared to non-patterned substrates. Thus, this finding has led us to the rational for the proposed research, which will validate LMP to regulate MSC into DA differentiation. The aims of study are: 1) Improve the LMP substrates and choose optimal substrates to increase MSC into DA differentiation; 2) To verify the effects of LMP on differentiation to DA in MSC. The present data showed LMP depress ANSC proliferation when compared to non-patterned substrates (control). Meanwhile, LMP can significantly enhance MSC differentiation to DA. The smaller the feature size is, the better upregulation applies to the differentiation; 3) The underlying mechanisms of topography-enhanced DA differentiation are further revealed by directing suppression of mitogen activated protein kinase/extracellular signaling-regulated kinase (MAPK/Erk) signaling pathway in MSC using U0126, known to inhibit the activation of Erk. The currently data suggest MAPK/ Erk pathway is partially involved in topography-induce differentiation. Furthermore, the therapeutic effect with DA induced by LMP will be investigated in PD animal model. Taken together, successful completion of these studies will therefore form a basis for algorithms to help elucidate mechanism of MSC regulating DA neuron differentiation by nano-substracts, to provide the strategies aimed at laying the foundation for further PD clinical studies.

Disclosures: L. Qi: None. **X. Zhang:** None.

Poster

217. Therapeutics of Parkinson's Disease: Target Validation

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Topic: C.03. Parkinson’s Disease

Support: French Research Agency (ANR-14-RARE-0001-01)

ERA-Net for Research on Rare Diseases

LABEX BRAIN ANR-10-LABX-43

Title: Reducing C-terminal truncation mitigates synucleinopathy and neurodegeneration in a transgenic model of multiple system atrophy

Authors: *W. MEISSNER[1], F. BASSIL[1], P.-O. FERNAGUT[1], E. BEZARD[1], Q. HOANG[2], D. RINGE[3], G. PETSKO[4];
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Abstract: Multiple system atrophy (MSA) is a sporadic orphan neurodegenerative disorder. No treatment is currently available to slow down the aggressive neurodegenerative process and patients die within a few years after disease onset. The cytopathological hallmark of MSA is the accumulation of alpha-synuclein (α-syn) aggregates in affected oligodendrocytes. Several studies point to α-syn oligomerization and aggregation as a mediator of neurotoxicity in synucleinopathies including MSA. C-terminal truncation by the inflammatory protease caspase-1 has recently been implicated in the mechanisms that promote aggregation of α-syn *in vitro* and in neuronal cell models of α-syn toxicity. We here present an *in vivo* proof of concept of the ability of the caspase-1 inhibitor prodrug VX-765 to mitigate α-syn pathology and to mediate neuroprotection in PLP-SYN mice, a transgenic mouse model of MSA. PLP-SYN and age-matched wild-type mice were treated for a period of 11 weeks with VX-765 or placebo. VX-765 prevented motor deficits in PLP-SYN mice compared to placebo controls. More importantly, VX-765 was able to limit the progressive toxicity of α-syn aggregation by reducing its load in the striatum of PLP-SYN mice. Not only did VX-765 reduce truncated α-syn but also decreased its monomeric and oligomeric forms. Finally, VX-765 showed neuroprotective effects by preserving tyrosine hydroxylase positive neurons in the substantia nigra of PLP-SYN mice. In conclusion, our results suggest that VX-765, a drug that was well tolerated in a six-week-long phase 2 trial in patients with epilepsy, is a promising candidate to achieve disease modification in synucleinopathies by limiting α-syn accumulation.

Disclosures: **W. Meissner:** None. **F. Bassil:** None. **P. Fernagut:** None. **E. Bezard:** None. **Q. Hoang:** None. **D. Ringe:** None. **G. Petsko:** None.

Poster

217. Therapeutics of Parkinson's Disease: Target Validation

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Support: SAF2010-17167

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Fundación Ramón Areces

AFA Försäkring

VR Swedish Research Council

Title: Acquisition of a complete dopaminergic a9-subtype electrophysiological phenotype and synaptic integration. of human ventral mesencephalic neural stem cells in a rodent hemiParkinsonian model

Authors: *T. RAMOS-MORENO[1], M. P. PEREIRA[2], N. AVALIANI[1], A. NELKE[2], M. KOKAIA[1], A. MARTINEZ-SERRANO[2];
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Abstract: Cell replacement therapy in Parkinson's Disease still miss a study addressing electrophysiological properties of human grafted neural stem cells and its correlation with behavioral recovery after transplantation in parkinsonian animal models. Here we study the electrophysiological profiles of two grafted ventral mesencephalic (VM) human Neural Stem Cells (hNSCs) clonal lines that are forced to overexpress Bcl-XL to preserve their neurogenic capacity, named C30-Bcl-XL and C32-Bcl-XL, onto the parkinsonian model of coronal striatal slices. Electrophysiological recordings show that these C30-Bcl-XL and C32-Bcl-XL cells integrate, mature and are able to achieve a complete A9-subtype phenotype 6 weeks after grafting into the striatal slice. Their electrophysiological properties include spontaneous pacemaker-like action potentials, highly regular firing pattern, long duration action potentials, high spike threshold and a sag component. This result is the first communication of a complete A9-subtype electrophysiological profile of DAn derived from any human stem cell in a PD model. In addition, we transplanted C30-Bcl-XL and C32-Bcl-XL cells in hemiparkinsonian animals to study behavioral recovery at 7 weeks post grafting and immune reaction of the host tissue towards the transplanted cells up to 12 weeks post grafting. A significant behavioral improvement was observed for C30-Bcl-XL transplanted animals at the short time point of 7 weeks. Our data also show a low immune reaction that would allow migration and integration of our human cell lines in the host tissue in future long-term studies.

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Poster

218. Mitochondrial and Other Neuronal-Glial Mechanisms in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: Krembil Foundation

Brain Canada Foundation

Parkinson Society Canada

Title: Elevated mitochondrial bioenergetics and axonal arborization size are key contributors to vulnerability of dopamine neurons in Parkinson's disease

Authors: *N. GIGUERE[1], C. PACELLI[1], M.-J. BOURQUE[1], M. LÉVESQUE[2], R. S. SLACK[3], L.-É. TRUDEAU[1];
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Abstract: Although the mechanisms underlying the loss of neurons in Parkinson's disease are not well understood, impaired mitochondrial function and pathological protein aggregation are suspected as playing a major role. Why dopamine (DA) neurons and a select small subset of brain nuclei are particularly vulnerable to such ubiquitous cellular dysfunctions is presently one of the key unanswered questions in Parkinson's disease research. One intriguing hypothesis is that their heightened vulnerability is a consequence of their elevated bioenergetic requirements. Here we used mouse DA neurons in primary culture to show for the first time that vulnerable nigral DA neurons differ from less vulnerable DA neurons such as those of the VTA (ventral tegmental area) by having a higher basal rate of mitochondrial OXPHOS (oxidative phosphorylation) and a smaller reserve capacity, as measured using a Seahorse bioanalyzer. We also find that nigral DA neurons have a higher density of axonal mitochondria, an elevated level of basal oxidative stress and a considerably more complex axonal arborization. Furthermore, we demonstrate that reducing axonal arborization by acting on axon guidance pathways with semaphorin 7A reduces in parallel the basal rate of mitochondrial OXPHOS and the vulnerability of nigral DA neurons to the neurotoxic agents MPP+ (1methyl4phenylpyridinium) and rotenone. Blocking L-type calcium channels with isradipine shows a similar effect, although it does not protect against rotenone. Our data provide the most direct demonstration to date in favor of the hypothesis that the heightened vulnerability of DA neurons in Parkinson's disease is directly due to their particular bioenergetic and morphological characteristics.

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Poster

218. Mitochondrial and Other Neuronal-Glial Mechanisms in Parkinson's Disease

Location: Hall A

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Topic: C.03. Parkinson's Disease

Support: Michael J. Fox Foundation Target Validation Award

Dartmouth SYNERGY Scholars Award

The Hitchcock Foundation

Title: A role for Nlrp3 in mediating rotenone-induced neuroinflammation

Authors: *M. C. HAVRDA[1], J. SULLIVAN[2], L. WANG[3], Y. PATANKAR[1], B. BERWIN[1], S. LEE[1], M. FELDMAN[1], A. YOUNG[1];
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Abstract: Pathologic examination of post-mortem tissues obtained from Parkinson's disease (PD) patients, animal models of PD and large scale epidemiologic studies implicate neuroinflammation in the development and progression of PD. Exposure to the pesticide rotenone is associated with the development of PD in agricultural workers and is the basis of rodent models of PD. In spite of these findings, the cellular and molecular mechanisms of rotenone-induced neuroinflammation remain unclear. Zhou and others identified a pro-inflammatory function for rotenone in blood cells where rotenone induced ROS-dependent activation of the Nlrp3 inflammasome. The Nlrp3-inflammasome is an intracellular mediator that can initiate an inflammatory cascade in response to cellular stress that is known to be active in Alzheimer's disease. We do not yet know if the Nlrp3 inflammasome mediates neuroinflammation associated with rotenone exposure in mice or during the progression of PD. To understand whether ingestion of small doses of rotenone could translate into neuroinflammation we exposed mice to rotenone, using intragastric gavage, over an extended time period. Using this model system, we observed progressive behavioral and histopathologic symptoms of Parkinsonism. Associated with PD symptomology we identified neuroinflammatory changes including an elevated pro-inflammatory cytokine profile and evidence of leukocyte recruitment in the CNS. Implicating *Nlrp3* in these neuroinflammatory changes, flow cytometry and immunohistologic studies indicated that peripheral innate immune cells, specifically $CD11b^{+}$, $Ly6G^{-}$, $Ly6C^{lo}$ "patrolling" monocytes, were recruited into the CNS of WT mice ingesting rotenone but not into the brains of rotenone-treated $Nlrp3^{-/-}$ mice. Further implicating Nlrp3 in mediating rotenone-induced neuroinflammation, we observed a sustained elevation of the canonical *Nlrp3*-dependent cytokines IL1b and IL6 in brain extracts obtained from wild-type mice ingesting rotenone but not in similarly treated $Nlrp3^{-/-}$ mice. Seeking a cellular origin for these cytokines in the CNS, we observed that in primary mixed glial cultures, $CD11b^{+}$ microglia were the sole source of rotenone-induced Nlrp3-dependent IL1b. Planned studies will extend these findings to evaluate the activation of the NLRP3 inflammasome in post-mortem tissues obtained from patients diagnosed with sporadic PD. The completion of these studies is expected to have broad reaching implications for our understanding of neuroinflammation occurring as the result of both long-term exposure to environmental toxins and PD.

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Poster

218. Mitochondrial and Other Neuronal-Glial Mechanisms in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: Fapesp 2012/15495-2

CNPq 240703/2012-0

Title: Impaired mitochondria trafficking and autophagy in hiPSC-derived dopaminergic neurons harboring three copies of SNCA gene

Authors: *M. FERRARI[1], T. Q. MELO[1,2], K. C. VAN ZOMEREN[2], S. C. V. M. COPRAY[2], E. W. G. M. BODDEKE[2];
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Abstract: Background: Protein aggregates containing α-synuclein, increased oxidative stress due mitochondria dysfunction and disturbances in autophagy are hallmarks of Parkinson's disease. The increased levels of α-synuclein can impair mitochondria function and autophagy. Mitochondria are essential organelles for neuronal function; their dynamics is dependent of proper trafficking to cell body and terminals. Alterations in the mitochondria transportation can precede the presence of aggregates and contribute to cell death. However, the link between alteration in mitochondrial transport and protein aggregation involved in neurodegenerative diseases is still unresolved. Human induced pluripotent stem cells (iPSCs) derived from patients suffering from neurodegenerative disorder allows the investigation of the cell pathogenesis process. Objective: The objective of this study is to analyze mitochondrial trafficking and autophagy in the human dopaminergic neurons derived from iPSCs of a Parkinson patient with a triplication of the alpha-synuclein gene (SNCA3) and of a healthy age matched control. . Design/methods: hiPSCs were differentiated into dopaminergic neurons based on dual SMAD inhibition following the protocol of Kriks et al. in 2011 (doi: 10.1038/nature10648.). Dopaminergic neurons were analyzed by immunocytochemistry to confirm the phenotype. Mitochondrial mobility and autophagy were analyzed in living cells using Mitotracker and Lyso-tracker respectively using confocal microscopy and 63x objective after 60 and 90 days of differentiation. Parallel to the differentiation of hiPSCs into dopaminergic neurons, neuroblastoma cells (SHSY5Y) containing α-synuclein wild-type or A30P or A53T were differentiated into DA neuron-like cells by exposure to retinoic acid during 5 days. Mitochondria mobility was analyzed using pDSRED2-mito after 4, 6 and 8 days of differentiation. Results: Mitochondria trafficking was decreased (50%) after 90 days of differentiation in human dopaminergic neurons derived from iPSC expressingSNCA3. In addition, lisossomes were accumulated (100%) after 90 days of differentiation in human dopaminergic neurons derived

from iPSC containing SNCA3. Retrograde trafficking was preferentially decreased (50%) after 6 days of differentiation in SHSY5Y containing α-synuclein A53T. Mitochondria is also fragmented after 8 days of differentiation in SHSY5Y containing α-synuclein A53T. Conclusion: The overexpression of alpha-synuclein as well as the expression of the mutant A53T can disturb mitochondria dymanics and autophagy, which is a mechanism that may trigger cell death during the course of Parkinson's disease.

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Poster

218. Mitochondrial and Other Neuronal-Glial Mechanisms in Parkinson's Disease

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 218.04/C87

Topic: C.03. Parkinson's Disease

Support: Fritz Thyssen Foundation

Title: Loss of mitochondrial chaperone protein mortalin in a *Drosophila* model of Parkinson's disease causes functional defects which are rescued by downregulation of Autophagy

Authors: *S. B. HANNAN[1], J. Y. ZHU[2], T. M. RASSE[3];
[1]Hertie Inst. of Clin. Brain Res., Tübingen, Germany; [2]Hertie Inst. of Clin. Brain Res., Tuebingen, Germany; [3]German Cancer Res. Inst., Heidelberg, Germany

Abstract: Impairments in mitochondrial function, lysosomal degradation pathways and synaptic transmission are cardinal features of Parkinson's disease (PD) pathogenesis. Genetic studies have identified mutations in mitochondrial chaperone protein Mortalin/Hsc70-5/GRP75 in PD patients. Mortalin levels are reduced in the brains of PD patients and disease-associated rare mutated genetic variants failed to rescue impaired mitochondrial integrity in cellular mortalin knockdown models. Recent studies in a *Drosophila* model of PD showed that pan-neuronal knockdown of Mortalin *in vivo* causes synaptic mitochondrial depletion due to enhanced mitophagy, reduced ATP levels, shortening of lifespan as well as demonstrating cardinal features of *Drosophila* models of PD including impaired body posture and climbing defects. In order to gain an insight into the physiological consequences of mortalin-knockdown, we performed electrophysiological analysis at the *Drosophila* neuromuscular junction following pan-neuronal knockdown of Mortalin. Our studies reveal that knockdown of Mortalin causes functional impairments in synaptic transmission characterized by defects in basal synaptic transmission as well as increased failure rates following sustained stimulation at a high frequency compared to control animals. Next, by utilizing *Drosophila* genetic tools, we performed an RNAi screen to identify genes that can modify loss-of-mortalin associated phenotypes. Our screen revealed a

number of enhancers and suppressors that could modify the pattern of temperature dependent paralysis caused by mortalin knockdown. Interestingly, 5 members of the autophagy machinery were identified as modifiers namely Atg1, Atg5, Atg7, Atg12, and Atg101. These genes are involved in autophagy induction (Atg1 and Atg101) and expansion (Atg5, Atg7 and Atg12). Down regulation of Atg1, Atg5, Atg7, Atg12, and Atg101 respectively ameliorated the temperature-sensitive paralysis in flies impaired for mortalin function. Protective effects of knocking down of Atg1, Atg5, Atg7, Atg12, and Atg101 were also observed to rescue mortalin-knockdown associated climbing defects, abnormal wing posture and importantly ATP levels suggesting the rescue of mitochondrial function.

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Poster

218. Mitochondrial and Other Neuronal-Glial Mechanisms in Parkinson's Disease

Location: Hall A

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Topic: C.03. Parkinson’s Disease

Support: JSPS KAKENHI Grant Number 15K09308

Title: Mitochondrial impairment-induced Ser129-phosphorylation of alpha-synuclein through intracellular calcium concentration change

Authors: A. SASAKI[1], H. SATO[1], T. KATO[1], *S. ARAWAKA[2];
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Abstract: One of the characteristic findings in the Parkinson’s disease (PD) pathology is the presence of intracellular α-synuclein (a-syn) aggregates, called Lewy bodies (LBs), in the surviving neurons. In the PD brains, a-syn deposited in LBs is known to be highly phosphorylated at the Ser129 residue. In contrast, only 4%, or less, of the total amount of a-syn is phosphorylated in the normal brains. This difference suggests that Ser129-phosphorylation of a-syn is a pathological event in the neurodegenerative process of PD. However, it is unknown what factors accelerate this event. In this study, we first tested whether alteration in intracellular calcium concentration affects Ser129-phosphorylation of a-syn using human dopaminergic SH-SY5Y cells stably expressing wild-type a-syn (wt-aS/SH) and rat primary cortical neurons. When cells were treated with calcium ionophore A23187, the amounts of Ser129-phosphorylated a-syn were significantly increased in an A23187 concentration- or incubation time-dependent manner. This increase was suppressed by addition of calcium chelator, EGTA or BAPTA-AM. In addition, the A23187-induced elevation was inhibited by addition of calmodulin inhibitor, W-7 or calmidazolium. Then, we tested whether mitochondrial complex I inhibitors alter Ser129-

phosphorylation of a-syn. In wt-aS/SH cells, treatment with MPP^+ or rotenone enhanced the phosphorylation of a-syn in a concentration-dependent manner. In rat primary cortical neurons, treatment with rotenone also enhanced the phosphorylation of a-syn. These increases were blocked by addition of either EGTA or BAPTA-AM. Our results showed that intracellular calcium concentration and calmodulin were factors to modulate Ser129-phosphorylayion of a-syn. The results also suggested that mitochondrial impairment enhanced Ser129-phosphorylation of a-syn by causing influx of extracellular calcium, because EGTA is a non-membrane permeable chelator and decreases extracellular calcium. Calcium dysregulation may link mitochondrial impairment to a-syn neurotoxicity in PD.

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Poster

218. Mitochondrial and Other Neuronal-Glial Mechanisms in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: NIH Grant NS078338

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Harvard Stem Cell Institute

Title: Mitochondrial dysfunction and ER stress response in human iPSC-derived neurons carrying LRRK2 G2019S mutation

Authors: ***J. A. KORECKA**[1], S. LEVY[1], M. L. TERPSTRA[1], D. C. DINESH[1], D. P. CHRISTENSEN[1], T. M. OSBORN[1], P. J. HALLETT[1], F. M. JODELKA[2], M. L. HASTINGS[2], O. ISACSON[1];
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Abstract: The Leucine-Rich Repeat Kinase (LRRK2) G2019S gain of function gene mutation can contribute to Parkinson disease (PD) pathogenesis. Previous work from our lab (Cooper et al., 2012, Sanders et al., 2014) and others indicate that the LRRK2 G2019S mutation can influence mitochondrial health, axon outgrowth, intracellular trafficking and autophagy. To further explore the function of LRRK2 and G2019S mutation-driven increased kinase activity we aim to investigate the neuronal ability to cope with 1. mitochondrial dysfunction induced by mitochondrial membrane depolarization (through valinomycin toxicity) and 2. endoplasmic reticulum (ER) stress response induced by inhibition of ER calcium influx and increase in

cytosol calcium concentration (through thapsigargin toxicity). We detected 2-fold higher nitric oxide levels but no elevated super oxide production in induced pluripotent stem cell (iPSC)-derived neurons from PD patients carrying the LRRK2 G2019S mutation when compared to healthy controls. In addition, we found a decreased ER stress response in these neurons by using lentiviral reporter constructs driven to quantitatively assess the activation of ER stress signal transduction pathways. Preliminary live cell imaging data showed altered mitochondrial subcellular distribution and translocation in LRRK2 G2019S neurons after mild mitochondrial toxicity. To determine the rate of mitochondrial lysosome dependent clearance, we are now exploring the real time dynamics of mitophagy in neurons using a lentiviral driven overexpression of a dual-fluorescence mitophagy specific bio-probe. As controls for our experiments we are using isogenic zinc finger nuclease based gene corrected neurons, antisense oligonucleotides targeting LRRK2 G2019S mutation, or pharmacological LRRK2 kinase inhibition. The altered mitochondrial function and ER stress response in neurons derived from patients carrying LRRK2 G2019S mutation sheds more light on the increased LRRK2 kinase activity and our understanding of the specific subcellular organelle dysfunction in PD pathogenesis.

Disclosures: **J.A. Korecka:** None. **S. Levy:** None. **M.L. Terpstra:** None. **D.C. Dinesh:** None. **D.P. Christensen:** None. **T.M. Osborn:** None. **P.J. Hallett:** None. **F.M. Jodelka:** None. **M.L. Hastings:** None. **O. Isacson:** None.

Poster

218. Mitochondrial and Other Neuronal-Glial Mechanisms in Parkinson's Disease

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Program#/Poster#: 218.07/C90

Topic: C.03. Parkinson’s Disease

Support: NMRC/CBRG/0042/2013

Title: S-Nitrosylation of divalent metal transporter 1 increases Iron deposition and contributes to loss of dopaminergic neurons after LPS-induced inflammation

Authors: ***C. LIU**[1], C.-W. ZHANG[2], K. C. CHEW[1], B. TAN[1], A. SUPPERMPOOL[1], K.-L. LIM[2], T. SOONG[1];
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Abstract: Iron elevation in substantia nigra is constantly reported in Parkinson’s disease (PD). Recently, a selective iron chelator was reported to improve PD-motor outcomes in a randomized clinical trial. Nitrosative stress has also been proposed as a major upstream event in PD pathogenesis. Nitric oxide has been linked with iron metabolism, while DMT1 has been

implicated in neurodegeneration via iron transport in animal models of PD. However, whether DMT1 function is modulated by nitrosative stress is unknown. Here we report that DMT1 could be nitrosylated when heterologously expressed in HEK 293 cells, and the DMT1 currents were increased by nitrosylation. The iron uptake and calcein quenching assays showed that nitrosylation of DMT1 increased its iron transport function. Using LPS-induced nigral inflammation model, we showed that DMT1 was nitrosylated *in vivo*. Iron uptake was increased in the LPS-injection site, which led to loss of dopaminergic neurons. Thus we showed a potential role of NO-mediated increase in iron uptake via DMT1 in inflammation related neurodegeneration.

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Poster

218. Mitochondrial and Other Neuronal-Glial Mechanisms in Parkinson's Disease

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Topic: C.03. Parkinson’s Disease

Support: 5R01NS070898

Title: MicroRNA-7 inhibits mitochondrial permeability pore function and confers neuroprotection

Authors: *A. DATTA CHAUDHURI, D.-C. CHOI, S. KABARIA, E. JUNN;
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Abstract: Mitochondrial dysfunction and electron transport chain failure are major contributors to neurodegeneration in Parkinson’s disease (PD). Voltage dependent anion channel 1 (VDAC1) is a transmembrane protein located on the outer mitochondrial membrane, which forms the mitochondrial permeability transition pore (PTP) along with cyclophilin D and adenine nucleotide translocase (ANT). Under conditions of cellular stress, mitochondrial depolarization leads to opening of the PTP, triggering mitochondrial swelling, release of pro-apoptotic proteins, ultimately resulting in cell death. MicroRNA-7 (miR-7) is a small non-coding RNA that has been found to be protective in several cellular models of PD. In the present study, we performed gene ontology (GO) analysis of proteomic profiles that are downregulated by miR-7, revealing that mitochondrial proteins are significantly overrepresented. Accordingly, miR-7 overexpression prevented MPP+-induced mitochondrial depolarization, increase in mitochondrial calcium and cytochrome c release from mitochondria as well as ROS generation. Among the mitochondrial proteins that were downregulated by miR-7, we found that VDAC1 3’UTR harbors a conserved miR-7 target site. Overexpression of miR-7 led to downregulation of VDAC1 mRNA and protein

levels, while inhibition of endogenous miR-7 led to an increase. Additionally, we demonstrated that miR-7 directly targets VDAC1 3'UTR by performing 3'UTR luciferase reporter assays. Similar to miR-7 overexpression, knockdown of VDAC1 by siRNA led to neuroprotection and prevention of pathological changes in the mitochondria upon exposure to MPP+. Furthermore, knockdown of VDAC1 reversed the anti-miR-7-induced sensitization of cells to MPP+ toxicity. Therefore this study identifies VDAC1 as a direct miR-7 target that contributes to its neuroprotective effect.

Disclosures: **A. Datta Chaudhuri:** None. **D. Choi:** None. **S. Kabaria:** None. **E. Junn:** None.

Poster

218. Mitochondrial and Other Neuronal-Glial Mechanisms in Parkinson's Disease

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 218.09/C92

Topic: C.03. Parkinson's Disease

Support: NIH Grant 1R01 NS065338-01A2

Title: Effects of acute neurotoxicant administration on ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) activity in MN9D cell line and mouse striatal synaptosomes

Authors: ***B. M. WINNER**[1], K. J. LOOKINGLAND[1], J. L. GOUDREAU[2];
[1]Pharmacol. and Toxicology, [2]Michigan State Univ., East Lansing, MI

Abstract: In Parkinson disease (PD), relatively selective degeneration of the substantia nigra pars compacta (SN) results in a loss of dopamine (DA) released in the striatum (ST) and leads to the motor disturbance symptoms in PD. We can recapitulate the loss of DA by administering acute 1-methyl-4-phenyl -1,2,3,6-tetrahydropyridine (MPTP). With acute MPTP administration, ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) protein is decreased in the SN after 24 h while mRNA for UCH-L1 is unchanged. UCH-L1 is a deubiquitinating enzyme linked to inheritable PD which functions in the ubiquitin proteasome system (UPS), a major pathway for protein degradation. UCH-L1 removes ubiquitin (Ub) from small proteins and thereby replenish the pool of monomeric Ub to be reused in the UPS. The loss of UCH-L1 protein is postulated to be associated with susceptibility of the SN to acute neurotoxic insult and perhaps neurodegeneration in PD. Similarly, in MN9D cells (a mesencephalic-derived DA cell line), UCH-L1 protein decreases in a time and concentration-dependent manner with administration of MPP+, the active form of MPTP which is readily taken up by dopamine transporter. Decrease in UCH-L1 protein in MN9D cells with MPP+ is also associated with loss of intracellular DA in the cells without significant cytotoxicity. Although the decrease in UCH-L1 protein in the SN and MN9D cells suggests that UCH-L1 may play an important role in susceptibility to neurotoxicant, the activity of the enzyme has not been determined in MN9D cells or striatal

synaptosomes isolated from mouse brain. It is currently unknown whether UCH-L1's hydrolase activity is compromised with acute MPTP treatment, and so the goal of the current study was to establish an assay to measure activity of UCH-L1 using an *in vitro* fluorogenic probe conjugated to ubiquitin (Ub-AMC). This substrate allows for kinetic readings to measure UCH-L1 activity *ex vivo* and determine if UCH-L1 function is impaired in MPTP-treated mouse brain. For this study, crude synaptosomes isolated from the ST of mice were collected at 4, 6, 8 12, and 24 h post-MPTP and UCH-L1 hydrolase activity was measured. The results of this study reveal an association between expression of UCH-L1 protein and hydrolase activity as a function of time after MPTP exposure in mouse brain and MN9D cell extracts.

Disclosures: B.M. Winner: None. **K.J. Lookingland:** None. **J.L. Goudreau:** None.

Poster

218. Mitochondrial and Other Neuronal-Glial Mechanisms in Parkinson's Disease

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 218.10/C93

Topic: C.03. Parkinson's Disease

Title: Investigation of PINK1/Parkin-dependent mitophagy and its relation to mitochondrial biogenesis and mt-UPR in neurons

Authors: *M. JACOUPY[1,2], F. MOUTON-LIGER[1,2], S. AZEGGAGH[1,2], G. BERTOLIN[1,2], F. BONELLO[1,2,3], C. GAUTIER[1,2], A. BRICE[1,2], O. CORTI[1,2];
[1]ICM, Brain and Spine Inst., Paris, France; [2]Sorbonne Université, UPMC Univ. Paris 06, UMR S 1127, Inserm U1127, CNRS 7225, Paris, France; [3]IPSEN, Paris, France

Abstract: Mutations in genes encoding Parkin (PARK2) and PINK1 (PARK6) cause autosomal recessive forms of Parkinson's disease. These proteins regulate jointly several processes relevant to maintenance of mitochondrial quality. We previously showed that Parkin interacts with PINK1 at the TOM machinery (Translocase of the Outer Membrane), a protein complex responsible for the mitochondrial import of the vast majority of the mitochondrial proteins. We provided evidence that the degradation of key subunits of the TOM complex initiates Parkin-dependent mitophagy. Here, we explored the relevance of these findings in primary cortical neurons from wild type and PARK2 KO mice. By confocal and FRET microscopy, we show that following mitochondrial depolarization triggered by the protonophore CCCP, PINK1 accumulates and recruits Parkin in proximity of the TOM complex. These events were impaired in PARK2 KO cells, suggesting that Parkin plays a role in stabilizing PINK1 on the outer mitochondrial membrane. Using an image-based quantitative analysis of markers of different mitochondrial subcompartments at various time points after CCCP treatment, mitochondrial loss was observed in wild type but not PARK2 KO cells. Components of the TOM complex were lost earlier than other markers, confirming that this machinery is an early target for Parkin-dependent

degradation. In parallel, we investigated the relationship between PINK1/Parkin-mediated mitochondrial degradation and other mitochondrial stress response pathways, including mitochondrial biogenesis and the unfolded protein response (mtUPR). We explored expression of master genes of these processes, as well as downstream targets, by quantitative RT-PCR. Our results show different transcriptional activation profiles between wild type and PARK2 KO mice after CCCP treatment. We also describe the development and validation of a genetically encoded reporter for the evaluation of mitochondrial import efficiently through the TOM complex. Our preliminary results suggest alteration of this aspect of mitochondrial biogenesis in cells with PARK2 mutations. Altogether our studies suggest that loss of mitochondrial protein import efficiency is coupled with other mitochondrial quality control mechanisms that respond to Parkin deficiency in primary neurons.

Disclosures: **M. Jacoupy:** None. **F. Mouton-Liger:** None. **S. Azeggagh:** None. **G. Bertolin:** None. **F. Bonello:** None. **C. Gautier:** None. **A. Brice:** None. **O. Corti:** None.

Poster

218. Mitochondrial and Other Neuronal-Glial Mechanisms in Parkinson's Disease

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Topic: C.03. Parkinson’s Disease

Support: K99ES024570

P50 AG025688

P30 NS055077

Title: Genome-wide epigenomic alterations in Parkinson’s disease

Authors: ***A. I. BERNSTEIN**, M. GEARING, J. MULLE;
Emory Univ., Atlanta, GA

Abstract: Parkinson’s disease (PD), the most common neurodegenerative movement disorder, is characterized by degeneration of the nigrostriatal dopaminergic pathway and other monoaminergic regions and the formation of cytoplasmic inclusions. The majority of cases of PD are sporadic (not caused by an inherited monogenic mutation). While the etiology of these sporadic cases remains unclear, it is thought to involve an interaction between genetic and environmental factors. Environmental risk factors have been identified and epidemiological studies suggest that exposure to environmental toxicants, especially persistent organic pollutants, increase the risk of PD. Many of these compounds have been found in post-mortem human PD brains and many groups have shown that these compounds cause oxidative stress and disrupt expression and function of dopaminergic-related and PD-related proteins, resulting in increased

susceptibility of dopaminergic neurons. It has been proposed that epigenetic modulations could serve as an intermediate process that imprints dynamic environmental experiences on the "fixed" genome, resulting in stable alterations in phenotype. Therefore, it is likely that these factors converge upon the epigenome. Recent work has also revealed a role for regulation of the transcriptome and the epigenome in PD and in the response to toxic exposures. Many lines of evidence suggest a role for epigenetic regulation, and, in particular, cytosine modifications in PD. Methylation of cytosine to 5-methylcytosine in genomic DNA is carried out by DNA methyltransferases. 5hmC, highly enriched in the nervous system, is generated from 5mC by Tet proteins. 5hmC displays temporal and spatial changes during neurodevelopment and aging. Aberrant gene methylation of PD-related genes and deficiencies in microRNAs have been observed in post-mortem PD brains. However, these studies have largely focused on the individual genes responsible for familial PD and not genome-wide changes or in regions or tissues not affected in PD. Moreover, it is not known how these changes in the epigenome are related to changes in neuronal vulnerability. It is possible that epigenomic changes contribute to neuronal vulnerability by altering the expression of proteins within the dopaminergic system. Here, we mapped genome-wide changes in cytosine modifications using DNA isolated from cingulate and parietal cortices of post-mortem early- and late-stage PD and age-matched controls. We identified regions that are differentially modified in PD and analyzed the genes they map to. Together, this analysis suggests that cytosine modifications play an important role in PD pathogenesis.

Disclosures: **A.I. Bernstein:** None. **M. Gearing:** None. **J. Mulle:** None.

Poster

218. Mitochondrial and Other Neuronal-Glial Mechanisms in Parkinson's Disease

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Alzheimer's Association NIRG-12-242135

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Title: Mitochondrial accumulation of α-synuclein in Parkinson's disease causes impaired protein import via tom40 release/degradation

Authors: ***V. VASQUEZ**[1,2,3], J. MITRA[3], P. M. HEGDE[3], K. S. RAO[1], M. L. HEGDE[3,4]; [1]INDICASAT-AIP, Panama, Panama; [2]Biothecnology, Acharya Nagarjuna Univ., Guntur, India;

[3]Radiation Oncology and Neurol., Houston Methodist Res. Inst., Houston, TX; [4]Weil Med. Col. of Cornell Univ., New York, NY

Abstract: α-synuclein is a presynaptic protein, whose intracellular aggregation in brain has been etiologically implicated in Parkinson's disease (PD), a progressive neurodegenerative disorder, affecting 4.9-19 per 100,000 people per year worldwide. Accumulation of mis-folded α-synuclein in the substantia nigra of the brain leads to the formation of Lewy body deposits, one of the histopathological features of PD, which also involves oxidative stress, pro-oxidant metal toxicity and increased nuclear genome damage. More recent studies have implicated significant mitochondrial pathology in PD brain, including accumulation of dysfunctional mitochondria with high levels of mitochondrial DNA (mtDNA) deletions/damage, and altered outer mitochondrial membrane (OMM) proteins, which also correlates with increased α-synuclein in mitochondria. However, the role of α-synuclein toxicity in mitochondria is unclear. Here we demonstrate that α-synuclein stably interacts/co-localizes with mitochondrial membrane translocases involved in the import of nuclear-coded proteins into mitochondria, TOM40 (outer membrane) and TOM20 (inner membrane) in normal neurons. Furthermore, we made a surprising observation that in α-synuclein overexpressing iPSC-derived neuronal cell model of PD, TOM40 is distinctly released/degraded in mitochondria, while TOM20 is not affected. Thus α-synuclein pathology in PD causes defective protein import via TOM40 release in mitochondria of dopaminergic neurons leading to imbalance in mtDNA damage/repair and anti-oxidant machinery. These data are consistent with evidence of increased mitochondrial DNA damage in PD affected human brain. Comprehensive molecular events involved in this phenomenon and potential prevention strategies will be discussed.

Disclosures: V. Vasquez: None. **J. Mitra:** None. **P.M. Hegde:** None. **K.S. Rao:** None. **M.L. Hegde:** None.

Poster

218. Mitochondrial and Other Neuronal-Glial Mechanisms in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: NS065789

AG026389

Title: Sensitivity of complex I to rotenone is activity-dependent in neurons

Authors: *C.-H. J. CHIANG[1], L. SANDERS[2], J. CALLIO[3], E. HOWLETT[2], C. T. CHU[3]; [1]UPMC Neuropathology, Pittsburgh, PA; [2]Pittsburgh Inst. for Neurodegenerative Dis., Pittsburgh, PA; [3]Univ. of Pittsburgh Sch. of Med. Dept. of Pathology, Pittsburgh, PA

Abstract: Neuronal and systemic decrease in the activity of mitochondrial respiratory complex I, the main entry point of the electron transport chain for oxidative phosphorylation, has been identified in both sporadic and hereditary forms of Parkinson disease (PD) and animal models of PD. More interestingly, systemic complex I inhibition by toxins such as rotenone generates parkinsonism in both human and model organisms. It remains unclear why neurons are particularly sensitive to complex I dysfunction and why certain populations of neurons are more severely affected to generate specific phenotypes such as parkinsonism. Mammalian complex I consists of 14 core subunits and 31 accessory subunits. Although complex I has been extensively studied in non-neuronal systems including heart, liver and muscle, its regulation is largely unknown in the nervous system. Here we show that in primary mouse cortical neurons the sensitivity of complex I to toxin inhibition was changed in response to neuronal and electron transport chain activities and substrate availability. There was a positive correlation between the oxygen consumption rates of the neurons and the sensitivity to rotenone, the commonly used complex I inhibitor. Furthermore, excitatory NMDA receptor (NMDAR) activation increased the activity of immunocaptured complex I from neurons. The changes induced by NMDAR activation correlated with the presence of significantly increased oxidative stress within the neurons. Our previous studies have implicated mitochondrial dysregulation in LRRK2 mutation-linked PD. Here we also show that neurons with LRRK2 R1441G mutation had decreased complex I activity and the capacity of oxidative phosphorylation. Ongoing studies are aimed at identification of activity-dependent changes in subunit composition and/or post-translational modifications of complex I in the nervous system, and how PD-linked LRRK2 mutations exert negative impacts. Understanding the regulation of complex I in neurons and the impacts of genetic risk factors may shed light on novel therapeutics for neurodegenerative diseases.

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Poster

218. Mitochondrial and Other Neuronal-Glial Mechanisms in Parkinson's Disease

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Support: AU forskningsfond NEURODIN Ideas Center (Denmark)

M. J. Fox Foundation for Parkinson's disease (USA)

Health Faculty at Aarhus Univ. (Denmark)

Title: Evaluation of the role of CD163+ macrophages in the neurodegeneration of Parkinson’s disease

Authors: *N. TENTILLIER[1], A. ETZERODT[1], K. SHRIVASTAVA[1], S. K. MOESTRUP[1], G. HALLIDAY[2], M. ROMERO-RAMOS[1];
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Abstract: Parkinson's disease (PD) is characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra (SN) and the presence of intraneuronal aggregated alpha-synuclein (a-syn) in Lewy bodies. Among other factors, inflammation seems to play a role in PD neurodegeneration. CD163 is a scavenger receptor normally expressed on peripheral monocytes/macrophages, which increases with inflammation. In brain, CD163 expression is limited to perivascular macrophages. We have observed infiltration of CD163+ macrophages into the area of neurodegeneration in the 6-hydroxydopamine (6-OHDA) PD model and the alpha-synuclein viral vector based PD model. We hypothesized that the migration of CD163+ macrophages into the brain-injured area in PD may influence local microglia. Therefore we aimed to deplete selectively the CD163+ macrophages vs. unspecific circulating macrophages. To do so we used CD163-targeted liposomes, via a specific antibody, loaded with doxorubicin vs clodronate untargeted liposomes, a well-known macrophages depletion strategy used by many before. Animals were injected twice per week for three weeks starting the day of the intra-striatal toxin injection. We are currently analyzing dopaminergic degeneration as well as the microglia response in the animals in order to determine the specific role of the circulating macrophages in the 6OHDA-induced toxicity. To complement our animal studies; in parallel we immunostained human brain tissue (posterior putamen) from PD patients and we are evaluating possible CD163+ cells infiltration during disease.

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Poster

218. Mitochondrial and Other Neuronal-Glial Mechanisms in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: Vicerrectoria de Investigación y Estudios de Posgrado-BUAP NAT 2014

Vicerrectoria de Investigación y Estudios de Posgrado-BUAP NAT 2015

Title: Chronic L-DOPA administration induces astrogliosis in rats with intra-nigral 6-OHDA-lesion

Authors: *G. RAMÍREZ GARCÍA[1,2], V. PALAFOX SÁNCHEZ[2], I. LIMON PEREZ DE LEON[2];

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Abstract: Parkinson's disease (PD) is characterized by the death of dopaminergic neurons in the substantia nigra pars compacta (SNpc) with the consequent decrease of dopamine (DA) in the striatum and prefrontal cortex (PFC). L-3,4-dihydroxyphenylalanine (L-DOPA) is administered for long term to restore DA levels and improve motor functions in PD patients. Is widely known that chronic L-DOPA could establish an oxidant environment for neuron, however, currently the impact of the L-DOPA treatment on astrogliosis is unknown. Besides has been showed that astroglia activation could relate with an inflammatory process. In this work, we evaluated the effect of chronic L-DOPA administration in rats with intra-nigral 6-hydroxydopamine (6-OHDA)-lesion on astrogliosis induction in the nigro-striatal-cortical pathway. After lesion, the animals were divided into four experimental groups, two groups received orally isotonic saline (SSI) administration and another two groups received orally L-DOPA/Carbidopa (100 mg/kg) for 20 days. After administration period, tyrosine hydroxylase (TH) and Glial Fibrillary Acidic Protein (GFAP) immunoreactivity was evaluated by immunohistochemistry in the SNpc, dorsal striatum and PFC. Our results showed that lesion with 6-OHDA induce a substantial astrocytic activation but the chronic L-DOPA administration induced an increment of immunoreactivity of GFAP. Also, the astrogliosis occurs in areas with great dopaminergic denervation, where the DA release is deregulated, making impossible DA reuptake, which contributes to induce an oxidative stress and inflammatory process. These facts suggest that astrogliosis in the striatum and PFC increased by L-DOPA treatment may be related with motor and cognitive complications that occur in PD patients. Support VIEP-BUAP NAT 2014 and 2015 to ID. See more information about this results: Neuroscience. 2015; 290:492-508. doi:10.1016/j.neuroscience.2015.01.047. Epub 2015 Jan 30.

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Poster

218. Mitochondrial and Other Neuronal-Glial Mechanisms in Parkinson's Disease

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Topic: C.03. Parkinson’s Disease

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Title: Behavioral characterization of mice lacking of PKR-like endoplasmic reticulum kinase (PERK) in dopaminergic neurons

Authors: *F. LONGO, E. SANTINI, E. KLANN;
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Abstract: Several neurodegenerative disorders, including Parkinson's Disease (PD) are associated with protein misfolding and the formation of distinct aggregates. Accumulation of misfolded proteins results in the endoplasmic reticulum (ER) stress and leads to the activation of an intracellular signaling pathway termed the unfolded protein response (UPR), which typically has a neuroprotective role. As a crucial component of the UPR, PERK activation results in phosphorylation and inhibition of eukaryotic initiation factor 2α (eIF2α), a translation factor that controls the initiation step of protein synthesis and has been implicated in long-term synaptic plasticity and memory formation. Paradoxically, the phosphorylation of eIF2α also results in the increased synthesis of transcription factors that contain unread open reading frames (uORF) in the 5'UTR of their mRNAs, including activating transcription factor 4 (ATF4), which is involved in the expression of several UPR target genes. Although the UPR serves a neuroprotective role via regulation of general and gene-specific translation, it also is responsible for promoting apoptotic cell death following sustained activation, which occurs in neurodegenerative disorders, such as PD. Growing evidence from studies on post-mortem tissue has shown expression of PERK and increased phosphorylation of eIF2α in dopaminergic neurons of PD patients compared to age-matched controls. Our central hypothesis is that a prolonged UPR results in inhibition of general protein synthesis via PERK-dependent phosphorylation of eIF2α, resulting in the loss of synaptic proteins, synaptic failure, and ultimately, neuronal death in dopaminergic neurons. To investigate the involvement of PERK in neurodegeneration and to determine whether the deletion of PERK in dopaminergic neurons results in motor disturbances and cognitive symptoms observed in PD, we generated mice selectively lacking either one or both copies of the PERK gene in dopaminergic neurons (DAT-PERK+/- and DAT-PERK -/-, respectively). We examined the DAT-PERK mutant mice in a set of complementary motor behavioral tests specific for akinesia (BAR test), bradykinesia (DRAG test), and overall gait ability (rotarod test, foot print test, and open field test), as well as in a series of cognitive tasks (water maze test, novel object recognition test, and associative fear conditioning). We found that the reduction of PERK in dopaminergic neurons results in the alteration of multiple motor and cognitive abilities in mice. These results support our overall hypothesis and are consistent with previous studies (Ma et al. 2013; Moreno et al., 2013).

Disclosures: **F. Longo:** None. **E. Santini:** None. **E. Klann:** None.

Poster

218. Mitochondrial and Other Neuronal-Glial Mechanisms in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

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Title: Fipronil-induces apoptosis via modulation of mitochondrial function and alteration in protein degradation machinery in SH-SY5Y cells

Authors: S. KANURI[1], H. JIN[1], A. VELLAREDDY[1], A. KANTHASAMY[1], *A. KANTHASAMY[2];
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Abstract: Chronic environmental exposure to pesticides and insecticides had been implicated in the etiopathogenesis of Parkinson's Disease (PD). Wide spread application of insecticides can gain entry into the food chain thereby increasing the risk of potential toxic effects. In this context fipronil (FPN), a broad spectrum insecticide has been shown to elicit its effects via the disruption of Gamma aminobutyric regulated chloride (GABA) channel thereby leading to the impairment in neuromuscular transmission. Recent studies have demonstrated that fipronil-induces neurotoxicity; however, the cellular mechanisms underlying fipronil-induced neurotoxicity remain poorly understood. In this study we systematically characterized the mechanistic basis of FPN-induced neurotoxicity using human neuroblastoma SH-SY5Y cells. Exposure of SH-SY5Y cells to FPN-induced a dose and time dependent increase in apoptotic cell death as assessed via DNA fragmentation assay. Additionally a similar scenario was evidenced when cell viability was assessed via MTT assay. Furthermore FPN-induced neuronal cell death was preceded by the dissipation of mitochondrial membrane potential (MMP), depletion of GSH levels and activation of caspase 3. Additionally, Western blot analysis revealed that FPN (60 uM)-induced a time dependent increase in beclin-1 expression, phosphorylation and cleavage of PKC-delta; and accumulation of ubiquitin positive aggregates. Collectively, these findings suggest that FPN can induce dopaminergic neurotoxicity by promoting caspase-3 mediated mitochondria dependent cell death signaling with a concomitant impairment in protein degradation machinery.

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Poster

218. Mitochondrial and Other Neuronal-Glial Mechanisms in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: NIH RO1-NS076772

Title: Real-time measurements of oxidative stress during chronic L-DOPA treatment for Parkinson's disease

Authors: *L. R. WILSON[1], C. A. LEE[2], N. D. RHODES[2], C. F. MASON[3], S. PANDA[4], L. A. SOMBERS[2];
[2]Chem., [3]Life Sci., [4]Biomed. Engin., [1]North Carolina State Univ., Raleigh, NC

Abstract: Parkinson's disease (PD) is a chronic neurodegenerative disorder characterized by the preferential loss of dopaminergic neurons stemming from the midbrain's substantia nigra pars compacta (SNpc) and innervating the dorsal striatum. The substantial decreases in striatal dopamine (DA) result in devastating hypokinetic movements and motor disturbances. Demonstrating an absence in understanding what initiates and drives this degeneration, the standard treatment strategy of dopaminergic replacement therapy via administration of Levodopa (L-DOPA; L-3,4 dihydroxyphenylalanine) has remained unchanged for several decades, despite a majority of patients exhibiting "off-peak" undesirable hyperkinetic and dyskinetic motor complications. One potential contributor to processes involved in the initiation, progression, and maintenance of Parkinsonian symptoms is increased generation of reactive oxygen species, such as hydrogen peroxide (H2O2). We aim to investigate how striatal H2O2 and DA dynamics underlie behavioral changes that result from chronic L-DOPA administration in a rodent model of PD (unilateral 6-OHDA lesion) using fast-scan cyclic voltammetry (FSCV), an electrochemical technique that affords precise spatial and temporal resolution. Specifically, carbon-fiber microelectrodes are used to simultaneously quantify rapid H2O2 and DA fluctuations at single recording sites in the dorsal striatum over several weeks of L-DOPA administration. These studies will aid in our understanding of how oxidative stress modulates nigrostriatal DA signaling, and will demonstrate how these signals correspond with the development of dyskinetic movements in the treatment of PD.

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Poster

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Title: Characterization of the mitochondrial enzymes activity and the lipidomic profile of Parkinsonian monkeys using cell membranes microarrays

Authors: *G. BARREDA-GÓMEZ[1], A. PÉREZ-VALLE[1], M.-D. GARCÍA-FERNÁNDEZ[1,2], J. GARATE[3], R. FERNÁNDEZ[3], T. TOLENTINO-CORTEZ[1], J. A. FERNÁNDEZ[3], E. ASTIGARRAGA[1];
[1]Dept. of Res. and Develop., IMG Pharma Biotech, Derio, Spain; [2]Pharmacol., UPV/EHU, Fac. of Med. and Dent., Leioa, Spain; [3]Physical-Chemistry, UPV/EHU, Fac. of Sci. and Technol., Leioa, Spain

Abstract: Parkinson´s disease (PD) is characterized by the selective loss of dopaminergic neurons, in which the mitochondrial dysfunction seems to play an important role. In this context, one of the first evidences appeared when it was found that long exposure to MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), an inhibitor of complex I of mitochondrial electron transport chain, generated parkinsonism in humans and monkeys. However, it is not entirely clear yet the effects induced by the chronic exposure to this inhibitor not only on the density and function of mitochondrial electron transport chain proteins but also on the lipid composition of the cellular membrane. For this purpose, the density of the complex I (using [3H]-Dihydrorotenone), the succinate dehydrogenase activity (complex II) and the cytochrome c oxidase activity (complex IV) were evaluated in different brain areas and tissues of control and MPTP-treated non-human primate (Macaca fascicularis) using cell membrane microarrays. To determine the lipid profile, MALDI imaging mass spectrometry (MALDI-IMS) was combined with cell membrane microarrays and artificial neuron networks were employed to data handling and analysis. MPTP treatment induced an increase of the succinate dehydrogenase activity in olfactory bulb, putamen, caudate and substantia nigra, where it was observed a decrease in cytochrome c oxidase activity. This treatment also produced an alteration of the lipid profile in these areas, being especially relevant the reduction in the phosphatidylserine (38:1) content. However, in the cerebellum, where a decrease of both enzymatic activities was recorded, this phosphatidylserine (38:1) was enhanced. Therefore, the MPTP treatment not only modulates the mitochondrial electron transport chain enzymes, but also seems to alter other mitochondrial enzymes that regulate the lipid metabolism such as the phosphatidylserine decarboxylase.

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Poster

218. Mitochondrial and Other Neuronal-Glial Mechanisms in Parkinson's Disease

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 218.20/D7

Topic: C.03. Parkinson's Disease

Title: Mitochondrial quality control in microglial cells and astrocytes and their regulation by the Parkin-dependent pathway

Authors: *F. MOUTON-LIGER, M. JACOUPY, J. SEPULVEDA, F. BONELLO, S.-M. HASSOUN, A.-L. PRIVAT, Z. ERPAPAZOGLOU, P. MICHEL, A. BRICE, J.-C. CORVOL, O. CORTI;
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Abstract: Parkinson's disease (PD) is a common, severe neurodegenerative disorder due to the preferential and progressive degeneration of the dopaminergic neurons of the substantia nigra, which may be intrinsically vulnerable to oxidative stress. The loss of these neurons is associated with a glial response composed mainly of activated microglial cells and, to a lesser extent, of reactive astrocytes. This glial response may be the source of trophic factors and can protect against reactive oxygen species. On the other side, the glial response can induce deleterious events related to the production of pro-inflammatory cytokines. Although most cases of PD appear to be sporadic, several gene mutations have been linked to familial forms of PD. The discovery of some of the proteins encoded by these genes, including Parkin (PARK2) and PINK1 (PARK6), at the mitochondria offered a new perspective on the involvement of mitochondria in PD. Specifically, these proteins are involved in the maintenance of a healthy pool of mitochondria by regulating their turnover by mitochondrial autophagy, also known as mitophagy. Most studies have examined this process in immortalized cell lines overexpressing high levels of Parkin. More recently, few researches have addressed the role of Parkin/PINK1-dependent mitophagy in primary cultured neurons. However, whether or how endogenous Parkin contributes to mitophagy in central nervous system glial cells remain unclear. In this study, by using new innovative protocols for highly-enriched isolation of astrocytes and microglia, we have observed in primary cultured cells derived from Parkin knockout (Parkin-/-) and wild-type (WT) mice, the impact of mitochondrial membrane depolarization induced by 24 or 48 hours of CCCP (Carbonyl cyanide m-chlorophenyl hydrazine) on the mitochondrial quality control mechanisms mediated by Parkin. Our results show that astrocytes and microglial cells derived from WT mice undergo strong mitophagy following CCCP treatment and that this process is significantly impaired in cells from PARK2-/- mice. Interestingly, these defects are associated with alteration of glial cells morphology, activation and in pro-inflammatory cytokines production. Finally, to analyze whether the functionally and morphologically mitochondrial impairment in astrocytes or microglia exhibit a disturbed interaction with neurons and contributes to neuronal loss, Parkin-/- and WT glial cells are currently being co-cultured with WT neurons. These results should allow a better understanding of Parkin-dependent mitochondrial dysfunction in neuronal and glial physiology and their impact on neurodegeneration in PD.

Disclosures: F. Mouton-Liger: None. **M. Jacoupy:** None. **J. Sepulveda:** None. **F. Bonello:** None. **S. Hassoun:** None. **A. Privat:** None. **Z. Erpapazoglou:** None. **P. Michel:** None. **A. Brice:** None. **J. Corvol:** None. **O. Corti:** None.

Poster

218. Mitochondrial and Other Neuronal-Glial Mechanisms in Parkinson's Disease

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 218.21/D8

Topic: C.03. Parkinson's Disease

Support: Harvard Stem Cell Institute

Consolidated Anti-Aging Foundation

Title: Lysosomal glycolipid changes in Parkinson's disease are mirrored by normal aging of the human and mouse brain

Authors: *P. HALLETT[1], E. N. ROCHA[1], M. HUEBECKER[2], G. A. SMITH[1], D. A. PRIESTMAN[2], F. M. PLATT[2], O. ISACSON[1];
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Abstract: *GBA1* encodes for glucocerebrosidase (GCase), a lysosomal lipid hydrolase involved in glycosphingolipid metabolism, and mutations in *GBA1* cause Gaucher disease (GD), which is the most common autosomal recessive lysosomal storage disease. A reduction in lysosomal GCase activity causes accumulation of the corresponding glycosphingolipid substrates, glucosylceramide (GluCer) and glucosylsphingosine (GluSph). *GBA1* haploinsufficiency can result in a 30-50% reduction in GCase activity and increases the risk for developing PD dramatically. We recently made the observation that there is a reduction of GCase in sporadic PD patients without the *GBA1* mutation (nonGBA1-PD), and a progressive age-dependent reduction in GCase activity and increase in glycolipids in healthy subjects in the brain regions most affected in PD (Rocha EN et al., 2015 *Ann. Clin. Trans. Neurol.* doi: 10.1002/acn3.177). This age-dependent decline in GCase activity and rise in GluSph eventually becomes similar to sporadic nonGBA1-PD patients, and by the time an individual reaches the 7^{th}-8^{th} decade of life, GCase levels in the substantia nigra and putamen are reduced to the same extent as subjects with sporadic PD. We therefore hypothesize that aging mirrors changes to the GBA pathway similar to those that occur in genetic (*GBA1* haploinsufficiency) and sporadic (nonGBA1) forms of PD, and that disrupted GBA and lysosomal homeostasis occurring with normal aging may accelerate degenerative processes in vulnerable neurons and lower the threshold for developing PD. In order to further explore and model these age-dependent changes in the GBA pathway in animals, we have measured levels of glycosphingolipids in whole brain homogenates from wildtype (FVB) mice aged between 7-17 months of age. Our results show that levels of GluCer are significantly correlated with age ($r=0.3732$, $p<0.05$) and that by the latest timepoint studied (17 months), GluCer is increased to 136% of younger (7 month) mice, whereas levels of gangliosides GD1b ($r=-0.5054$, $p<0.01$) and GT1b ($r=-0.3442$, $p<0.05$), are negatively correlated with age. Measurements of GCase activity and other lysosomal hydrolases, as well as alpha-synuclein

levels, will further determine alterations in GBA and protein degradation pathways. Determining changes in the activity of GCase and other lysosomal hydrolases and their associated glycosphingolipids in normal aging and in PD, will shed light on underlying pathophysiological mechanisms. Furthermore, assays for specific lysosomal hydrolases and glycosphingolipids may in the future be essential both as biomarkers and pharmacodynamic for PD.

Disclosures: **P. Hallett:** None. **E.N. Rocha:** None. **M. Huebecker:** None. **G.A. Smith:** None. **D.A. Priestman:** None. **F.M. Platt:** None. **O. Isacson:** None.

Poster

218. Mitochondrial and Other Neuronal-Glial Mechanisms in Parkinson's Disease

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 218.22/D9

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH Grant NS081746

Title: Mito-function of DJ1 in Parkinson's disease

Authors: *R. CHEN, J. WU, P. MIRANDA, K. N. ALAVIAN, E. A. JONAS;
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Abstract: Parkinson’s disease (PD), which includes Familial PD (10%) and Sporadic PD (90%), is a progressive neurodegenerative disorder with limited therapeutic options. PD is characterized by loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc). Genetic studies of PD have identified several related genes, including LRRK2, Parkin/PINK-1 and DJ1. Most PD genes are necessary for normal mitochondrial function. However, the functional mechanism of DJ1 is poorly understood. Here, we investigated the function of DJ1 and its two disease-associated point mutants, M3 (A104T) and M5 (L166P). We found that overexpression of WT but not M3 or M5 in HEK293 cells significantly increased ATP levels. Recombinant WT DJ1 protein also strikingly enhanced ATPase activity of purified ATP synthase complex, measured as the rate of decrease in NADH fluorescence, whereas mutants had no effect. In response to ATP hydrolysis, WT but not mutant DJ1 protein efficiently decreased the inner membrane leak, measured during the movement of the H+ ions into sub-mitochondrial vesicles enriched in F1FO ATP synthase complex (SMVs). Patch clamp recordings of WT SMVs and SMVs from DJ1 -/- mice confirmed that WT DJ1 but not mutant DJ1 protein decreases inner membrane conductance. Our evidence indicates that the differences in activity between WT and mutant DJ1 proteins was not due to differences in interaction with Bcl-xL or with the F1FO ATP synthase beta-subunit. Interestingly, we found that DJ1 depletion affected the expression of several mitochondrial-related genes in mice, especially in midbrain and in tissues with high ATP demand. We also established methods for efficient dopaminergic (mesDA) neuronal culture of

WT and DJ1-/- mouse midbrain cells. We found that DJ1-/- mesDA neurons have low survival rate. Thus, our study uncovered an important role of DJ1 in Parkinson's disease by affecting mitochondrial inner membrane properties, cell metabolism and death.

Disclosures: **R. Chen:** None. **J. Wu:** None. **P. Miranda:** None. **K.N. Alavian:** None. **E.A. Jonas:** None.

Poster

219. Motor Neuron Disease

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Bernice Ramsay Discovery Grant (ALS Canada)

Title: Rescue of neuromuscular junction integrity in two SOD1 mice models by chronic *in vitro* blockade of glial muscarinic receptors

Authors: ***D. ARBOUR**, É. MARTINEAU, E. TREMBLAY, R. ROBITAILLE;
Univ. De Montréal, Montréal, QC, Canada

Abstract: Amyotrophic lateral sclerosis (ALS) is a non-cell autonomous neurodegenerative disease characterized by progressive loss of motoneurons and the destruction of the neuromuscular junctions (NMJ). Despite the importance of NMJ malfunction and the reported involvement of other glial cells in ALS, the contribution of glial cell at the NMJ remains unknown. Perisynaptic Schwann cell (PSCs), glial cells at the NMJ, influence structural stability, integrity and repair of the NMJ and synaptic activity. Of particular importance is the PSCs muscarinic signaling that must be reduced to allow morphological plasticity and repair of the NMJ. However, our recent data suggest that this signaling is enhanced in a SOD1 mouse model. Hence, we hypothesize that enhanced PSC muscarinic receptor (mAChRs) function may deter NMJ repair and remodelling in ALS and that chronic *in vivo* blockade of PSC mAChRs should restore the state of NMJ repair in ALS and allow glial rescue to proceed. SOD1 mice (G93A or G37R) were injected 3 times a week to target the soleus muscle either with a saline solution or pirenzepine, an antagonist of M1, 3 and 5 subtypes known to be present on PSCs. A combination of techniques was used in the SOD1G37R mice to obtain a good read-out of the PSCs functions. More specifically, we performed Ca2+ imaging to assess PSCs properties, electrophysiological recordings for synaptic properties and immunostaining for the NMJs morphological analysis. Pirenzepine treatment was efficient at reducing PSC mAChRs activation since the Ca2+ response evoked by the nerve stimulation or local muscarine application were significantly reduced, while Ca2+ responses evoked by local application of ATP were unchanged. Interestingly, the synaptic properties (quantal release and spontaneous activity) were unchanged. Importantly, reduction of

the mAChRs activity in PSCs restored their ability to repair and maintain NMJs structural integrity as suggested by the reduction in the level of denervation in the pirenzepine-treated mice. An NMJ healthyness index reflecting the quality of innervation also revealed a better general condition of the NMJs in the pirenzepine group. Taken together, these results suggest that reducing PSCs mAChRs activation could be beneficial in a context of ALS. This intrinsic PSC property could represent an important and novel therapeutic target in ALS.

Disclosures: D. Arbour: None. **É. Martineau:** None. **E. Tremblay:** None. **R. Robitaille:** None.

Poster

219. Motor Neuron Disease

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Program#/Poster#: 219.02/D11

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Fondazione Italiana di ricerca per la SLA (AriSLA), ALSsiMO PGTR 10/2012

Title: Morpholino antisense oligomer against SOD1 for amyotrophic lateral sclerosis therapy

Authors: C. SIMONE, M. NIZZARDO, F. RIZZO, G. ULZI, A. RAMIREZ, M. BUCCHIA, A. BORDONI, G. COMI, *S. CORTI;
Univ. of Milan, Milan, Italy

Abstract: Neurotoxicity from accumulation of misfolded/mutant proteins is thought to drive pathogenesis in neurodegenerative diseases. Mutations in superoxide dismutase 1 (SOD1) are linked to familial amyotrophic lateral sclerosis (FALS) resulting in progressive motor neuron death through one or more acquired toxicities. Interestingly wild-type SOD1 has been associated also to sporadic ALS (SALS), as misfolded SOD1 has been reported in affected tissues of sporadic patients. For these reasons, it represents a promising therapeutic target for antisense oligonucleotides. We now report slowed disease progression, ameliorated neuromuscular function and increased survival in an ALS *in vivo* model following therapeutic delivery of morpholino (MO) oligonucleotides designed to reduce the synthesis of ALS-causing human SOD1. Neuropathological analysis demonstrated an increased motor neuron and axon number, an ameliorated muscle trophism and a reduced micro and macrogliosis-mediated inflammation. Moreover, MO yield robust SOD1 suppression, in particular of misfolded form, not only *in vivo* in ALS rodent, but also in human patient samples, setting the stage for MO-mediated therapy in human clinical trials.

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Poster

219. Motor Neuron Disease

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Support: The Milton Safenowitz Post-Doctoral Fellowship ALSA (JHJ)

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Les Turner ALS Foundation (PHO)

Wenske Foundation (PHO)

NIH Grant 1R01NS085161-01 (PHO)

Title: Specific transduction of corticospinal motor neurons by AAV2 upon direct motor cortex injection

Authors: *J. H. JARA[1], M. J. STANFORD[1], Y. ZHU[2], C. G. BROOKS[1], W. W. HAUSWIRTH[3], M. C. BOHN[4], S. H. DEVRIES[2], P. OZDINLER[1,5,6];
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Abstract: The application of adeno-associated virus (AAV) in gene therapy has multiple advantages due to its long-term expression in the central nervous system (CNS) and low immunoreactivity in humans. Gene therapy strategies in CNS include Canavan's disease, Alzheimer's disease and motor neuron diseases such as amyotrophic lateral sclerosis (ALS). Targeting only the vulnerable neuron populations without affecting other neuron types within the cerebral cortex is a major obstacle for translational neuroscience. This applies to ALS, in which the corticospinal motors neurons (CSMN; a.k.a upper motor neurons) show selective vulnerability and progressive degeneration. In this study, seven different AAV serotypes that harbor the eGFP gene were tested for their ability to transduce CSMN upon direct injection into the layer V of the motor cortex. CSMN transduction was confirmed by Ctip2 immunocytochemistry and by presence of red fluorescent microsphere in the CSMN after retrograde labeling by injection into the corticospinal tract (CST). Large pyramidal neurons located in layer V showed higher tropism for AAV2-2. In an effort to increase the selective transduction of CSMN by AAV, we used capsid proteins that are engineered, and different promoters to drive the eGFP expression. Our results suggest that the choice of the promoter is critically important to enhance selectivity of gene expression in CSMN. Furthermore, specific

transduction of CSMN was feasible in mouse models with progressive CSMN degeneration. Identification of AAV serotypes that transduce only a select set of neuron populations at symptomatic stage of disease is critically important to develop effective and long-term gene therapy approaches in the cerebral cortex.

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Poster

219. Motor Neuron Disease

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 219.04/D13

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CDMRP DOD AL130079

Title: Expression of glial cell line-derived neurotrophic factor (GDNF) in the muscle using AAV does not slow disease progression the G93A SOD1 rat model of ALS

Authors: ***V. J. GARCIA**, G. GOWING, M. GODOY, P. SUEZAKI, P. AVALOS, D. RUSHTON, O. SHELEST, L. GARCIA, C. CHIU, I. ORELLANA, C. N. SVENDSEN; Regenerative Med. Institute/ Biomedica Sci., Cedars-Sinai, West Hollywood, CA

Abstract: Amyotrophic lateral sclerosis (ALS) is a complex and devastating disorder characterized by the degeneration of motor neurons in the cortex, brainstem and spinal cord leading to muscle weakness, paralysis and death within 1-5 years of diagnosis. Numerous studies have demonstrated a direct beneficial effect of growth factors, such as glial cell line-derived neurotrophic factor (GDNF) on motor neuron survival and function in experimental models of ALS. Specifically, the expression of GDNF at the muscle has been shown to promote motor neuron survival and function. Here, we performed direct intramuscular injection of Adeno-Associated Virus (1, 5, 6 or 9) encoding GDNF in the SOD1G93A rat model of ALS. Surprisingly, despite high expression of GDNF in injected muscles, the administration of AAV-GDNF had no effect on motor function when compared to controls. Quantification of motor neurons and muscle innervation are currently underway to determine the effect, if any, of AAV-GDNF on motor neuron pathology.

Disclosures: **V.J. Garcia:** None. **G. Gowing:** None. **M. Godoy:** None. **P. Suezaki:** None. **P. Avalos:** None. **D. Rushton:** None. **O. Shelest:** None. **L. Garcia:** None. **C. Chiu:** None. **I. Orellana:** None. **C.N. Svendsen:** None.

Poster

219. Motor Neuron Disease

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 219.05/D14

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Minimally invasive stereotactic surgical device for spinal cord injection of therapeutics

Authors: *S. K. SUCKOW[1], P. AVALOS[2], M. J. BAKER[1], B. EDIN[1], D. DRAZIN[3], M. DRLIK[4], J. GROVE[4], C. N. SVENDSEN[2];
[1]Due North Innovation, Portland, OR; [2]Regenerative Med. Inst., [3]Neurosurg. Dept., Cedars-Sinai Med. Ctr., Los Angeles, CA; [4]StarFish Med., Victoria, BC, Canada

Abstract: Physicians frequently require an incredible degree of accuracy when they perform surgical procedures around anatomical sites, especially in and around the spine. There is a need for stabilized surgical instruments and advanced work to optimize safety and usability during surgery. To meet this need, research was conducted in order to design a minimally invasive stereotactic surgical device that provides a stable and easily controlled interface for precise positioning of surgical instruments, such as a cannula or needle, within a surgical space. For this application, the minimally invasive stereotactic surgical device was designed for research relating to spinal cord injections. As such, an accessory cannula was designed to attach to the minimally invasive stereotactic surgical device to help deliver therapeutics, such as stem cells, to the dorsal horn of the spinal cord. Further, the minimally invasive stereotactic surgical device attaches to a third-party surgical retractor system, such as the Mast Quadrant Retractor, which is placed into a small incision site. To ensure steadiness of the surgical retractor system, stabilizing arms of the retractor system are affixed to the surgical table, which secures the surgical retractor in place. Design development of the minimally invasive stereotactic surgical device and accessory cannula included bench testing using a surrogate model of a spine, simulation testing including observational analysis related to human factors using a synthetic human model and animal testing including observational analysis related to usability. During simulation and animal testing, the minimally invasive stereotactic surgical device and accessory cannula were shown to move with the breathing motions of the animal and/or simulation model without invasively attaching the device beyond its connection to the surgical retractor.

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Poster

219. Motor Neuron Disease

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NRF-2010-0020408

NRF-2014R1A2A1A11052042

Title: Environmental enrichment after transplantation of mesenchymal stem cells enhance angiogenic effect in chronic hypoxic-ischemic brain injury

Authors: *S. WI[1,2], J. SEO[1,2], J. YU[1,2], Y. SHIN[1,2], S. CHO[1,2,3,4];
[2]Brain Korea 21 PLUS Project for Med. Sci., [3]Rehabil. Inst. of Neuromuscular Dis., [1]Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [4]Yonsei Stem Cell Res. Ctr., Avison Biomed. Res. Ctr., Seoul, Korea, Republic of

Abstract: Objective: Recent studies have shown that transplantation of mesenchymal stem cells(MSCs)have paracrine effects. Some studies are shown that environmental enrichment(EE)results in improved sensory motor function and stimulated endothelial cell proliferation. Astrocytes are activated by both MSCs and EE. Activated astrocytes produce not only angiogenic factors but also neurogenesis factors. We investigated angiogenic effects of astrocyte activation by EE and MSCs transplantation on neurobehavioral function in an animal model of chronic HI brain injury. **Methods:**HI brain damage was induced in 7-day-old CD-1®(ICR) mice by unilateral carotid artery ligation and exposure to hypoxia(8% O_2 for 90min). At 6 weeks of age, the mice were randomly injected with either MSC ($1x10^5$/2μl) or phosphate buffered saline(PBS) into the striatum and assigned to either EE or standard cages(SC), comprising MSC-EE, MSC-SC, PBS-EE, PBS-SC. Whereas SC controls were housed for the same duration in a standard cag(27×22.5×14 cm), EE mice were housed in a huge cage(86×76×31 cm) containing novel objects for up to 2 months. Rotarod, forelimb-use asymmetry, grip strength, and openfield tests were performed to evaluate neurobehavioral function. We confirmed the fate of transplanted cells, the levels of endogenous angiogenesis, activated astrocytes and glial scar using immunohistochemistry. To identify growth factors that are regulated by MSC transplantation and/or EE, neostriata separated from brain, a array-based multiplex ELISA assay was used to determine which of the following 10 cytokines were detectable in the neostriata. Expression of angiogenic and neurotrophic factors using western blot. **Results:**EE and MSCs transplantation synergistically improved rotarod latency, forelimb-use asymmetry, and grip strength compared to those of the other groups. The number of engrafted MSCs and $CD31^+$ endothelial cells were significantly higher in mice in EE than those in SC. Two weeks after EE and MSC transplantation increased density of $CD\text{-}31^+$ and a-smooth muscle $actin^+$ cells coupled with increased $GFAP^+$ astrocytic density and fibroblast growth factor-2 (FGF-2) but not the level of $CS\text{-}56^+$ glial scarring in the striatum. The fate of transplanted cells and the levels of endogenous angiogenesis, astrocyte activation and angiogenic factors were also measured. **Conclusions:**EE and MSC transplantation synergistically improved neurobehavioral functions. The underlying mechanisms of this synergism included enhanced repair processes such as higher engraftment of the transplanted MSCs, increased endogenous angiogenesis and astrocyte activation coupled with upregulation of FGF-2.

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Poster

219. Motor Neuron Disease

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: The Judith and Jean Pape Adams Foundation

Title: Genotype specific impact of nuclear factor E2-related factor 2 (Nrf2) treatment in animal models of amyotrophic lateral sclerosis

Authors: ***W. NANDAR**[1], E. B. NEELY[1], Z. SIMMONS[2], J. R. CONNOR[1];
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Abstract: H63D HFE gene variant, found in approximately 30% of ALS patients, impacts disease processes implicated in amyotrophic lateral sclerosis (ALS). We generated double transgenic mice (SOD1/H67D) and show the addition of H67D HFE (homologous to human H63D) to the SOD1 mice shortens survival and accelerates disease progression. We found elevated oxidative stress associated with decreased Nrf2 levels is one mechanism contributing to accelerated disease in double transgenic mice. The Nrf2 signaling pathway is the major cellular defense mechanism against oxidative stress and regulates expressions of many endogenous antioxidant genes. Neuroprotective effects of the Nrf2 activator (2-Cyano-3,12-Dioxooleana-1,9-Dien-28-Oic acid trifluoroethylamide or CDDO-TFEA) are reported in preclinical models of neurodegenerative disease. We evaluated the impact of HFE genotype on the effect of CDDO-TFEA using double transgenic and SOD1 mice. We hypothesize a genotype-dependent response to treatment in ALS. At 97 days of age, double transgenic and SOD1 mice were fed either control or diet supplemented with CDDO-TFEA (400mg/kg body weight). We evaluated effects on disease onset, disease progression and survival. Disease onset was determined by motor performance on the rotarod apparatus. A gripstrength meter that measures forelimb and hindlimb strength was used to evaluate disease progression. Disease end-stage was defined as the inability of an animal to right itself within 30 seconds after being placed on its side. We found diet supplemented with CDDO-TFEA significantly prolonged survival in double transgenic mice, but had no effect in SOD1 mice. The CDDO-TFEA had no effect on age at onset and disease duration in double transgenic or SOD1 mice. Moreover, we separately evaluated nutritional effects and found mice fed an animal-based diet containing cholesterol survived longer than those fed a vegetarian-based diet lacking cholesterol, an effect more pronounced in double transgenic mice. Thus, nutritional interventions including specific nutritional supplements could have therapeutic values in ALS. Our findings indicate a genotype specific response to treatment

in ALS and provide support for stratifying patients by genotype. Therefore, findings from double transgenic mice, which are relevant to the 30% of ALS patients carrying H63D HFE, could impact clinical practice and evaluation of treatment strategies.

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Poster

219. Motor Neuron Disease

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ALS Therapy Alliance Grant 2013-F-030

Title: The role of nuclear export in TDP43-mediated neurodegeneration

Authors: *H. ARCHBOLD[1], X. LI[1], S. TAMIR[4], S. BARMADA[1,2,3];
[1]Dept. of Neurol., [2]Cell. & Mol. Biol. Program, [3]Neurosci. Program, Univ. of Michigan, Ann Arbor, MI; [4]Karyopharm Therapuetics, Newton, MA

Abstract: Nuclear exclusion and cytoplasmic deposition of the RNA binding protein TDP43 (transactive response element DNA binding protein of 43 kDa) is a pathological hallmark of the related neurodegenerative disorders amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Mutations in the TDP43 gene associated with familial ALS cause a redistribution of the protein from the nucleus to the cytoplasm, and we have previously shown that cytoplasmic TDP43 deposition closely predicts cell death, making the nucleocytoplasmic shuttling of TDP43 an attractive therapeutic target. TDP43 contains putative nuclear export signal (NES) and nuclear localization signal (NLS) motifs, as well as an M9-like domain, which is sufficient for karyopherin-mediated transport of related RNA binding proteins. Here, we find that selective inhibitors of nuclear export (SINE) compounds - designed to inhibit the interaction of exportin 1 (Xpo1/CRM1) with its cargo substrate proteins - improve cellular survival in primary neuron models of ALS and FTD involving TDP43. Preliminary data indicate that SINE compounds decrease levels of endogenous, but not exogenously expressed, TDP43. TDP43 negatively regulates its own production at the RNA level, suggesting that nuclear sequestration of TDP43 may promote a negative feedback loop that protects neurons from death by decreasing nuclear and whole cell levels of TDP43. In support of this hypothesis, steady state TDP43 levels are dose-dependently related with neurodegeneration, and alternative methods for reducing TDP43 protein levels have proven to be potent neuroprotective therapies in cellular models of

ALS and FTD. However, SINE compounds affect the distribution and activity of several transcription factors important for neuronal survival, and an indirect effect upon TDP43 levels and/or neurodegeneration remains a possibility. Given that related SINE compounds are currently undergoing evaluation in clinical trials for other indications, we expect that validation of their neuroprotective effects in neuronal models of ALS and FTD such as these will facilitate and accelerate their translation to humans with disease.

Disclosures: **H. Archbold:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); KPT compounds provided by Karyopharm Therapeutics Inc.. **X. Li:** None. **S. Tamir:** A. Employment/Salary (full or part-time):; Full time employee of Karyopharm Therapeutics, Inc.. **S. Barmada:** None.

Poster

219. Motor Neuron Disease

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Agencia Nacional de Investigación e Inovación (ANII) - FCE 2011_7342

Title: Effect of the tyrosine kinase inhibitor masitinib in transgenic rats expressing SOD1G93A mutation

Authors: ***E. TRIAS**[1], S. IBARBURU[1], R. BARRETO-NÚÑEZ[1], T. MACIEL[2], P. DÍAZ-AMARILLA[3], P. CASSINA[4], L. MARTÍNEZ-PALMA[4], J. BABDOR[2], C. MANSFIELD[5], A. MOUSSY[5], I. MOURA[2], J. S. BECKMAN[6], O. HERMINE[2,7], L. BARBEITO[1]; [1]Neurodegeneration, Inst. Pasteur De Montevideo, Montevideo, Uruguay; [2]Dept. of Hematology, Imagine Institute, Hôpital Necker, Paris, France; [3]Neurobiología Celular y Mol., Inst. de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay; [4]Dept. de Histología, Facultad de Medicina, Univ. de la República, Montevideo, Uruguay; [5]AB Sci., Paris, France; [6]Dept. of Biochem. and Biophysics,, Linus Pauling Institute, Oregon State Univ., Corvallis, OR; [7]Ctr. national de référence des mastocytoses (CEREMAST), Paris, France

Abstract: Neuronal degeneration in amyotrophic lateral sclerosis (ALS) appears to begin as a focal process that spreads contiguously through the upper and lower motor neurons. Such disease progression leads to increasing paralysis. It likely implicates an acquired pathogenic mechanism maintained by interaction of damaged neurons and inflammatory cells including astrocytes, microglia, mast cells and T cells. We have previously showed evidence for aberrant glial cells (AbA cells) emerging around damaged motor neurons. Because AbA cells exhibit a high toxicity for motor neurons, they may be considered as a cell type specifically associated to disease progression in ALS. As part of the ALS neuroinflammatory reaction, mast cells appear as a key

cell type associated to chronic inflammation. In this context, two studies have shown evidence for increased mast cell infiltration in the postmortem samples of ALS patients. Masitinib mesilate (Mb) is a selective, tyrosine kinase inhibitor that targets c-Kit, Lyn and Fyn pathways. By combined targeting of c-Kit, Lyn and Fyn, Mb is particularly efficient in controlling mast cell survival, differentiation, and degranulation. Our hypothesis establishes that Mb indirectly decrease the appearance of inflammatory AbA cells through the down-regulation of activated mast cells. To further explore our hypothesis we assessed whether Mb can slow disease progression in the SOD1G93A rat model of ALS. Mb (30 mg/kg/day) was orally administered from disease onset until complete paralysis. Mb treatment significantly prolonged life span of ALS SOD1G93A rats. To further determine the mechanism of action of Mb we analyzed the neuroinflammatory components in the degenerating spinal cord after the treatment. The histopathological analysis performed suggested that Mb provided a significant protection against progressive motor neurons loss and degeneration when compared with control animals. Furthermore, all parameters of neuroinflammation, including the degree of astrocytosis, the number of AbA cells and microgliosis were dramatically reduced in Mb-treated rats with respect to control. Taken together, the present findings provide convincing evidence for a protective role of Mb in an inherited model of ALS. Additionally, the treatment regime implemented by these studies reflects a clinically plausible scenario, i.e. therapeutic settings. Mb is currently being evaluated in two phase 3 clinical trials for ALS, administered as either a single-agent or in combination with riluzole.

Disclosures: **E. Trias:** None. **S. Ibarburu:** None. **R. Barreto-Núñez:** None. **T. Maciel:** None. **P. Díaz-Amarilla:** None. **P. Cassina:** None. **L. Martínez-Palma:** None. **J. Babdor:** None. **C. Mansfield:** None. **A. Moussy:** None. **I. Moura:** None. **J.S. Beckman:** None. **O. Hermine:** None. **L. Barbeito:** None.

Poster

219. Motor Neuron Disease

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 219.10/D19

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: EVMS-William and Mary Collaborative Grant

Title: Pur alpha: a potential therapy for ALS due to the C9ORF72 expanded repeat

Authors: *E. W. GODFREY[1], J. W. ORIANS[2], T. E. CRIST[2], E. M. JOHNSON[3], D. C. DANIEL[3];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that results in paralysis due to loss of motor neurons in the cortex and spinal cord. The most common genetic cause of ALS is an expanded hexanucleotide repeat (GGGGCC) in a non-coding region of the C9ORF72 gene, which encodes a protein involved in endocytosis and autophagy. Patients with this mutation have hundreds to thousands of copies of this repeat sequence. Repeat sequence RNA aggregates in nuclear foci that can be detected by fluorescent *in situ* hybridization (FISH). The mechanism by which the C9ORF72 repeat causes ALS is unknown, but the repeat sequence in the RNA sequesters RNA-binding proteins. Disturbing the content of RNA-binding proteins in the cell can affect transcription, translation, and splicing of many mRNAs, resulting in expression of altered RNAs and reduction in RNA content. Pur-alpha, an abundant RNA- and DNA-binding protein that binds to GGGGCC repeats, or a peptide with a generic Pur family repeat, may be therapeutic in C9ORF72 ALS. Pur alpha binds to the GGGGCC sequence with high affinity and has been colocalized with the nuclear RNA foci. Overexpression of Pur alpha in *Drosophila* or neuronal cells with 30 copies of the repeat resulted in a reduction in neurodegeneration (Xu et al., 2012). We used virally transformed lymphocytes from C9ORF72 ALS patients to test the ability of Pur alpha to reduce cellular pathology. We labeled autophagosomes of cells from patients and controls with an antibody to p62, a protein in the autophagosome membrane. These organelles are involved in removal of proteins that misfold and aggregate in the cytoplasm of neurons in ALS. We also quantitated the number of autophagosomes and autolysosomes using a fluorescent LC3 probe. Lymphoblasts from C9ORF72 patients had significantly more autophagosomes and p62 protein than control cells. Transfection of Pur alpha or treatment of cells with the Pur peptide resulted in a significant reduction in p62-positive puncta in C9ORF72 lymphoblasts. Pur alpha and the Pur peptide reduced the number of autophagosomes almost to the level in control cells. Our results suggest that Pur proteins and the peptide stimulate autophagy and ameliorate cellular pathology in this form of ALS. Experiments are underway to test the efficacy of the peptide in reducing cellular pathology in neurons differentiated from iPS cells of C9ORF72 ALS patients.

Disclosures: **E.W. Godfrey:** None. **J.W. Orians:** None. **T.E. Crist:** None. **E.M. Johnson:** None. **D.C. Daniel:** None.

Poster

219. Motor Neuron Disease

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: The Health and Medical Research Fund (HMRF), Food and Health Bureau, Hong Kong Special Administrative Region Government (Ref. No: 01122026)

Title: Protective effects of heat shock protein 27 on anti-ganglioside antibody-mediated neuropathy in a mouse model of Guillain-Barré syndrome

Authors: *P. ASTHANA[1], G. KUMAR[1], R. C. C. CHANG[2,3], G. ZHANG[4], K. A. SHEIKH[4], C. H. E. MA[1,5];
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Abstract: Guillain-Barré syndrome (GBS) is a serious autoimmune disease affecting the peripheral nervous system. Majority of the GBS patients experienced neurological symptoms such as paresthesia, muscle weakness, persistent pain and areflexia. In GBS, immune cells start attacking the major complex gangliosides present on the peripheral nerves resulting in nerve injury or demyelination. Clinical studies showed that high levels of anti-ganglioside antibodies were detected in the sera of the GBS patients. Our previous studies reported that heat shock protein (Hsp) 27 accelerated the axonal regeneration in mice after peripheral nerve injury. In the current *in vitro* and *in vivo* experiments, we showed that forced expression of a human (h) Hsp27 could overcome anti-ganglioside mediated nerve regeneration inhibition. Passive transfer of anti-ganglioside antibodies (GD1a/GT1b-2b) into transgenic (Tg) mice overexpressing hHsp27 and their littermates (LM) to study their functional recovery. Sensory function recovery assessed by pinprick test was ten days earlier in hHsp27 Tg mice as compared to LM controls. Motor function recovery was restored to baseline in Tg mice as observed by grip strength, toe spread and sciatic functional index. Our electromyography and histology data showed that Tg mice demonstrated a mark improvement of muscle function in terms of increasing compound muscle action potential and neuromuscular junction reinnervation. Taken together, our data indicates that Hsp27 could be a potential therapeutic target for GBS prevention. Further investigation is needed to elucidate the molecular mechanism involved by examining some of the key genes in axonal regeneration by single cell PCR.

Disclosures: **P. Asthana:** 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 Health and Medical Research Fund (HMRF), Food and Health Bureau, Hong Kong Special Administrative Region Government (Ref. No: 01122026). **G. Kumar:** None. **R.C.C. Chang:** None. **G. Zhang:** None. **K.A. Sheikh:** None. **C.H.E. Ma:** None.

Poster

219. Motor Neuron Disease

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 219.12/D21

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CIRM DR2A-05320

Title: Neural progenitor cells secreting glial cell line-derived neurotrophic factor (GDNF) for the treatment of amyotrophic lateral sclerosis

Authors: *G. M. GOWING, B. SHELLEY, P. AVALOS, J. LATTER, A. HURLEY, L. GARCIA, K. STAGGENBORG, M. CHEN, M. GODOY, P. SUEZAKI, D. RUSHTON, R. PARADIS, J. ZELAYA, A. LIN, L. SHUE, C. CHANG, C. CHIU, K. NISHIMORI, J.-P. VIT, C. N. SVENDSEN;
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Abstract: Astrocytes releasing the powerful growth factor glial cell line-derived neurotrophic factor (GDNF) have been shown to slow motor neuron degeneration in the SOD1G93A rat model of amyotrophic lateral sclerosis (ALS). In the current study we have generated a process comparable line of human neural progenitors engineered to secrete GDNF (hNPC-CNS10GDNF). These have been used to perform full dose ranging, bio-distribution studies, along with preliminary toxicity and tumorogenicity studies in a rodent model of ALS, immune compromised animals and pigs. We show that at all doses the transplanted cells survive, migrate, release GDNF and do not form tumors. Grafted cells provided significant protection to dying motor neurons in this model, although no dose could protect the motor neuron connection to muscle and thus did not prevent paralysis in the animals. Together this body of work shows that hNPC-CNS10 GDNF can be grown under Good Manufacturing Procedure (GMP)-like conditions and safely delivered to both rodents and pigs where they protect dying motor neurons. This combined stem cell and gene therapy approach is now being moved forward into final pre-clinical enabling studies and submitted as an investigational new drug (IND) to treat ALS.

Disclosures: G.M. Gowing: None. **B. Shelley:** None. **P. Avalos:** None. **J. Latter:** None. **A. Hurley:** None. **L. Garcia:** None. **K. Staggenborg:** None. **M. Chen:** None. **M. Godoy:** None. **P. Suezaki:** None. **D. Rushton:** None. **R. Paradis:** None. **J. Zelaya:** None. **A. Lin:** None. **L. Shue:** None. **C. Chang:** None. **C. Chiu:** None. **K. Nishimori:** None. **J. Vit:** None. **C.N. Svendsen:** None.

Poster

219. Motor Neuron Disease

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 219.13/D22

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Fapesp

Title: Tempol treatment reduces microglial reaction in late stages of disease in SOD1 mutant mice

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Abstract: A hallmark of amyotrophic lateral sclerosis (ALS) is the progressive loss of motoneurons. Preservation of synapses together with modulation of gliosis may contribute to decrease or delay neuronal degeneration. This may not be achieved with riluzole alone, that is the only drug currently available. Thus, new substances must be evaluated, given the fact that the oxidative stress greatly contributes to the disease progression. In this scenario, 4-hydroxy-TEMPO (tempol) is a potent nitric oxide scavenger and superoxide dismutase (SOD) mimetic, putatively capable of neuroprotection. Therefore, we investigated neuroprotection and glial reaction in SOD1 G93A mice orally treated with tempol. Male and female siblings were distributed in the following groups: control (absence SOD1 mutation); vehicle treated SOD1 mutant mice; riluzole (8mg/kg) treated; tempol (24mg/kg) treated. Treatment began in 70 days old mice and was kept every other day for twenty days. From that point, drug administration was carried out twice a week until euthanasia at degree four of disease. Following fixative perfusion, the lumbar spinal cord was dissected out and processed for Nissl staining (neuronal survival) and immunohistochemistry, to evaluate glial reaction (GFAP and Iba1) and spinal synapse preservation (synaptophysin, GAD65 and VGLUT1). Immunolabeling was accessed in three spinal cord regions: ventral horn (lamina IX), intermediated region and dorsal horn (laminae I to III). The body weight, onset of the disease, motor score and survival were also analyzed. Nissl staining revealed that ALS led to degeneration of more than fifty percent of all motoneurons, and different treatments were unable to reverse such scenario. SOD 1 mutant mice also displayed loss of more than half of synapses in the ventral horn, being more prominent in glutamatergic (VGLUT1-positive, 87%) than in GABAergic inputs (55%). Uniform loss of such inputs took place in other spinal cord regions. Astrogliosis, evaluated by GFAP labeling, was increased in all SOD1 mutant groups, appearing more intense in the ventral horn. Tempol and riluzole were effective in decreasing microglial reaction, seen by Iba1 labeling, particularly in the dorsal region, that receives most of the primary afferent inputs, contributing, among other functions, to the sensorimotor integration. Nevertheless, no statistical differences among groups regarding the onset of the disease, loss of body mass, progression and survival endpoint were achieved. Taken together, the results indicate that tempol is unable to reverse the fate of SOD1 mutant mice, although it shows potentially beneficial effects regarding microglial reaction.

Disclosures: G. Chiarotto: None. **A.B. Spejo:** None. **A.L.R. Oliveira:** None.

Poster

219. Motor Neuron Disease

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: AurimMed Pharma, Inc.

NIH grant 8P20GM103464

Nemours Foundation

Title: Identification of novel inducers of SMN2 expression using the Privileged Structure Platform

Authors: *M. E. R. BUTCHBACH[1,2,3], A. W. HARRIS[1], A. PESYAN[4];
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Abstract: Spinal muscular atrophy (SMA), a leading genetic cause of infant death worldwide, is an autosomal recessive motor neuron disease caused by the loss of *SMN1* but retention of *SMN2*. The number of copies of *SMN2* inversely correlates with disease severity in both SMA patients as well as in mouse models for SMA. There is currently no successful treatment for SMA; however, much attention has been focused on *SMN2* as a target for therapeutics development. AurimMed Pharma, Inc. has developed a focused library of potent central nervous system (CNS) active compounds based on their Privileged Structure Platform (PSP). In this study, we screened this PSP library for compounds that modulate *SMN2* expression using reporter assay cell lines as well as fibroblasts derived from SMA patients. To monitor changes in *SMN2* promoter activity in response to drug treatment, we used a β-lactamase reporter construct under the control of the 3.4 kb *SMN2* promoter expressed in motor neuron-like NSC-34 cells. 64 compounds from the PSP library were screened for SMN2 promoter induction activity; 26 of these compounds showed enhanced *SMN2* promoter activity relative to vehicle (DMSO)-treated cells. In fact, 3 of these compounds induced *SMN2* promoter activity at a level similar to that for our positive control, the C5-substituted 2,4-diaminoquinazoline D156844. We also tested 7 PSP compounds for their ability to increase SMN localization to subnuclear gems in SMA cells. Type II SMA fibroblasts were treated with these compounds for 5 days and then immunolabeled for SMN. Of the 7 test compounds, 6 increased gems counts relative to vehicle-treated SMA fibroblasts in a dose-dependent manner. 2 of these compounds increased gem counts to those observed in healthy, carrier fibroblasts. Some of the gem inducing compounds did not significantly increase *SMN2* promoter activity. We have identified CNS active compounds that increase *SMN2* promoter activity and/or SMN protein localization to gems. Because some of the gem inducing PSP compounds did not increase *SMN2* promoter activity, different compounds may be regulating SMN2 expression at different levels of gene regulation. Future work will determine the

mechanisms by which these compounds are increasing *SMN2* expression. Furthermore, these compounds will be moved forward into preclinical drug studies in mouse models of SMA.

Disclosures: **M.E.R. Butchbach:** 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.; AurimMed Pharma, Inc.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AurimMed Pharma, Inc.. **A.W. Harris:** None. **A. Pesyan:** A. Employment/Salary (full or part-time):; AurimMed Pharma, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AurimMed Pharma, Inc..

Poster

219. Motor Neuron Disease

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 219.15/D24

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Boehringer Ingelheim Ulm University BioCenter (BIU): N2

Title: Monoacylglycerol lipase inhibiton is therapeutically effective in chronic mouse models of amyotrophic lateral sclerosis and Parkinson's disease

Authors: N. PASQUARELLI[1], C. PORAZIK[1], J. HANSELMANN[1], P. WEYDT[1], B. FERGER[2], *A. WITTING[1];
[1]Univ. Ulm, Ulm, Germany; [2]Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach/Riß, Germany

Abstract: For the development of therapeutic strategies towards neurodegenerative diseases, the endocannabinoid system is a valuable approach. Especially the 2-arachidonoyl glycerol (2-AG)-degrading enzyme monoacylglycerol lipase (MAGL) represents a promising target as inhibition of MAGL may induce neuroprotective and anti-inflammatory effects by increasing 2-AG and decreasing arachidonic acid and prostaglandins. To study the therapeutic potential of MAGL in neurodegenerative diseases, we pharmacologically inhibited MAGL by the highly selective inhibitor KML29 in chronic mouse models of amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD). For ALS, we orally treated 150-day-old low-copy B6SJL-Tg(SOD1*G93A)dl1Gur/J (SOD1G93A) mice with KML29 (0, 0.5, 5, 10 mg/kg, 3x/week) and monitored disease onset, progression and survival as well as running wheel activity and body weight. For PD, we orally treated mice from the chronic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine/probenecid (MPTP, 10 mg/kg; probenecid 250 mg/kg; 2x/week) mouse model of PD with KML29 and the fatty acid amide hydrolase (FAAH) inhibitor PF3845 (10

mg/kg, 5x/week) over five weeks and quantified dopamine and metabolites by HPLC/ECD in the striatum. In addition we quantified the expression of cannabinoid receptors and MAGL on mRNA and protein level and of endocannabinoids, arachidonic acid and prostaglandins by LC-MS/MS. To investigate the effect of MAGL inhibitors on neuroprotection and inflammation, we quantified cytokines and neurotrophins on mRNA and protein level and performed immunohistochemistry. In SOD1G93A mice, KML29 delayed the disease onset, the occurrence of first pareses and the disease end-stage in a dose-dependent manner. Furthermore, KML29 significantly delayed the disease-associated loss in body weight and running wheel activity. Post mortem, KML29 increased 2-AG levels in a cumulative manner during the disease course and decreased arachidonic acid without enhancing anandamide levels. In addition, KML29 induced an increase in MAGL, CB1 and BDNF expression, suggesting a neuroprotective mechanism. In MPTP mice, KML29, but not PF3845, was able to restore striatal dopamine levels by 20 %, further highlighting a neuroprotective mode of action of KML29. In conclusion, our therapeutic application of KML29 evidences MAGL as a valuable target for the treatment of neurodegenerative diseases like ALS and PD and warrants further investigation on a mechanistic level.

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Poster

219. Motor Neuron Disease

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Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CIRM Grant DR2A-05320

Institutional

Title: Use of a new minimally invasive intraparenchymal spinal delivery system for the injection of stem cells into the spinal cord

Authors: ***P. AVALOS**[1], G. GOWING[1], B. C. SHELLEY[1], D. DRAZIN[2], L. GARCIA[1], M. J. BAKER[3], S. SUCKOW[3], B. EDIN[3], C. N. SVENDSEN[1];
[1]Regenerative Med. Inst., [2]Neurosurg., Cedars-Sinai Med. Ctr., Los Angeles, CA; [3]Due North Innovation, Portland, OR

Abstract: A number of novel treatment approaches for diseases, such as amyotrophic lateral sclerosis (ALS) and spinal cord injury, require intraparenchymal delivery of a therapeutic into the spinal cord. ALS is a neurodegenerative disease that affects motor neurons leading to

paralysis and death 1 to 5 years following diagnosis. There is no effective treatment for ALS. Direct transplantation of stem cells into the spinal cord parenchyma has been shown to have beneficial effects in ALS animal models. However, spinal transplantation is a relatively new procedure and currently available systems require extensive training, involve cumbersome and complex devices and use delivery systems that may lead to increased risk of injury. We have developed a new spinal delivery system that can safely, successfully and precisely target the injection of human neural progenitor cells (hNPC) secreting the powerful growth factor glial cell line-derived neurotrophic factor (GDNF) to the spinal cord of Yucatan mini-pigs. Preliminary studies have shown survival and appropriate targeting of grafted cells. Moreover, evaluation of post-operative locomotor function of mini-pigs shows that they return to the pre-surgical baseline within 5 days post-surgery, demonstrating the safety of this approach. In conclusion, this new device appears efficient, safe and simple to use. As such, an extensive Good Laboratory Practice (GLP) safety study in Yucatan mini-pigs is ongoing in order to validate the device for use in a Phase I trial for the transplantation of hNPC secreting GDNF into the lumbar spinal cord of ALS patients.

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Poster

219. Motor Neuron Disease

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Program#/Poster#: 219.17/D26

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: CIBERNED

TerCel

Title: CSF1R signalling has a pivotal role in amyotrophic lateral sclerosis

Authors: ***A. MARTÍNEZ-MURIANA**[1], R. MANCUSO[1], I. FRANCOS-QUIJORNA[1], A. OLMOS-ALONSO[2], R. OSTA[3], V. PERRY[2], X. NAVARRO[1], D. GOMEZ-NICOLA[2], R. LÓPEZ-VALES[1];
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Abstract: Inflammation is a common neuropathological feature in many neurological disorders, including amyotrophic lateral sclerosis (ALS). In the present work we studied the contribution of CSF1R signalling to inflammation in ALS. We found that microglial cell expansion in the spinal cord of SOD1G93A transgenic mice showed a similar progression than the expression of CSF1R

and its ligand CSF1. Administration of GW2580, a selective CSF1R inhibition, reduced microglial cell proliferation in this ALS mouse model suggesting the importance of CSF1-CSF1R signalling in microgliosis in ALS. Moreover, GW2580 slowed disease progression and death of SOD1G93A mice. Electrophysiological assessment revealed that that GW2580 reduced muscle denervation prior to its effects on microglial cells. Interestingly, we found that macrophages invaded the peripheral nerve of ALS mice before microgliosis occurred, and that treatment with GW2580 attenuated macrophages influx into the nerve, in part, due to the difficulty of bone marrow cells to produce monocytes upon CSF1R inhibition. Overall, our findings provide clear evidence that CSF1R signalling regulates inflammation in the central and peripheral nervous system in ALS, and highlight the importance of nerve macrophages in the course of ALS pathology, supporting therapeutic targeting of CSF1R in ALS

Disclosures: A. Martínez-Muriana: None. **R. Mancuso:** None. **I. Francos-Quijorna:** None. **A. Olmos-Alonso:** None. **R. Osta:** None. **V. Perry:** None. **X. Navarro:** None. **D. Gomez-Nicola:** None. **R. López-Vales:** None.

Poster

219. Motor Neuron Disease

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 219.18/D27

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: ALS Worldwide

Title: Assessing the neuroprotective effects of MicroNeurotrophins in the SOD1 mouse model of Amyotrophic Lateral Sclerosis

Authors: *K. E. GLAJCH[1], K. MUELLER[1], L. RYCYNA[1], C. VANDERBURG[1], A. GRAVANIS[2], G. SADRI-VAKILI[1];
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder caused by loss of motor neurons. ALS patients experience rapid deterioration in muscle function with an average lifespan of 3-5 years after diagnosis. Currently, the most effective therapeutic for the treatment of ALS only extends lifespan by a few months. Neurotrophic factors (NTFs) are a group of molecules involved in neuronal development, maintenance, and survival. NTF treatment has previously shown efficacy in pre-clinical ALS models. However, clinical trials using NTFs produced no major improvements in ALS patients, due in part to the limited penetration of NTFs across the blood brain barrier (BBB). Microneurotrophins (MNTs) are small neurotrophic-like compounds that cross the BBB and bind to tyrosine kinase receptors mimicking the pro-survival effects of endogenous neurotrophic factors. In this study, we sought

to determine the potential therapeutic efficacy of the MNT BNN27 in a mouse model of ALS expressing the G93A mutation in the superoxide dismutase (SOD1) gene. Previous preliminary studies from our group showed that administration of BNN27 resulted in trends towards the improvement of disease phenotypes, delayed disease progression, and increased motor neuron number in the lumbar spinal cord in male and female mutant SOD1 mice. We will present results from our large pre-clinical study on the effects of BNN27 treatment on motor neuron survival, motor behavior, disease progression, and survival. These findings could reveal a novel potential therapeutic for the treatment of ALS.

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Poster

219. Motor Neuron Disease

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Program#/Poster#: 219.19/D28

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Intra spinal delivery of human neural progenitors releasing ciliary neurotrophic factor (CNTF) ameliorate functional decline in the G93A rat model of amyotrophic lateral sclerosis

Authors: *M. GODOY, G. GOWING, P. SUEZAKI, D. RUSHTON, K. STAGGENBORG, P. AVALOS, L. GARCIA, O. SHELEST, R. PARADIS, C. CHIU, C. CHANG, C. SVENDSEN; Board of Governors Regenerative Med. Inst., Cedars-Sinai Med. Ctr., Los Angeles, CA

Abstract: Amyotrophic lateral sclerosis (ALS) is a complex and devastating disorder characterized by the degeneration of motor neurons in the cortex, brainstem and spinal cord, resulting in muscle weakness, paralysis and death within 1-5 years of diagnosis. Glial cells, specifically Schwann cells and astrocytes in the CNS and PNS, express ciliary neurotrophic factor (CNTF), which has been demonstrated to protect neurons, including motor neurons, following injury. Here we used an ex-vivo gene therapy approach with the transplantation of human neural progenitors expressing CNTF into the spinal cord of G93A SOD1 rats in order to see if they could extend motor neuron survival and function. We have demonstrated that neural progenitors expressing CNTF survive unilateral transplantation into the lumbar spinal cord and secrete CNTF. Preliminary behavioral results have shown that female rats treated with CNTF-expressing neural progenitor cells show a significant difference in rate of motor function decline on the ipsilateral versus contralateral side. Further analysis is underway to confirm the effect of transplanting CNTF-expressing neural progenitor cells on motor neuron survival and function. In conclusion, our results suggest a therapeutic benefit of CNTF in the ALS rodent animal model.

Disclosures: **M. Godoy:** None. **G. Gowing:** None. **P. Suezaki:** None. **D. Rushton:** None. **K. Staggenborg:** None. **P. Avalos:** None. **L. Garcia:** None. **O. Shelest:** None. **R. Paradis:** None. **C. Chiu:** None. **C. Chang:** None. **C. Svendsen:** None.

Poster

219. Motor Neuron Disease

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 219.20/D29

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NHMRC project 1044407

Title: Alternative anaplerotic fuel in the hSOD1G93A mouse model of Amyotrophic Lateral Sclerosis

Authors: ***K. BORGES**, T. TEFERA, Y. WONG, K. TAN, T. MCDONALD, S. NGO; Univ. of Queensland, St Lucia, Australia

Abstract: Amyotrophic lateral sclerosis (ALS) is characterized by degeneration of motor neurons in the motor cortex and spinal cord resulting in muscle weakness and degeneration, paralysis and ultimately death usually due to respiratory failure. There is mounting evidence that there is impaired energy metabolism in patients with ALS and in animal models that contributes to the disease. There is an increased energy requirement as a result of hypermetabolism (higher energy expenditure), reduced food intake and weight loss. Alternative metabolic substrates such as triheptanoin, the triglyceride of heptanoate, are ideally suited to alleviate many of the known metabolic problems in ALS. Triheptanoin is already being tested in clinical trials for energy metabolism and other neuromuscular disorders. Being tasteless, it is mixed with food and quickly provides heptanoate, which diffuses freely into the mitochondria of all tissues, including motor neurons and muscle. Heptanoate is beta-oxidized to acetyl-CoA, the main substrate for the tricarboxylic acid (TCA) cycle, and propionyl-CoA. Propionyl-CoA refills C4 intermediates of the TCA cycle (anaplerosis) as it is metabolized to succinyl-CoA and then oxaloacetate. Our quantitative real time PCR data show that when compared to healthy wild type mice, the mRNA levels of several enzymes involved in glycolysis and the TCA cycle were significantly reduced in the gastrocnemius muscle of $hSOD1^{G93A}$ mice at 10 and 25 weeks of age, including the dehydrogenases for pyruvate (Pdha1), 2-oxoglutarate (Ogdh) and succinate (Sdha) and the main muscle glutamic pyruvic transaminase 2. 35% of calories provided as triheptanoin prevented the reduction in Pdha1 and Sdha mRNA levels seen in $hSOD1^{G93A}$ mice, illustrating protective effects of triheptanoin on the TCA cycle. Also, we found several differences in maximal activities for glycolytic and other enzymes in muscle at different disease stages in $hSOD1^{G93A}$ mice, indicating impaired energy metabolism. Initiating triheptanoin to $hSOD1^{G93A}$ mice at P35

delayed the onset of motor symptoms by 2.5 weeks, as well as onset of body weight loss and motor balance loss (all $p<0.01$**)** and seems to delay motor neuron loss at 10 weeks.

Disclosures: **K. Borges:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Ultrgenyx Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ultrgenyx Pharmaceuticals. **T. Tefera:** None. **Y. Wong:** None. **K. Tan:** None. **T. McDonald:** None. **S. Ngo:** None.

Poster

219. Motor Neuron Disease

Location: Hall A

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Program#/Poster#: 219.21/D30

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: PHS Grant No. 5 T32 NS 41234-14

Foglia Family Foundation

Title: Atypical electropherogram patterns are observed among C9orf72 (G4C2) expansion carriers in a familial and sporadic ALS and ALS/FTD North American cohort study

Authors: *J. L. LOWRY, J. YAN, L. KINSLEY, N. SIDDIQUE, H.-X. DENG, T. SIDDIQUE; Neurol., Northwestern Univ., Chicago, IL

Abstract: A hexanucleotide (G_4C_2) repeat expansion mutation on Chromosome 9 open reading frame 72 (C9orf72; C9) is currently identified as the most common cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). We have been actively genotyping one of the largest North American ALS and ALS/FTD cohorts since originally establishing genetic linkage at chromosome 9p21.3–9p21.1 in families with concomitant FTD and ALS (Yan J., *et al.* (2006) *Neurology* 67, 186). To date, we have screened more than 500 FALS and 1400 SALS cases with and without FTD for the C9 expansion mutation using the repeat primed-polymerase chain reaction (RP-PCR) method. This cohort consisted of patients that have not been previously found to carry other known ALS-causing mutations. Of those, ~50% FALS and ~5% SALS cases appear to be C9 expansion carriers evident by >23 repeats. Typically, electropherograms from expanded C9 carriers are described as displaying a characteristic saw-tooth pattern with ≥21 peaks, where each successive peak differs in one (G_4C_2) unit that decreases in signal intensity (SI) as fragment size increases. Interestingly, we identified that ~40% of our expanded C9 cases do not generate electropherograms displaying a typical saw-tooth pattern. Instead, we detect an unreported atypical pattern that is characterized by a series (<21) of equally robust peaks, followed by several additional peaks (>21) of low SI that requires manipulation of the SI range for adequate detection. We have confirmed that these samples are indeed expanded using a

non-radioactive Southern blot method that utilizes a digoxigenin-labeled C9 probe. What causes a difference in electropherogram pattern among C9 expansion carriers is currently unknown and is being investigated by our lab. It is possible that atypical expansion carriers have interruptions present in the (G_4C_2) tract as seen in other expansion disorders. Additionally, several other labs have reported the presence of mutations in the low complexity sequence region immediately following the (G_4C_2) tract. Mutations with in or following the (G_4C_2) tract may prevent efficient primer annealing and/or elongation of the product during the PCR reaction resulting in premature reaction termination. It is interesting to speculate whether variances in the expansion pattern correlate with differences in disease phenotype. Ultimately, the overarching goal of our studies is to gain a more thorough understanding of the pathogenic mechanisms associated with the C9 (G_4C_2) repeat expansion mutation, with the intention of identifying disease-causing pathways that may one day be exploited for therapeutic intervention.

Disclosures: J.L. Lowry: None. **J. Yan:** None. **L. Kinsley:** None. **N. Siddique:** None. **H. Deng:** None. **T. Siddique:** None.

Poster

219. Motor Neuron Disease

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 219.22/D31

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: Identification of modifiers of C9orf72-associated dipeptide toxicity in a new *C. elegans* model

Authors: *S. T. LAMITINA[1], J. MONAGHAN[2], U. PANDEY[2], K. MARSHALL[1], C. SNOZNIK[1];
[1]Dept of Pediatrics and Cell Biol., [2]Dept of Pediatrics, Childrens Hosp. of Pittsburgh of UPMC, Pittsburgh, PA

Abstract: Nucleotide repeat expansions are a common cause of multiple age-related neurodegenerative diseases. An expansion of the intronic hexanucleotide repeat GGGGCC in the C9orf72 gene was recently found to be associated with two major neurodegenerative diseases – amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Both repeat containing RNA and repeat encoded dipeptide proteins are associated with neurotoxicity and death in *Drosophila* and mammalian cell culture. To gain additional insights into the mechanisms of C9orf72 associated dipeptide toxicity, we generated transgenic *C. elegans* expressing dipeptides fused to GFP. We focused solely on the effects of dipeptide toxicity by varying codon usage, which preserves the dipeptide coding sequence but eliminates potential RNA repeat induced toxicity. Dipeptide expression was controlled by promoters that are active in muscle, all neurons, or specific motor neurons. Similar to previous reports, expression of GR

and PR, but not GA and PA, was extremely toxic under all conditions, producing various phenotypes (lethality, short lifespan, motility defects) depending on the site of expression. Since there are no known modifiers of dipeptide toxicity, we used these phenotypes to perform biased and unbiased modifier screens. One such modifier was a mutant that activates the insulin signaling pathway, an evolutionarily conserved modifier of longevity and proteotoxicity from *C. elegans* to humans. This pathway is known to reduce toxicity in other *C. elegans* ALS models, suggesting that dipeptide toxicity and other forms of ALS may share a common mechanism(s) of toxicity that is opposed by the target(s) of insulin signaling. Genetic screens to identify additional modifiers of C9orf72-associated dipeptide toxicity are ongoing.

Disclosures: S.T. Lamitina: None. **J. Monaghan:** None. **U. Pandey:** None. **K. Marshall:** None. **C. Snoznik:** None.

Poster

219. Motor Neuron Disease

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 219.23/D32

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Target ALS-Columbia University

NIH Grant RO1-NS074886

Title: iPS astrocytes derived from amyotrophic lateral sclerosis patients up regulate efflux transporters in endothelial cells

Authors: *H. QOSA, S. MARKANDAIAH, J. LICHTER, P. PASINELLI, D. TROTTI; Thomas Jefferson Univ., Philadelphia, PA

Abstract: Previous studies showed that riluzole, the only FDA-approved drug for treatment of amyotrophic lateral sclerosis (ALS) and other investigational ALS therapeutics are substrates for drug efflux transporters; P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP). These transporters can restrict the brain access of ALS therapeutics and enhance their extrusion from the brain. In addition, gradual increase in the expression and activity levels of these transporters over disease progression have been observed in the spinal cord of mouse model of ALS. Upregulation of P-gp and BCRP was also observed in postmortem spinal cord tissues of ALS patients, supporting the premise of development of pharmacoresistance in ALS. Therefore, the overall goal of this research is to identify the CNS-specific mechanisms of P-gp/BCRP overexpression that are activated in ALS in order to develop a therapeutic approach that can decrease the activity of these transporters and enhance ALS therapeutics brain level. First specific aim of this work is to evaluate the effect of astrocytes on endothelial expression of P-gp and BCRP. To achieve this aim, we used in-vitro blood-brain barrier (BBB) model that is

composed from endothelial cells and astrocytes derived from human induced pluripotent stem cells (iPS) that are obtained from ALS patients. Second specific aim is the identification of the signaling cascade(s) that contribute significantly to P-gp/BCRP up-regulation in ALS. We used our in-vitro BBB model to evaluate the activity of NF-κB-dependent pathways and xenobiotic-nuclear receptors pathways in endothelial cells after exposure to ALS-astrocytes. Our results showed significant increase in the endothelial expression of P-gp but not BCRP after co-culture with ALS-astrocytes. Increase of P-gp expression was associated with activation of NF-κB-dependent pathway but not xenobiotic-nuclear receptors in endothelial cells. Our findings suggest the contribution of ALS-astrocytes to the development of drug pharmacoresistance through activation of NF-κB-dependent pathways.

Disclosures: H. Qosa: None. **S. Markandaiah:** None. **J. Lichter:** None. **P. Pasinelli:** None. **D. Trotti:** None.

Poster

219. Motor Neuron Disease

Location: Hall A

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Program#/Poster#: 219.24/D33

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: NIH 5U54NS091046-02

Title: Establishing the molecular signatures of motor neuron cultures derived from ALS, SMA and control induced pluripotent stem cells

Authors: *C. N. SVENDSEN[1], L. ORNELAS[1], D. SAREEN[1], B. SHELLEY[1], M. BARCH[2], J. OSTERLOH[2], S. SAMSI[2], P. MILANI[3], N. L. PATEL-MURRAY[3], E. MENDEZ[4], R. SATTLER[4], E. CROWGEY[1], A. MATLOCK[1], M. CASALE[5], R. LIM[5], J. WU[5], S. FINKBEINER[2], E. FRAENKEL[3], J. D. ROTHSTEIN[4], J. VANEYK[1], L. THOMPSON[5]; [1]Regenerative Med., Cedars-Sinai Med. Ctr., West Hollywood, CA; [2]Gladstone, San Francisco, CA; [3]MIT, Cambridge, MA; [4]Johns Hopkins, Baltimore, MD; [5]Univ. of California Irvine, Irvine, CA

Abstract: Subtle variations amongst different cell types in the central nervous system (CNS) remain undefined, which has complicated the successful discovery of disease-modifying therapies for neurodegenerative diseases. There is a critical need to define the state and predict the behavior of healthy and diseased human cells in the CNS. Our knowledge of the CNS and the ability to intervene rationally in disease would be dramatically advanced by generating quantitative molecular phenotypes_essentially cell signatures_of human neurons, astrocytes and oligodendrocytes from healthy people and from patients with CNS disorders such as the motor neuron diseases. Despite this desperate need, the inaccessibility of human brain cells has made

studying them difficult until the hallmark discovery of cellular reprogramming and the induced pluripotent stem cell (iPSC) technology. The NeuroLINCS consortium has generated 16 total iPSC lines from amyotrophic lateral sclerosis (ALS) patients with the C9orf72 (4) or SOD1 mutations (4), spinal muscular atrophy patients (4) and control subjects (4). Motor neurons, the primary cell type affected in these diseases, were generated from the iPSCs under specific differentiation protocols. Transcriptomics, epigenomics, whole genome sequencing, proteomics, high content imaging, high throughput longitudinal single cell analysis and other cell-based assays are all in progress using standardized and parallel cultures. Specific "omics" profiles are associated with these different motor neuron diseases (or "genetic perterbagens"). Integrated signatures are currently being generated using bioinformatics, statistics and computational biology to establish patterns that may lead to a better understanding of the underlying mechanisms of disease. We are also developing innovative software tools and approaches that will make the comprehensive signature generating process faster, and more reliable. All of this data will be made available through the NIH LINCS program for the entire scientific community to utilize.

Disclosures: **C.N. Svendsen:** None. **L. Ornelas:** None. **D. sareen:** None. **B. shelley:** None. **M. Barch:** None. **J. Osterloh:** None. **S. Samsi:** None. **P. Milani:** None. **N.L. Patel-Murray:** None. **E. mendez:** None. **R. Sattler:** None. **E. Crowgey:** None. **A. Matlock:** None. **M. Casale:** None. **R. Lim:** None. **J. Wu:** None. **S. Finkbeiner:** None. **E. Fraenkel:** None. **J.D. Rothstein:** None. **J. VanEyk:** None. **L. Thompson:** None.

Poster

219. Motor Neuron Disease

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 219.25/D34

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: The environmental neurotoxin β-methylamino-L-alanine: Modeling mother-to-infant transfer using human cell lines

Authors: *L. ERSSON[1], M. ANDERSSON[2], U. BERGSTRÖM[2], I. BRANDT[2], E. B. BRITTEBO[1];
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Abstract: The environmental neurotoxin β-N-methylamino-L-alanine (BMAA) has been implicated in the etiology of human neurodegenerative disease, presumably following dietary exposure. BMAA is a developmental neurotoxin that induce long-term learning and memory deficits, as well as neurodegeneration and intracellular fibril formation in the hippocampus of adult rats exposed neontally.We have also reported that BMAA is rapidly transported into the mammary gland of lactating mice, secreted in breast milk and subsequently transported to the

brain of the suckling pups, reaching higher concentrations in the neonatal than the maternal brain. Mother's milk is consequently proposed as a major exposure pathway for BMAA in neonatal rodents. Being a zwitterion, BMAA needs specific transporters to be transported via mammary epithelium, neonatal intestinal epithelium, and through cell membranes of neurons and glia cells to enter the neonatal brain. To model this transport in humans, we examined the uptake of ^{14}C-BMAA in four human cell lines; i.e. MCF7 mammary epithelial cells, Caco 2 intestinal cells, SH-SY5Y neuroblastoma cells and U343 astroglioma cells. BMAA was transported in all cell types. The uptake of BMAA was more rapid in the neuronal and astrocyte cell lines than in the mammary cell line and the Caco 2 cells. Competition experiments with other amino acids indicated that BMAA is taken up by transporters other than the large amino acid transporters 1 and 2 (LAT 1 and LAT 2) in SH-SY5Y and U343 cells. Taken together, the results suggest that mother-to-infant transport of BMAA to neonatal brain will occur also in humans.

Disclosures: L. Ersson: None. **M. Andersson:** None. **U. Bergström:** None. **I. Brandt:** None. **E.B. Brittebo:** None.

Poster

219. Motor Neuron Disease

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 219.26/D35

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Brain Research Trust

Title: Investigating the contribution of β-amyloid1-42 mediated toxicity to motor neuron degeneration in models of amyotrophic lateral sclerosis (ALS)

Authors: *J. BRYSON[1], D. BUI[1], C. BARCELLOS-MACHADO[2], I. LIEBERAM[2], L. GREENSMITH[1];
[1]Sobell Dept. of Motor Neurosci. and Movement Disorders, UCL Inst. of Neurol., London, United Kingdom; [2]MRC Ctr. for Developmental Neurobio., King's Col. London, London, United Kingdom

Abstract: Motor neuron degeneration is the primary pathological hallmark underlying the progressive deterioration of motor function that occurs in patients with amyotrophic lateral sclerosis (ALS). Importantly, recent evidence indicates that motor neurons also undergo degeneration in other neurological disorders in which amyloid precursor protein (APP) has been implicated, including Niemann Pick type-C (NPC) disease[1] and Alzheimer's disease (AD)[2]. In animal models of AD, overexpression of human β-amyloid$_{1-42}$ (Aβ$_{42}$), a neurotoxic cleavage product of APP, has been shown to be sufficient to cause motor neuron degeneration. Previous research from our group demonstrated that genetic ablation of APP in the SOD1^{G93A} mouse

model of ALS resulted in a significant improvement of multiple disease parameters, including muscle innervation, motor function and motor neuron survival[3]. Additionally, we demonstrated a ~3-fold upregulation of endogenous murine $A\beta_{42}$, which accumulates within motor neurons and astrocytes in $SOD1^{G93A}$ mice. Taken together, this evidence strongly suggests that APP contributes to pathology in $SOD1^{G93A}$ mice and that the toxicity may be caused by enhanced amyloidogenic processing of APP in the setting of an ALS-like environment. In the current study, we have developed a long-term culture system using a combination of mouse embryonic stem cell (ESC) derived motor neurons (ESC-MNs) and astrocytes (ESC-ACs), which enables functional maturation of motor neurons *in vitro*[4]. Traditional methods of culturing primary motor neurons do not permit long-term maintenance and maturation of motor neurons, which typically remain viable for only 1-2 weeks. Using this ESC-derived system, we investigated the direct toxicity of oligomeric $A\beta_{42}$ ($_oA\beta_{42}$) to 'mature' motor neurons. Our results show that mature motor neurons are indeed susceptible to $_oA\beta_{42}$-mediated toxicity. We are now examining whether elevated levels of human $_oA\beta_{42}$ (as opposed to endogenous murine $A\beta_{42}$ which is thought to be less toxic) exacerbate motor neuron degeneration in $SOD1^{G93A}$ mice. Given the enormous efforts to develop pharmacological inhibitors of $A\beta_{42}$ production as a therapy for AD, evidence of a direct contribution of $A\beta_{42}$ to motor neuron degeneration in ALS could lead to new therapeutic approaches for this devastating disease. **References:** 1 Maulik M, Ghoshal B, Kim J, et al. (2012) *Hum Mol Genet*. Nov 15;21(22):4857-75. 2 Seo JS, Leem YH, Lee KW, et al. (2010) *J Alzheimers Dis.* 21(1):263-76. 3 Bryson JB, Hobbs C, Parsons MJ, et al. (2012) *Hum Mol Genet.* Sep 1;21(17):3871-82. 4 Bryson JB, Machado CB, Crossley M, et al. (2014) *Science.* Apr 4;344(6179):94-7.

Disclosures: J. Bryson: None. **D. Bui:** None. **C. Barcellos-Machado:** None. **I. Lieberam:** None. **L. Greensmith:** None.

Poster

219. Motor Neuron Disease

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 219.27/D36

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: AFM 16465

Title: Electrophysiological properties of human motoneurons derived from iPSc of patients with Amyotrophic Lateral Sclerosis

Authors: *B. LAMOTTE D'INCAMPS[1], C. LEFEBVRE[2], C. DALLE[3], C. NICAISE[4], F. SALACHAS[5], L. LACOMBLEZ[5], S. MILLECAMPS[2], S. BLANCHARD[6], D. BOHL[2,6]; [1]Ctr. For Neurophysics, Physiol. and Pathology, Paris, France; [2]ALS causes and mechanisms of motor neuron degeneration, [3]Plateforme d'Electrophysiologie, Inst. du Cerveau et de la Moelle

épinière - ICM- INSERM U 1127 - CNRS UMR-7225 - UPMC-Université Paris 6, Paris, France; [4]Lab. Neurodegeneration and Regeneration, URPhyM-NARILIS, Univ. of Namur, Namur, Belgium; [5]Maladies du Système Nerveux, Ctr. de référence maladies rares SLA, Hôpital Pitié-Salpêtrière, Paris, France; [6]Neurosciences, Unité Biothérapies pour les Maladies Neurodégénératives - Inst. Pasteur, Paris, France

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disorder characterized by progressive degeneration and death of motoneurons. It is a fatal disorder with no effective therapy and patients have only 2 to 5 years of life expectancy after diagnosis. The majority of ALS cases are sporadic while around 10% are familial cases. Until recently, only one mouse model recapitulated the disease, but this model represented only 1% of all ALS cases. Despite several hypotheses regarding the mechanisms leading to ALS, little is known today and this is mostly due to disease heterogeneity and the impossibility to have access to human affected motoneurons. By providing such an access to human cells, induced pluripotent stem cells (iPSc) offers today a unique opportunity to study motoneurons of patients and to learn more about what triggers their death. In the present study, we have investigated the electrophysiological properties of iPS-derived motoneurons generated from ALS patients with mutations in one of the three main genes responsible for ALS (C9ORF72, SOD1, TDP-43) and healthy subjects of different ages (11, 33 and 69 years old). Motoneuron differentiation was performed as described in Maury et al. (Nat. Biotechnol., 2015) allowing the production of synchronized differentiated motoneuron cultures devoid of proliferative cells. HB9-positive and Islet1-positive motoneurons were produced as soon as day 17 and both control and ALS motoneurons survived in the presence of neurotrophic factors for more than 13 weeks. We currently analyze the formation of protein aggregates in these aged motoneurons. To examine electrophysiological properties of individual motoneurons, cultures were transduced with a lentiviral vector expressing RFP under the control of the HB9 promoter and only the largest RFP-positive neurons were recorded at 7 and 12 weeks after plating. We observed that at both time points all recorded control and ALS neurons were electrically active and able to fire at least one action potential in response to a pulse of depolarizing current. These data are in contrast with the recently published results by Devlin et al. (Nat Commun., 2015) and need to be confirmed. We then compared the Na+ and K+ currents developing during voltage steps of various amplitudes and could not find evidence for a decrease of INa+, with the cells aging from 7 to 12 weeks. The electrical behavior of the three cell lines of ALS-iPSc-derived motor neurons was comparable but a small tendency towards reduced excitability with respect to the aged-matched control iPSc-derived motor neurons was observed. Experiments are in progress to validate these results.

Disclosures: **B. Lamotte D'Incamps:** None. **C. Lefebvre:** None. **C. Dalle:** None. **C. Nicaise:** None. **F. Salachas:** None. **L. Lacomblez:** None. **S. Millecamps:** None. **S. Blanchard:** None. **D. Bohl:** None.

Poster

219. Motor Neuron Disease

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 219.28/D37

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: China Scholarships Council/University of Edinburgh Scholarships

Title: Studying glia-neuronal interaction in C9ORF72 repeat expansion mediated amyotrophic lateral sclerosis using a human induced pluripotent stem cell based *in vitro* model

Authors: *C. ZHAO[1,2], A.-C. DEVLIN[3], B. T. SELVARAJ[2], A. SERIO[4], S. BOROOAH[1], E. M. CLEARY[2], K. BURR[2], C. E. SHAW[5], G. B. MILES[3], S. CHANDRAN[1,2];
[1]Ctr. for Clin. Brain Sci., [2]Euan MacDonald Ctr. for Motor Neurone Dis. Res., Univ. of Edinburgh, Edinburgh, United Kingdom; [3]Sch. of Psychology and Neurosci., Univ. of St. Andrews, St. Andrews, United Kingdom; [4]Dept. of Materials, Imperial Col. London, London, United Kingdom; [5]Med. Res. Council Ctr. for Neurodegeneration Res., King's Col. London, London, United Kingdom

Abstract: Background: Recent advances in the genetics of amyotrophic lateral sclerosis (ALS) show that GGGGCC hexanucleotide repeat expansion on C9ORF72 is the most common genetic cause of ALS. Understanding the pathogenic mechanisms underlying this mutation may hold the key to developing potential therapeutic treatments. In addition, accumulating experimental and human pathological evidence implicates non-cell autonomous mechanisms in the aetiopathogenesis of ALS. Astrogliosis and glial pathology has long been described in ALS but until recently has been assumed to be secondary and / or reactive. Human stem cell technologies allow the *in vitro* study of cellular autonomy with a focus on astrocytes. Results: Induced pluripotent stem cells (iPSCs) were generated from 3 patients carrying the GGGGCC hexanucleotide expansion on C9ORF72 as well as 2 healthy controls. Highly enriched (>90%) functional astrocytes were derived from all iPSC lines following a well-established protocol. Expansion carrying astrocytes recapitulate pathological features of C9ORF72 mediated ALS: a) dysregulation of C9ORF72 transcripts and b) presence of GGGGCC RNA foci in nuclei. Additionally, using longitudinal live imaging and Kaplan-Meier survival analysis, an increased risk of cell death was revealed in expansion carrying astrocytes when compared to controls. Mutant astrocytes also showed increased cell vulnerability under autophagy inhibition. In order to assess the non-cell autonomous contribution of repeat expansion carrying astrocytes, cell viability as well as electrophysiological profile of motor neurons cocultured with astrocytes is currently being examined. Discussion and Conclusion: Our work has established a platform to investigate the glial pathology and potential non-cell autonomous toxicity of astrocytes in C9ORF72 repeat expansion related ALS.

Disclosures: **C. Zhao:** None. **A. Devlin:** None. **B.T. Selvaraj:** None. **A. Serio:** None. **S. Borooah:** None. **E.M. Cleary:** None. **K. Burr:** None. **C.E. Shaw:** None. **G.B. Miles:** None. **S. Chandran:** None.

Poster

219. Motor Neuron Disease

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Program#/Poster#: 219.29/D38

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: C9orf72 repeat-associated non-atg (ran) translation products induce tdp43 mislocalization and neurodegeneration in models of als and ftd

Authors: *B. FLORES[1], M. IVANOVA[2], A. KRANS[2], P. TODD[2], S. BARMADA[2];
[2]Neurol., [1]Univ. of Michigan, Ann Arbor, MI

Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by a loss of motor neurons and muscle weakness. Patients with ALS may also present behavioral, personality, or language abnormalities that are associated with frontotemporal dementia (FTD). The most common cause of mutation familial forms of both FTD and ALS is a hexanucleotide (GGGGCC) expansion in a noncoding region of the chromosome 9 open reading frame 72, or *C9ORF72*. Expanded *C9ORF72* transcripts undergo a unique mechanism of translation, known as repeat associated non-ATG (RAN) translation, in all reading frames, but the significance of these RAN translation products in ALS and FTD pathogenesis is unknown. For the majority of patients with ALS and FTD, including *C9ORF72*-mutation carriers, the most striking pathologic characteristic is the cytoplasmic accumulation of TDP43, a nuclear RNA binding protein. The connection between C9ORF72 RAN peptides, TDP43 metabolism, and neurodegeneration remains fundamentally unclear. Our goals are to investigate the association between C9ORF72 mutations, abnormal TDP43 deposition, and their respective contributions to neuronal toxicity and disease pathogenesis. Here, we show that synthetically-derived C9ORF72 RAN products — glycine-arginine (GR), glycine-alanine (GA), and glycine-proline (GP) dipeptides, depending on the translational reading frame of the (GGGGCC) repeat — aggregate *in vitro*, and they reduce viability in a mammalian cell line. To determine if the RAN products are neurotoxic, we utilized fully-automated longitudinal fluorescence microscopy to prospectively visualize large populations of live neurons and track their survival. We show that short (6mer) GR dipeptides selectively increase the risk of death in rodent primary cortical neurons, but as the length increases both GA and GR dipeptides display neurotoxicity. Consistent with their negative effects on survival, both GA and GR dipeptides are internalized by neurons, but not GP dipeptides. In primary neurons expressing constructs containing hexanucleotide *C9ORF72* expansion mutations associated with RAN translation are more toxic than those that do not support RAN translation. Furthermore, in neurons treated with the short (6mer) GA dipeptide, we observed a reduction in the nuclear/cytoplasmic ratio of TDP43, suggesting that neurotoxic RAN dipeptides accentuate TDP43 mislocalization. These initial findings are an important, first step, in making the connection between *C9ORF72* mutations, RAN dipeptides, and neurodegeneration in ALS and FTD.

Disclosures: **B. Flores:** None. **M. Ivanova:** None. **A. Krans:** None. **P. Todd:** None. **S. Barmada:** None.

Poster

219. Motor Neuron Disease

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 219.30/D39

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Support: Farber Family Foundation

Title: Arginine-rich sense and antisense dipeptides in post-mortem human tissues of c9orf72-als/ftd patients

Authors: ***T. R. WESTERGARD**[1], X. WEN[1], N. A. SCHNEIDER[2], P. PASINELLI[1], D. TROTTI[1];
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Abstract: The non-coding GGGGCC hexanucleotide repeat expansion in the C9ORF72 gene is the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The expansion sense and anti-sense RNA transcripts can be translated through repeat associated non-ATG (RAN) translation to form five dipeptide repeat proteins (DRPs). We, as well as other labs, have shown that the arginine-rich dipepetides, particularly Proline-Arginine (PR), are highly toxic. We have demonstrated this toxicity in primary cortical and motor neuron cultures, transgenic fly models, and in human iPS-derived neurons. Toxicity of these arginine-rich dipeptides is associated with aggregate formation of the dipeptides in the nucleus, with strong co-localization with nucleolin and fibrillarin, both nucleoli proteins. To further determine the human relevance of the observed dipeptide toxicity, we performed immunofluorescence (IF) imaging techniques on post mortem human tissues. Human tissues were collected from mutant C9ORF72 positive ALS patients, mutant C9ORF72 negative ALS patients, and non ALS patients. Analyses of the human tissue showed robust paranuclear and nuclear PR positive cells specifically in mutant C9ORF72 ALS positive patients. Interestingly, PR staining primarily showed aggregation in the nucleus that co-localized in nucleoli, similar to toxicity factors seen in the primary cell cultures.

Disclosures: **T.R. Westergard:** None. **X. Wen:** None. **N.A. Schneider:** None. **P. Pasinelli:** None. **D. Trotti:** None.

Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 220.01/D40

Topic: C.05. Aging

Title: Does MAO-A play a role in the development of depression for individuals with Alzheimer's disease

Authors: *Z. WEI[1], B. CHAHARYN[1], K. FEHR[1], K. CHEN[2], J. C. SHIH[2], D. D. MOUSSEAU[1];
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Abstract: The exact association between Alzheimer's disease (AD) and depression is unknown. One possibility is the biological changes caused by AD may predispose to depression. Another possibility is that depression-related changes in the brain occur first and subsequently lead to AD. Our research program focuses on monoamine oxidase- A (MAO-A), an enzyme that has been associated independently with both depression as well as AD. We chose to examine relevant mouse models for any evidence of the direction of causality. We first used a transgenic mouse model of AD, the 'J20' strain that expresses the human Amyloid Protein Precursor (APP) (Cg-Tg PDGF-APP SwInd). The mutated form of APP (carries the Swedish and Indiana mutations) is an aggressive model of amyloidosis. We tested these mice and their wildtype littermates in the Tail Suspension Test (TST), a test used as a reflection of depression-like behavior. The time the mice spent immobile (i.e. despaired) decreased in the wildtype mice as they aged: i.e. 179.4 sec ± 35.1 (at three months of age) and 147.3 sec ± 49.7 at 12-months of age. In contrast, immobility time was increased in older J20 mice: i.e. 143.8 sec ± 57.4 (at three months) and 183.4 sec ± 36.6 at 12 months of age. We then crossbred J20 mice with a strain of mice bearing a spontaneous mutation in the mao-A gene that results in a truncated and inactive MAO-A protein (a functional knock-out: KO). The four genotypes studied were MAO-A WT/APP-, MAO-A WT/APP+, MAO-A KO/APP-, and MAO-A KO/APP+. Using the TST, we observed that immobility time increased in MAO-A WT/APP+ mice as they aged when compared with their age-matched MAOA WT/APP- mice, and immobility time decreased in MAO-A KO/APP- mice compared with mild type mice. We did not observe any of the expected difference between MAO-A KO/APP- and MAO-A KO/APP+ mice. However, preliminary studies using the Novel Object Recognition revealed that the cognitive decline associated with the APP genotype was accelerated in an MAO-A KO background. Our data indicate that the expression of an AD-related APP mutation could predispose to a depression-like phenotype, and that the loss of MAO-A could accelerate the AD process. We continue to examine the mechanism(s) underlying this intriguing observation.

Disclosures: Z. Wei: None. **B. Chaharyn:** None. **K. Fehr:** None. **K. Chen:** None. **J.C. Shih:** None. **D.D. Mousseau:** None.

Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

Location: Hall A

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Topic: C.05. Aging

Support: NSF grant 1026958

Title: Guanine nucleotide exchange factor OSG-1 confers functional aging via dysregulated Rho signaling in Caenorhabditis elegans neurons

Authors: *F. SESTI, Z. DUAN;
Neurosci. and Cell Biol., Rutgers, Piscataway, NJ

Abstract: Rho signaling regulates a variety of biological processes, but whether it is implicated in aging, remains an open question. Here we show that a guanine nucleotide exchange factor of the Dbl family, OSG-1, confers functional aging by dysregulating Rho GTPases activities in *C. elegans*. Thus, gene reporter analysis revealed widespread OSG-1 expression in muscle and neurons. Loss of OSG-1 gene function was not associated with developmental defects. In contrast, suppression of OSG-1 lessened loss of function (chemotaxis) in ASE sensory neurons subjected to conditions of oxidative stress generated either during natural aging or by oxidative challenges or by genetic mutations. RNAi analysis showed that OSG-1 was specific toward activation of RHO-1 GTPase signaling. RNAi further implicated actin-binding proteins ARX-3 and ARX-5, thus the actin cytoskeleton, as one of the targets of OSG-1/RHO-1 signaling. Taken together these data suggest that OSG-1 is recruited in conditions of oxidative stress, a hallmark of aging and contributes to promote loss of neuronal function by affecting the actin cytoskeleton via altered RHO-1 activity.

Disclosures: F. Sesti: None. **Z. Duan:** None.

Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

Location: Hall A

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Program#/Poster#: 220.03/D42

Topic: C.05. Aging

Support: NSERC (RGPIN-2015-03958)

Title: RAGE induces abnormal mitochondrial dynamics in sympathetic neurons expose to high glucose

Authors: M. G. OTERO[1], X. LU[2], A. CHANDNA[2], Y. YAMAMOTO[3], T. FALZONE[1], *V. A. CAMPANUCCI[2];
[1]Inst. de Biologia Celular y Neurociencia, Buenos Aires, Argentina; [2]Physiology, Col. of Med., Univ. of Saskatchewan, Saskatoon, SK, Canada; [3]Grad. Sch. of Med. Sci., Kanazawa, Japan

Abstract: Mitochondria are dynamic and tubular organelles essential for normal neuronal physiology, and their dysfunction leads to the metabolic abnormalities observed in diabetes. In diabetic neuropathy, mitochondrial dysfunction is marked by decreased electron transport chain (ETC) activity, suboptimal rates of respiration and impaired calcium homeostasis. Although mitochondria normally produce ROS as by-products of ATP biosynthesis, hyperglycemia drives excessive electron donation to the ETC, resulting in the further production of ROS and leading to mitochondrial and cellular damage. Hyperglycemia also leads to the formation of advanced glycation endproducts (AGEs) which bind to their membrane receptor, RAGE. We have recently shown that RAGE signaling is required for malfunction of autonomic neurons in diabetes, and we hypothesize that RAGE signaling is linked to mitochondrial dysfunction. To test this hypothesis we investigated changes in mitochondrial dynamics in cultured autonomic neurons from the autonomic superior cervical ganglion (SCG) of mice and we exposed them to either control or high glucose conditions. To investigate the potential role of RAGE we used SCG neurons from RAGE knock-out mice(RAGE-/-) mice. To visualize and study live mitochondria, neurons were transfected with a vector containing enhanced green fluorescent protein (EGFP) fused to a mitochondrial signal peptide (MITO-EGFP). Our findings revealed that high glucose induces morphological changes in mitochondria in SCG neurons. Electron micrographs revealed an abundance of damaged mitochondria cristae and mitochondrial swelling in sections from STZ-diabetic mice, while control mice had preserver mitochondrial ultrastructure. Moreover, *in vitro* observation along neurites, showed that high glucose disrupted compensatory changes in mitochondrial dynamics in neurons from WT when compared to RAGE-/- mice. These changes in the WT neurons included a significant increase in the density and proportion only of mitochondria moving anterogradely, while in the RAGE-/- neurons there was a significant increase in the density and proportion of mitochondria moving both anterogradely and retrogradely. In addition, we found a significant reduction in the size of stationary mitochondria in neurons from WT but not from RAGE-/- mice. Therefore, since mitochondria in RAGE-/- neurons do not show changes in size/morphology, our data suggest that the observed changes in mitochondrial dynamics may be part of a compensatory response to high glucose, a task that is impaired by the activation of RAGE signaling.

Disclosures: M.G. Otero: None. **X. Lu:** None. **A. Chandna:** None. **Y. Yamamoto:** None. **T. Falzone:** None. **V.A. Campanucci:** None.

Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: C.05. Aging

Support: Fapesp

Capes

Cnpq

Title: Iron restriction induces alterations in dopamine metabolism and PrPC expression

Authors: *J. M. PINO[1], H. K. M. ANTUNES[2], S. Q. D. GIAMPÁ[2], K. S. LEE[3];
[2]Psicobiologia, [3]Biologia Mol., [1]Univ. Federal De São Paulo UNIFESP, Sao Paulo, Brazil

Abstract: Neurodegenerative diseases can be characterized by protein aggregation, and reactive oxigen species (ROS) seems to contribute to this fenomenon. ROS can be produced by several metabolic pathways including dopamin metabolism. Dopamine (DA) can be enzimatically degraded generating hydrogen peroxide (H_2O_2) or auto-oxidated generating dopamine quinone. Celular príon protein (PrP^C) is one of the proteins that can directly react with dopamine metabolites *in vitro*, but the physiological meaning of this reaction is not clear. PrP^C is highly expressed glycoprotein that carries out important physiological functions in central nervous system. Several studies showed neuroprotective effects of PrP^C against oxidative stress. Thus the reaction between PrPC and oxidative metabolites of dopamine might be one of the protective mechanisms to neutralize ROS and alteration of dopamine metabolism might influence PrPC expression. Therefore in this study, we disturbed the dopamine metabolism by iron restriction in diets to investigate the expression levels of PrP^C. Male C57/BL6 mice were divided in two groups, one treated with normal diet (CTL) and the other treated with iron restricted diet (IR) for one month. Iron deficiency in IR group was verified by reduced iron and ferritin levels in liver, serum and hippocampus. Iron restriction also reduced the level of DA and its metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in striatum. However this decrease doesn't seem to be caused by a diminished DA synthesis, as the expression of tyrosine hydroxylase (TH) was not altered. In the prefrontal cortex, the HVA levels were augmented in IR group. Moreover, HVA concentration was correlated with the TH levels, suggesting that this increase was due to the enhanced DA synthesis and its rapid metabolization. Interestingly both HVA and TH showed a positive correlation with the PrP^C expression in prefrontal cortex. Our data show that PrP^C expression was influenced by DA metabolism in a brain region-specific manner.

Disclosures: J.M. Pino: None. **H.K.M. Antunes:** None. **S.Q.D. Giampá:** None. **K.S. Lee:** None.

Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

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Topic: C.05. Aging

Support: FAPESP

CAPES

CNPq

NAPNA

NAP

Title: Effects of intermittent fasting on lipopolysaccharide-induced changes on glutamate-nitric oxide-Na,K-ATPase pathway during brain aging

Authors: *A. R. VASCONCELOS[1], P. F. KINOSHITA[2], L. M. YSHII[2], A. M. M. ORELLANA[2], A. E. BÖHMER[2], L. DE SÁ LIMA[2], R. ALVES[2], D. Z. ANDREOTTI[2], T. MARCOURAKIS[2], C. SCAVONE[2], E. M. KAWAMOTO[2];
[1]Univ. of Sao Paulo, Sao Paulo, Brazil; [2]Univ. of Sao Paulo, Sao Paulo, Brazil

Abstract: Introduction: Chronic neuroinflammation is a common characteristic of neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases, that may contribute to loss of function and cell death. The signaling of the endotoxin Lipopolysaccharide (LPS) is linked to glutamate-nitric oxide (NO)-Na,K-ATPase isoforms pathway in the central nervous system (CNS) and also causes neuroinflammation. Intermittent fasting (IF, every other day feeding) induces adaptive responses in the brain that can suppress inflammation, but the age-related effect of IF on LPS modulatory influence on NO-Na,K-ATPase isoforms pathway is unknown. Methods: This work compared the effects of an inflammatory stimulus induced by LPS (1mg/kg, i.v. bolus, 2 hours before euthanasia) on α1,α2,3-Na,K-ATPase activity, nitric oxide synthase (NOS) gene expression and/or activity, cyclic guanosine monophosphate (cGMP), 3-nitrotyrosine (3-NT)-containing proteins, and levels of thiobarbituric acid-reactive substances (TBARS) in CNS of young (4 months) and older (24 months) male Wistar rats submitted to the IF protocol for 30 days. Results: LPS induced an age-related effect in neuronal nitric oxide synthase (nNOS) activity, cGMP, and TBARS levels in rat hippocampus that was linked to changes in α2,3-Na,K-ATPase activity, 3-NT proteins, and inducible NOS gene expression. IF induced adaptative cellular stress-response signaling pathways that reverts LPS effects in rat hippocampus of young and older rats. Conclusion: The results suggest that IF in both ages would reduce the risk for brain function deficits and neurodegenerative disorders linked to inflammatory response in the CNS.

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Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

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Topic: C.05. Aging

Support: NRF Grant 2012-003338

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Title: The effects of LRRK2 genetic mutations on mitochondrial dynamics in Parkinson's disease model

Authors: ***J. KIM**, J. KIM, J. JANG, H. SEO;
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Abstract: Parkinson's disease (PD) is progressive neurodegenerative disease that shows substantial loss of dopaminergic neuron in substantia nigra pars compacta (SNpc). Genetic mutation, oxidative stress, mitochondrial abnormalities and aging are main cause of PD. From previous studies, it is known that leucine-rich repeat kinase 2 (LRRK2), which contains GTPase and kinase domains, controls mitochondrial fission-fusion proteins. In this study, we focused on the effects of LRRK2 genetic mutations on mitochondrial function depending on age. LRRK2 G2019S and R1441G mice at old age showed significant decrease in the latency to fall in rotarod test. Mitochondria complex II/III activity was decreased in LRRK2 R1441G mutant mice compared to littermates (LM). The expression levels of mitochondrial fusion-fission related genes were determined in the striatum of LRRK2 G2019S and R1441G PD model mice. Our results suggest that mitochondrial dynamics can be applied for future therapeutic approach for PD.

Disclosures: **J. Kim:** None. **J. Kim:** None. **J. Jang:** None. **H. Seo:** None.

Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

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Support: AA MNIRGDP-12-258900 (CH)

NARSAD 21069 (CH)

NIH F31 NS083277 (HW)

Sie Foundation (JL)

Title: Age-dependent effects of RCAN1 overexpression on memory and synaptic plasticity

Authors: *H. WONG[1], J. LEVENGA[2], B. ROTHERMEL[3], E. KLANN[1], C. HOEFFER[2]; [1]Neural Sci., New York Univ., New York, NY; [2]Inst. for Behavioral Genet., Univ. of Colorado, Boulder, CO; [3]Dept. of Cardiol., UT Southwestern Med. Ctr., Dallas, TX

Abstract: Patients with Down syndrome (DS) and DS model mice display 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. Interestingly, overexpression of RCAN1 has been observed in brain tissue from not only DS patients but also AD patients. To understand the potential contribution of chronically high RCAN1 levels in the progression of neurodegenerative disease, we generated transgenic mice that overexpress RCAN1 in the brain using the cre/lox system. We found that selective overexpression of a human RCAN1 isoform in the mouse forebrain leads to the development of behavioral and plasticity deficits with age. Aged but not young RCAN1-overexpressing mice display impaired hippocampus-dependent memory and synaptic plasticity. To explain these age-dependent effects, we investigated the biochemical differences between young and aged transgenic mice and found a role for oxidative stress. Overall, this work should provide insight into the molecular basis of the cognitive phenotypes and neurodegeneration associated with DS and AD and whether RCAN1 may be a therapeutic target for treatment of these neurological disorders.

Disclosures: H. Wong: None. **J. Levenga:** None. **B. Rothermel:** None. **E. Klann:** None. **C. Hoeffer:** None.

Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

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Topic: C.05. Aging

Support: NIH Grant AG028271

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Title: HMGB1 sensitizes microglia in the aged brain causing prolonged inflammation and cognitive impairments following infection

Authors: *L. K. FONKEN, M. M. KITT, M. G. FRANK, R. M. BARRIENTOS, L. R. WATKINS, S. F. MAIER;
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Abstract: Healthy aged individuals can experience precipitous cognitive decline, typically following events that induce peripheral inflammation (e.g. infection, surgery, or injury). Peripheral immune stimuli cause exaggerated and prolonged inflammatory responses in the aged brain and these changes likely underlie increased susceptibility to cognitive impairments. However, the mechanisms that prime the inflammatory response in the aged brain are poorly understood. Here we propose that HMGB1 activates the NLRP3 inflammasome in the aged brain, resulting in neuroinflammatory priming. Following an E. coli injection, young rats (3 mos FBN) resolve neuroinflammatory responses within 24 h, whereas at 4 d post-E. coli aged rats (24 mos) still show cognitive deficits and elevated pro-inflammatory cytokines (including IL-1β and IL-18) in the hippocampus. Mature IL-1β and IL-18 are produced by the NLRP3 inflammasome, which is basally elevated in the aged brain along with other key inflammatory genes such as MHCII, toll-like receptors, and HMGB1. Previous research indicates that sub-septic levels of HMGB1 do not necessarily induce a pro-inflammatory cytokine response in the brain; rather, HMGB1 potentiates the effects of a subsequent neuroinflammatory challenge. Thus, to test whether increased HMGB1 in aged rats elicits neuroinflammatory priming, we blocked endogenous HMGB1 activity with the antagonist Box-A. Intra-cisterna magna (ICM) injection of Box-A down-regulated expression of several inflammatory pathway genes - including NLRP3 - in the hippocampus of aged rats. Importantly, Box-A did not alter gene expression in young rats, suggesting it may not interfere with non-pathological immune responses in young animals. The administration of ICM Box-A to aged rats 24 h prior to an E. coli injection prevented the protracted hippocampal inflammatory response. Furthermore, aged rats displayed cognitive impairments in a fear-conditioning paradigm 4 days following E. coli injection, and these impairments were reduced by Box-A pretreatment. Finally, we investigated whether HMGB1 mediates inflammatory priming through action on microglia, the primary innate immune cells of the central nervous system. ICM pretreatment with Box-A prevented priming in *ex vivo* microglia stimulated with LPS. Overall, these experiments indicate that elevated HMGB1 in the aged brain may prime the NLRP3 inflammasome in microglia, thereby driving an exaggerated and prolonged neuroinflammatory response following peripheral immune challenge. Ongoing experiments will identify the cellular source of increased HMGB1 in the aged brain.

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Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: C.05. Aging

Support: School of Medical Sciences PhD Scholarship (VK)

Title: Differences in plasma and CNS cytokine levels in adult and ageing rats

Authors: ***I. P. JOHNSON**[1], V. KATHARESAN[1], M. D. LEWIS[2], R. VINK[3];
[1]Univ. of Adelaide, Adelaide, Australia; [2]Mind and Brain theme, South Australian Hlth. and Med. Res. Inst., Adelaide, Australia; [3]Div. of Hlth. Sci., Univ. of South Australia, Adelaide, Australia

Abstract: It has been proposed that an increase in inflammatory cytokines in the blood and the central nervous system (CNS) explains many aspects of ageing, including age-related neurodegeneration. In this study, we distinguished age-related changes in CNS cytokines from those occurring in the blood (systemically), by analysing CNS after flushing the blood from its vessels. Groups of 3-5 female Sprague-Dawley rats aged 3 months, 12-18 months and 24 months were used. The open-field test measured exploratory behaviour and general anxiety levels as total distance travelled using the Stoelting "ANY-maze" software. Rats were deeply anaesthetised and blood plasma was obtained and stored at -80°C. Transcardial perfusion with saline followed immediately, brainstem was then removed, trimmed at the mid-pons level, homogenised and the supernatant stored at -80°C. Protein estimation assays ensured the same amount of protein was loaded for each sample. The Bio-Plex Pro Rat 12plex cytokine assay kits were used to measure the concentration of twelve cytokines (IL-1α, IL-β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, TNFα, IFN-γ and GM-CSF) in both plasma and brainstem samples as duplicates for 3m and 12-18m rats and triplicates for 24m rats. The Magpix Luminex multiplexing platform was used to quantitate cytokines. A general linear model generated descriptive statistics following by multivariate ANOVA and Dunnett's post-hoc test to report statistical significance of $p<0.05$, $p<0.01$ and $p<0.001$. Both 3m- and 12-18m old rats showed significantly larger exploratory behaviour and higher anxiety levels compared to 24m old rats. 3m old rats had higher mean concentrations of IL-5, IL-6 and IFNγ compared to 12-18m rats in the brainstem with no significant differences in the plasma. However, 3m old rats had lower mean concentrations of IL-1α, IL-2, IL-4, IL-10 and TNFα compared to 24m old rats in the brainstem. Interestingly, 3m old rats had higher mean concentrations of IL-12p70 and TNFα in plasma compared to 24m old rats. In addition, 24m old rats have higher levels of IL-1α, IL-4, IL-

6, IL-13, TNF-α, IFNγ and GM-CSF in the brainstem compared to 12-18m old rats with no differences in plasma. Contrary to current assumptions, our results indicate that ageing is associated with a general decline in peripheral inflammatory cytokines but a general increase in central inflammatory cytokines. This may reflect an intrinsic loss in competency of peripheral immunity with age or alternatively be the result of a regulatory system where increased levels of central inflammation negatively feedbacks to the periphery.

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Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

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Topic: C.05. Aging

Support: NIH R37 AG008796

NIH T32 AG020506

Glenn/AFAR Scholarship for Research in the Biology of Aging

Title: Ameliorating age-related deficits in hippocampal function through viral overexpression of CREB protein

Authors: *X.-W. YU[1], D. M. CURLIK, II[1], M. M. OH[1], J. C. P. YIN[2], J. F. DISTERHOFT[1]; [1]Feinberg Sch. of Med., Northwestern Univ., Chicago, IL; [2]Genet., Univ. of Wisconsin, Madison, WI

Abstract: Humans and animals often display learning and memory impairments as they age, however the underlying mechanisms of these impairments are poorly understood. Identifying the molecular pathways that mediate these impairments will allow us to design therapeutics to prevent or reverse these deficits. One important brain region for learning and memory is the hippocampal CA1 region. The Disterhoft laboratory has shown that CA1 pyramidal neurons from cognitively-impaired aged rats are less excitable than those from young and cognitively-unimpaired aged animals. These and many other experiments suggest that an age-related decrease in the excitability of CA1 pyramidal neurons mediates age-related cognitive impairments. Of note, the nuclear transcription factor cAMP response element binding protein (CREB) has been found to be a modulator of neuronal excitability. Additionally, our recent experiments have revealed that basal levels of phosphorylated CREB (pCREB) are decreased in dorsal hippocampus with age, and that pCREB levels correlate with behavioral performance during a hippocampal-dependent spatial learning task. Previous studies in young adult animals have shown that increasing CREB levels can enhance both excitability of CA1 pyramidal

neurons, and learning and memory. Together, these results suggest that elevating CREB levels in aged animals will ameliorate deficits in both excitability, and learning and memory. Therefore, this project used an adeno-associated virus to overexpress CREB in CA1 of young and aged rats. Levels of CREB mRNA were increased in infected regions of CA1, confirming virally mediated overexpression of CREB. As predicted, we found that infected cells were more excitable than control cells. On-going experiments are being conducted to confirm that increases in total and activated CREB protein will ameliorate age-related learning and memory deficits. This work is supported by NIH R37 AG008796 and T32 AG020506, and the Glenn/AFAR Scholarship for Research in the Biology of Aging.

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Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

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Support: FAPESP 2013/10787-8

Title: Effects of the anti-aging hormone Klotho on AKT/FoxO signaling in the central nervous system

Authors: ***C. MAZUCANTI**, M. CARARO, T. SALA, L. YSHII, C. SCAVONE;
Univ. of Sao Paulo, Sao Paulo, Brazil

Abstract: INTRODUCTION: Klotho gene, a senescence associated gene, was originally identified by insertional mutagenesis in mice and codifies a single pass type I transmembrane protein, known as Klotho. Klotho protein has been considered an aging phenotype repressor, since its suppression in mice precipitates age-related characteristics, such as arteriosclerosis, osteoporosis, skin atrophy, infertility, early thymus involution and pulmonary emphysema, as early as 4 weeks old. Life expectancy of these mice is very low, normally not greater than 8 weeks. Membrane-bound Klotho acts as an obligatory FGF23 co-receptor, hence playing a crucial role on phosphate metabolism. By suffering a shedding process Klotho can be cleaved from its transmembrane domain and act as a regulatory hormone, acting upon ion channels (e.g. TRPV5 and ROMK1) and repressing Wnt and Insulin/IGF signaling. FoxO is a class of transcription factors known to be regulated by insulin signaling and that has been for quite some time related to anti-aging properties. OBJECTIVES: The objective of this work is to study the influence of Klotho protein upon insulin signaling in the hippocampus of mice lacking Klotho protein, tracking its effects on FoxO phosphorylation and activation state. METHODS: 2 months

old, male klotho knockout mice (and its genotype variants), were euthanized and its hippocampi dissected and collected for western blotting analysis. AKT, mTOR, FoxO1a and FoxO3 phosphorylation states were assessed by calculating the ratio between phosphorylated form and total form of such proteins. For astrocyte-rich cultures, 3 days old pups were decapitated and its cerebral cortices dissected and digested (both enzymatically and mechanically) for primary cell culture. Culture was maintained in a humidified CO2 incubator, in DMEM supplemented with FBS 10% until they reach 100% confluence. At this point, cultures were shaken in an orbital shaker, at 37oC, 180 RPM for 15 hours. Astrocytes, strongly attached to the bottom, were then pre-treated with recombinant Klotho (0,5nM) for 24 hours, and then challenged with H2O2 500µM for 30 minutes. Cell viability was assessed via MTT reduction. RESULTS: It is clear that in the hippocampi of Klotho knockout mice, AKT signaling is enhanced and its phosphorylation on Ser473 induces inactivation of both Foxo1a and FoxO3. Klotho-treated astrocytes displayed higher viability when challenged with H2O2, linking FoxO activity with anti-oxidant defense. CONCLUSIONS: Klotho can modulate insulin signaling and therefore modify FoxO activity. Anti-oxidant properties of this transcription factor may be correlated to its effects as an aging suppressor.

Disclosures: C. Mazucanti: None. **M. Cararo:** None. **T. Sala:** None. **L. Yshii:** None. **C. Scavone:** None.

Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 220.12/E3

Topic: C.05. Aging

Support: Academia Sinica, Investigation Award

Title: Activation of the A2A adenosine receptor protects PC12 cells from the H2O2-induced DNA damage by TRAX

Authors: *Y. CHERN[1], T. CHIEN[2], S.-Y. CHANG[3], H.-L. LAI[3];
[1]Inst. Biomed Sci., Taipei, Taiwan; [2]Inst. of Life Sci., National Defense Medical Center, Taipei, Taiwan; [3]Inst. of Neuroscience, Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract: Adenosine has been implicated in a wide variety of physiological functions via four adenosine receptors (A1, A2A, A2B and A3). The A2A adenosine receptor (A2AR) is a G-protein coupled receptor, which is located in many brain areas with the highest level in the striatum. We previously reported that the translin- associated protein X (TRAX) is an interacting protein of the C terminus of A2AR. TRAX was first identified as an interacting protein of a DNA/RNA binding protein (translin), which functions in controlling mRNA transport,

translation, DNA repair, and DNA recombination. It was shown earlier that, upon γ-irradiation, TRAX interacts specifically with C1D (an activator of DNA- dependent protein kinase), and might play an important role in DNA repair of double-strand breaks. In the present study, we demonstrated that stimulation of A2AR by an A2AR-selective agonist (CGS21680) markedly ameliorated the DNA double-strand breaks evoked by elevated oxidative stress through TRAX in a neuronal cell line (PC12). Moreover, stimulation of A2AR might facilitate the non-homologous end joining (NHEJ) repair activity by enhancing the activation and phosphorylation of DNA-dependent protein kinase (DNA-PK) at T2609. Collectively, TRAX might contribute to the activation of DNA-PK in the detection of DNA double-strand breaks induced by oxidative stress.

Disclosures: **Y. Chern:** None. **T. Chien:** None. **S. Chang:** None. **H. Lai:** None.

Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 220.13/E4

Topic: C.05. Aging

Support: NIH Grant AG031158

Title: Pattern recognition receptor activity increases NF-κB signaling in cerebellar granule cells: A potential mechanism for age-associated alterations in neuronal structure and function

Authors: ***N. W. DEKORVER**, J. ARIKKATH, S. J. BONASERA;
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Abstract: In a mouse model of age-associated locomotor decline, pattern recognition receptor (PRR) and immune molecule expression is increased in a region specific manner in the aging brain, and these changes do not localize to microglia. Many PRRs signal through conserved cellular pathways, one of which is nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). NF-κB dysregulation is involved in numerous disease processes and has been implicated in aging. We hypothesize that increased PRR expression and subsequent activation in the aging brain leads to dysregulation of NF-κB signaling and thereby alterations in neuron structure and function. To test this hypothesis we developed an enriched primary cerebellar granule cell culture (pCGC) system that we then characterized for PRR expression. The effect of PRR activity on NF-κB signaling was tested by stimulating pCGC cultures with agonists for select pattern recognition receptors expressed in culture and increased in the aging cerebellum. Stimulation of the PRR toll-like receptor 2 (Tlr2) with synthetic triacylated lipoprotein (Pam3CSK4) led to a time dependent increase in nuclear p65 and increased phosphorylated p65, both markers of active NF-κB. Additionally, stimulation of Tlr2 led to time dependent increases in NF-κB regulated transcriptional products including Tlr2, complement 3, beta-2 microglobulin,

Fc fragment of IgE, and nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha (IκBα), further demonstrating increased activation of NF-κB signaling. There was no increase observed in toll-like receptor 4 transcript, suggesting specificity for NF-κB mediated transcripts. Additionally, pretreatment of primary cerebellar granule cells with pyrrolidine dithiocarbamate (PDTC), an inhibitor of NF-κB activity, attenuates Pam3CSK4 mediated increases in phosphorylated p65 and partially attenuates transcriptional changes. These results demonstrate that stimulation of specific PRRs in cerebellar granule cell cultures leads to increased p65 translocation, phosphorylation, and production of NF-κB regulated transcriptional products. Many of the transcriptional products that increased with stimulation of Tlr2 are present at increased levels in the aged C57BL6 mouse and human cerebellum. It is our goal to use this system to further characterize the pathway from PRR stimulation to NF-κB activation, including mechanisms of NF-κB regulation in cerebellar granule cells. Understanding these mechanisms may provide neuron specific targets for pharmacological intervention in diseases with known NF-κB dysregulation.

Disclosures: N.W. Dekorver: None. **J. Arikkath:** None. **S.J. Bonasera:** None.

Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

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Program#/Poster#: 220.14/E5

Topic: C.05. Aging

Support: NIH Grant 1RO1AG044919

Title: Changes in nutrient sensing pathways alter bio-energetics and are upstream of age dependent changes in microglial phenotype

Authors: *A. FLOWERS[1], H. BELL-TEMIN[1], L. DALY[1], S. M. STEVENS[2], P. BICKFORD[3]; [1]Mol. Pharmacol. and Physiol., Univ. of South Florida, Tampa, FL; [2]Biochem., [3]Mol. Pharmacol. and Physiol., Univ. of South Florida, tampa, FL

Abstract: Microglia play a pivotal role in the homeostasis of the brain and their proper functioning requires dynamic control over migration, expansion/contraction, and proper response to environmental signals. The objective of this study was to identify age dependent changes in microglial cellular components that could potentially contribute to priming, a state where microglia are over-responsive to pro-inflammatory and under responsive to anti-inflammatory signals. To accomplish these objectives we used SILAC labeled mass spectrometry. We attained primary microglia from brains of 5 month and 22 month C57BL/6 mice. 250 proteins were differentially expressed ($p<0.05$) between young and old. Ingenuity Pathway Analysis was performed on all differentially expressed proteins with at least a 1.5 fold change and a $p<0.05$

statistical cut-off. Top canonical pathways were EIF2 signaling, Isoleucine degradation, ketolysis, ketogenesis, and Gluataryl-CoA Degradation. Analysis of upstream regulators predicted an inhibition of RICTOR, PSEN2, PSEN1, and MAPK1. Alterations in Rictor an essential component of the mTORC2 signaling complex were one explanation for the observed changes in amino acid metabolism. To further investigate we used siRNA specific for RICTOR and knocked down mTORC2 activity in a microglial cell line. Decreasing mTORC2 activity recapitulated the aged microglial phenotype resulting in an increased M1 polarization and a decreased response to M2 activators. We followed this experiment by examining the activity of the mTORC1 and mTORC2 pathway in young and aged microglia by examining phosphorylation of their downstream targets. Our findings indicate that Impairments in amino acid metabolism may represent a first hit to the downstream disruption bioenergetics. This disruption likely leads to an increased reliance on glycolysis for energy production, resulting in an increase in M1 polarization. Because chronic inflammation contributes to the progression of degenerative disease, understanding age-induced impairments in microglial polarization are key to the development of therapeutics. Our findings suggest that nutrient sensing pathways are altered with age and contribute to loss of immune homeostasis.

Disclosures: **A. Flowers:** None. **H. Bell-Temin:** None. **L. Daly:** None. **S.M. Stevens:** None. **P. Bickford:** None.

Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 220.15/E6

Topic: C.05. Aging

Support: NSERC grant MGM04321

Title: Neuroinflammation, dopaminergic neurodegeneration and behavioral impairment in hyperglycemic rats

Authors: K. DUFRESNE[1], J. RENAUD[1], N. ST-AMAND LUNA[1], C. LAVOIE[1], G. COSTA[2], N. SIMOLA[2], M. MORELLI[2], *M. MARTINOLI[1];
[1]Dept. of Med. Biol., Univ. Quebec, Trois-Rivieres, QC, Canada; [2]Dept. Biomed. Sci., Univ. di Cagliari, Cagliari, Italy

Abstract: It is now well known that hyperglycemia is a cause of oxidative stress reported to be harmful for the nervous system. Epidemiological evidence displays a relationship between diabetes and neurodegenerative disorders, including Alzheimer's disease [1] and Parkinson's disease [2]. We have recently shown that dopaminergic neurons in culture are vulnerable to physiologically sustainable high levels of glucose by exhibiting increased production of reactive

oxygen/nitrogen species, expression of pro-apoptotic markers and cell death [3,4]. In light of these results, this study aimed to characterize dopaminergic neurodegeneration in a streptozotocin-nicotinamide rat model of chronic hyperglycemia and metabolic impairment. Male Sprague-Dawley rats were injected with nicotinamide followed by streptozotocin, and metabolic parameters were monitored for five months. During the last three weeks, ultrasonic vocalizations and social behavior were recorded in pairs of unacquainted rats in a novel environment. At 5 months, rats were sacrificed, and brain and intestinal tissues were harvested for immunoblotting or immunohistochemical analyses. Metabolic measurements show that streptozotocin-nicotinamide-injected rats were hyperglycemic and metabolically impaired, as demonstrated by modulation of weight, glucose tolerance, plasma insulin levels, polyuria, polydipsia and polyphagia, as compared with control rats. In addition, ultrasonic vocalization patterns, which are critically regulated by dopaminergic pathways [5], were disrupted in pairs of hyperglycemic rats compared to pairs of control rats. Freezing and aggressive incidents were also notably increased in these rats compared to control animals. Further experiments were performed to detect dopaminergic neurodegeneration in hyperglycemic rat midbrains and mesenteric plexus, which houses up to 50% of total dopamine in the body. Our results show a definite modulation of dopaminergic function and neurodegeneration accompanied by neuroinflammation in hyperglycemic rats. Altogether, our data evoke a correlation between hyperglycemia and dopaminergic neurodegeneration with a component of neuroinflammation, which provides new insight on the higher occurrence of PD in diabetic patients. 1. Vignini A et al. (2013) Curr Diabetes Rev, 9:218-27. 2. Jagota P et al. (2012) J Neurol Sci, 314:5-11. 3. Bournival J et al. (2012) Rejuvenation Res, 15:322-33. 4. Renaud J et al. (2014) Neurotox Res, 25:110-23. 5. Thompson B et al. (2006) Behav Brain Res, 168: 64-73

Disclosures: **K. Dufresne:** None. **J. Renaud:** None. **N. St-Amand Luna:** None. **C. Lavoie:** None. **G. Costa:** None. **N. Simola:** None. **M. Morelli:** None. **M. Martinoli:** None.

Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

Location: Hall A

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Program#/Poster#: 220.16/E7

Topic: C.05. Aging

Support: Indian Council of Medical Research

Title: Establishing an animal model to study the neurobiology of obesity and ageing

Authors: ***J. K. SINHA**, S. GHOSH, M. RAGHUNATH;
Natl. Inst. of Nutr. (NIN), Hyderabad, India

Abstract: Purpose: Proportion of aged individuals is on the rise in general population and non-communicable diseases like obesity is a leading cause of death in older individuals. 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. Neuronal and glial changes that were observed in these rats were in line with other brain ageing studies. 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 that 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: **J.K. Sinha:** None. **S. Ghosh:** None. **M. Raghunath:** None.

Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 220.17/E8

Topic: C.05. Aging

Title: Modulation of metabolic parameters and antioxidant enzymes in diabetic aging female rat brain: Beneficial role of Metformin

Authors: *P. KUMAR, N. BAQUER;
Jawaharlal Nehru Univ., New Delhi, India

Abstract: Objective: The objective of this study was to investigate beneficial effects of metformin on membrane bound enzymes (monoamine oxidase, Na^+ K^+ ATPse,) and antioxidant enzymes (sueroxidase dismutase, glutathione S-transferases), lipid peroxidation, neurolipofuscin, DNA degradation in diabetic aging brain of female rats. Methods: Young (3 months) adult (12 months) and aged (24 months) rats will be diabetic by using alloxan monohydrate. Metformin

was administered i.p. at a dose of 200 mg/kg/day for 30 days to both control and diabetic aging rats. Learning was tested in a Morris water maze. A detailed study was carried on membrane linked enzymes, membrane fluidity, neurolipofuscin, antioxidant enzymes and DNA degradation to identify the antidiabetic and antiaging role of metformin using biochemical ,molecular and histiochemical study. Results: Present study shows that there was a similar pattern of increased lipid peroxidation, neurolipofuscin, DNA degradation and monoamine oxidase activity and a decrease in membrane fluidity, Na^+ K^+ ATPse, antixodant enzymes activities in brain of both aging and diabetes. Metformin was found to be an effective treatment in stabilizing and normalizing the membrane functions; therefore this therapy can be considered an alternative to be explored further as a means of diabetic and aged related disorders control. Metformin treatment also reversed the age related changes studied, to normal levels, elucidating an anti-aging, antidiabetic and neuroprotective action. Conclusions: The results of this study will be useful for pharmacological modification of the aging process and applying new strategies for control of age related disorders including metabolic syndrome.

Disclosures: P. Kumar: None. **N. Baquer:** None.

Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 220.18/E9

Topic: C.05. Aging

Title: Longevity manipulations differentially affect serotonin/dopamine level and behavioral deterioration in aging caenorhabditis elegans

Authors: *J.-A. YIN, X. LIU, J. YUAN, S. CAI;
Inst. of Neuroscience, SIBS, CAS, Shanghai, China

Abstract: Aging is accompanied with behavioral and cognitive decline. Changes in the neurotransmitter level are associated with the age-related behavioral deterioration, but whether well-known longevity manipulations affect the function of neurotransmitter system in aging animals is largely unclear. Here we report that serotonin (5-HT) and dopamine (DA) level decrease with age in *C. elegans*. The reduction results in down regulation of the activity of neurons controlled by 5-HT/DA signaling, and deterioration of some important behaviors, including pharyngeal pumping, food-induced slowing responses, and male mating. Longevity manipulations differentially affect the age-related decline in neuronal level of 5-HT/DA. The reduction and resultant behavioral deterioration occur in long-lived worms with defective insulin signaling [daf-2(e1370), age-1(hx546)] or mitochondria function [isp-1(qm150), tpk-1(qm162)], but not in long-lived worms with dietary restriction eat-2(ad1116). A reduced expression level of dopa decarboxylase BAS-1, the shared enzyme for 5-HT/DA synthesis, is responsible for the

decline in 5-HT/DA levels. RNAi assay revealed that the sustained 5-HT/DA level in neurons of aged eat-2(ad1116) worms requires PHA-4 and its effectors superoxide dismutases and catalases, suggesting the involvement of reactive oxygen species in the 5-HT/DA decline. Furthermore, we found that elevating 5-HT/DA ameliorates age-related deterioration of pharyngeal pumping,food-induced slowing responses,and male mating in both wild-type and daf-2(e1370) worms.Together,dietary restriction preserves healthy behaviors in aged worms at least partially by sustaining a high 5-HT/DA level, and elevating the 5-HT/DA level in wild-type and daf-2(e1370) worms improves their behaviors during aging.

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Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

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Topic: C.05. Aging

Support: CONICYT 21080629

FONDECYT 1110267

NIH-NIDA grant DA021213

Title: Inhibition of dopamine transporter by arachidonic acid oxidation is associated with γ-ketoaldehyde adduct formation in PC12 cells

Authors: ***J. A. PINO**[1], N. OSSES[2], T. B. BAUST[1], C. A. VALLE[3], J. G. REYES[2], G. E. TORRES[1];
[1]Neurobio., Univ. of Pittsburgh, Pittsburgh, PA; [2]Pontifical Catholic Univ. of Valparaiso, Valparaiso, Chile; [3]Univ. of Viña del Mar, Viña del Mar, Chile

Abstract: Oxidative stress and dysfunction of the dopamine transporter (DAT) underlie pathologies such as Parkinson's disease, a brain associated disorder where arachidonic acid (AA) is abundant as an acyl chain in phospholipids. AA oxidation produces γ-ketoaldehydes (γ-KAs) species, that can modify protein structure and function by forming adducts. Although, previous reports have shown that AA produces a decrease in DAT activity, the impact of AA lipid peroxidation, possible γ-KA-DAT adduct formation and the resulting effects on DAT functionality have not been examined. In this study, PC12 cells overexpressing DAT were incubated under different experimental conditions and the γ-KA-DAT adducts formation and DAT function were evaluated. When PC12 cells were incubated with AA under oxidative conditions or with synthetic γ-KAs, a significant increase in the amount of γ-KA-DAT adducts was observed compared to the control condition. In addition, the formation of γ-KA-DAT

adducts was associated to a significant decrease in the maximal velocity (Vmax) of DAT, an effect that could be reversed by the specific γ-KA scavenger, salicylamine. Consequently, our results strongly suggest that γ-KAs are covalent modifiers and inhibitors of DAT function in cells, and support a novel mechanism for oxidative stress-related DAT protein dysfunction and altered dopaminergic transmission.

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Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

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Program#/Poster#: 220.20/E11

Topic: C.05. Aging

Support: BBSRC EastBio DTP PhD student 4 year scholarship (2013-2017)

Title: The neuronal mitochondrial proteome and regulation of synaptic stability

Authors: ***L. C. GRAHAM**[1], S. L. EATON[2], D. J. LAMONT[3], P. J. BRUNTON[2], C. M. HENSTRIDGE[4], T. L. SPIRES-JONES[4], T. H. GILLINGWATER[4], G. PENNETTA[4], P. SKEHEL[4], T. M. WISHART[2];
[1]The Roslin Inst., Midlothian, United Kingdom; [2]The Roslin Inst., Edinburgh, United Kingdom; [3]Univ. of Dundee, Dundee, United Kingdom; [4]Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: Mitochondria are the 'power-houses' of all cells, generating ATP to fuel numerous pathways which are vital for cellular form and function [1]. Neuronal processes and synapses present a constant demand for ATP to maintain ionic gradients and neurotransmission events [2], promoting sub-populations of mitochondria to be enriched pre- and post-synaptically [3, 4]. These mitochondria display unique enzymatic [5], calcium buffering [6, 7] and antioxidant properties [8] and have thus been associated in the pathogenesis of a variety of neurodegenerative diseases where the synapse is the primary target. We have used label-free proteomics to characterise the proteomes of these synaptic and non-synaptic mitochondria at a basal level after following early biochemical isolation methods [5]. By utilizing this methodology we have generated a species-specific molecular fingerprint and demonstrated distinct proteomic profiles between the two sub-populations of mitochondria, dependent upon sub-cellular localisation. Quantitative fluorescent western blotting was used to validate the proteomic studies in a range of species, suggesting that the data may be an accurate reflection of the fingerprint for distinct mitochondrial populations. These results also suggest that mitochondrial neuronal sub-populations and their relative protein abundances are likely conserved between mammals. Following this, *in vivo* assays of mitochondrial candidates using

Drosophila larval fillet preparations were performed. Our data demonstrates that selective knock-down of intrinsic mitochondrial proteins alter synaptic morphology which may contribute to pathological processes during ageing and disease.

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Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

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Support: Canada Research Chair in Neurodegenerative Diseases

Canadian Institutes of Health Research

Fonds de recherche du Québec – Santé

University of Sherbrooke

Research Center on Aging

Title: The role of STK3 in neurodegeneration : Brain region-specific alterations in the aging process

Authors: *M. LESSARD-BEAUDOIN[1,3], M.-J. DEMERS[1,3], M. LAROCHE[1,3], G. GRENIER[2], R. GRAHAM[1,3];
[1]Dept. of pharmacology and physiology, [2]Dept. of Surgery, Univ. of Sherbrooke, Sherbrooke, QC, Canada; [3]Res. Ctr. on Aging, Sherbrooke, QC, Canada

Abstract: Neurodegenaration is observed in several neurological diseases including Alzheimer disease, and Huntington disease (HD), and in the aging process. Caspase-6 (casp6) activation and cleavage of several casp6 substrates is a key pathological event in many neurodegenarative diseases. In HD, casp6 cleavage of mutant huntingtin produces a neurotoxic fragment essential to the development of HD. Characterization of the casp6 interactome will improve our understanding of the causes and consequences of its overactivation and identify novel therapeutic targets for neurodegenerative diseases. Preliminary results reveal cleavage of Serine/Threonine Kinase 3 (STK3) by casp6. As a fragment of STK3 has previously been shown *in vitro* to have proapoptotic functions, the generation of a caspase cleaved STK3 fragment *in vivo* could influence neurodegenerative pathways.In order to define the function of STK3 and characterise its potential role in neurodegeneration and in aging process, the expression of STK3 and

caspases were analysed in the cortex, cerebellum and striatum of C57BL6 wild type male mice at 3,12,23-28 and >30 months of age. STK3 mRNA increases in the cerebellum ($p=0.057$) and striatum ($p<0.0001$), and decreases in the cortex ($p=0.003$) of C57BL6 male mice with age. STK3 protein expression (full-length and fragment levels) increases in all brain regions with age. Interestingly, a 2-3 fold increase is observed for the full-length ($p=0.0006$) and the fragment ($p=0.0002$) of STK3 in the cortex. Casp6 mRNA decreases in the cortex ($p=0.04$) and increases in the striatum ($p=0.0006$) and the cerebellum ($p=0.0009$) with age. However, casp6 protein expression increases in the cortex ($p=0.03$) and cerebellum ($p=0.012$) and decreases in the striatum ($p=0.06$) with age. Protein expression of caspase-3, which has previously been shown to also cleave STK3, decreases in the cerebellum ($p=0.03$) with aging. These results suggest that important dysregulation occurs in the expression and post-translational modifications of STK3 with aging. Moreover, the caspases known to cleave STK3 are dysregulated in a region-specific manner. This provides important information regarding their understanding in the aging process, and can serves as comparative data for HD and other age-associated diseases.

Disclosures: M. Lessard-Beaudoin: None. **M. Demers:** None. **M. Laroche:** None. **G. Grenier:** None. **R. Graham:** None.

Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

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Program#/Poster#: 220.22/E13

Topic: C.05. Aging

Title: Glyceraldehyde-3-phosphate dehydrogenase regulates c-jun N-terminal kinase activation under oxidative stress conditions

Authors: *K. SATO, M. ITAKURA, Y.-T. AZUMA, T. TAKEUCHI, H. NAKAJIMA; Osaka Prefecture Univ., Izumisanoshi, Japan

Abstract: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has diverse functions and introduce of cell death under oxidative stress. Mitogen-activated protein kinase (MAPK) is also established as a candidate of an important signaling pathway under oxidative stress. MAPK pathways lead to various cellular responses including cell proliferation, differentiation, and apoptosis. Recently, it has been reported that GAPDH interacts with apoptosis signal-regulating kinase1 (ASK1) which is upstream kinase of MAPK pathway. In addition, it is revealed that oxidative stress-induced ASK1 activation emerges cellular dysfunction evoked by oxidized GAPDH. It is, however, not clear how oxidized GAPDH is involved in MAPK pathways. Therefore, we examined the activation level of each typical MAPK such as an Extracellular Signal-regulated kinase, p38, or c-jun N-terminal Kinase (JNK) in GAPDH-knockdown HEK cells under exposure of oxidative stress. While treatments of cells with GAPDH-siRNA reduced

significantly the hydrogen peroxide (H2O2)-induced JNK activation, there is no influence of both Extracellular Signal-regulated kinase and p38 activation. We next investigated the protein-protein interaction between GAPDH and JNK using an immunoprecipitation assay. The GAPDH-JNK interaction was observed only by the treatment with H2O2, suggesting that its interaction is likely to depend on oxidized GAPDH. Moreover, we conducted an experiment using human GAPDH-knockdown cells transiently expressing rabbit wild type (WT) or the active site cysteine-substituted mutant GAPDH that lacks the ability to bind JNK. In GAPDH-knockdown cells, decline of JNK activation was restored by conducting exogenous rabbit WT-GAPDH, but was not by that of its mutant. Thus, oxidized GAPDH is essential for H2O2-induced JNK activation. Together, these findings suggest that GAPDH binds to JNK in an oxidative stress-dependent manner, resulting in JNK activation.

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Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

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Title: Role of surface MHCI in mediating negative effects of B2M and old blood on cognitive and regenerative function

Authors: *C. SNETHLAGE, L. SMITH, K. LIN, G. GONTIER, K. PLAMBECK, J. UDEOCHU, E. WHEATLEY;
Anat., UCSF, San Francisco, CA

Abstract: Aging drives cognitive and regenerative impairments in the adult brain, increasing susceptibility to neurodegenerative disorders in healthy individuals. Experiments using heterochronic parabiosis (in which the circulatory system of a young and old animal are joined) have demonstrated that age-related changes in the systemic environment drive aging phenotypes in the brain, indicating pro-aging factors in old blood. Previously we identify β2-microglobulin (B2M), a component of major histocompatibility complex class 1 (MHC I) molecules, as a pro-aging factor that negatively regulates cognitive and regenerative function in the adult hippocampus in an age-dependent manner. However, the specific mechanism by which B2M and old blood act on cognitive and regenerative function in the hippocampus is still unknown. We hypothesized that surface MHC I mediates the negative effects of B2M and old blood on

cognitive and regenerative function. Here we report that local hippocampal administration of exogenous B2M results in impairments in spatial learning and memory, as well as contextual fear conditioning following short-term recovery. Moreover, the negative effects of B2M on cognitive function were reversed after long-term recovery. To test if surface MHC I is involved in mediating cognitive effects of B2M, we used a genetic mouse model expressing reduced levels of surface MHC I (Tap1-/-), and detected no impairments in these mice after hippocampal B2M administration. Next we investigated the effects of B2M on regenerative function, and detected an MHC I-dependent decrease in neurogenesis after local exposure to B2M. Subsequently, we investigated whether reduced surface MHC I could also mitigate the negative effects of old blood elicited by heterochronic parabiosis. Consistent with previous reports we observed a decrease in neurogenesis in young wild type heterochronic parabionts. However, decreased neurogenesis was in part mitigated in young Tap1-/- heterochronic parabionts expressing reduced surface MHC 1. Collectively, these data show that surface expression of classical MHC I molecules plays an important role in regulating the negative effects of pro-aging factors in old blood, including B2M. Our work now identifies MHC I molecules as potential future therapeutic targets aimed at counteracting age-related cognitive and regenerative impairments.

Disclosures: **C. Snethlage:** None. **L. Smith:** None. **K. Lin:** None. **G. Gontier:** None. **K. Plambeck:** None. **J. Udeochu:** None. **E. Wheatley:** None.

Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 220.24/E15

Topic: C.05. Aging

Support: CIRM Predoctoral Fellowship

DP5-OD12178

Sandler Foundation

Title: Role of classical MHC I molecules H2-Kb and H2-Db in regulating adult neurogenesis and cognitive function with age

Authors: *K. LIN, L. K. SMITH, S. A. VILLEDA;
Anat., UCSF, San Francisco, CA

Abstract: Aging in the brain is a multifaceted process in which changes in regenerative capacity and functional integrity lead to cognitive decline and susceptibility to neurodegenerative diseases. Identifying methods to prevent or even reverse the effects of aging in the brain is thus

critical. Adult neural stem cells (NSCs) have been at the center of exciting attempts to curb age-related cognitive dysfunction. However, in order to use regenerative therapeutics to restore cellular and cognitive functions in the aging brain, we must first understand the mechanisms underlying changes in NSC function with age. Previously, using heterochronic parabiosis (in which the circulatory systems of young and old animals are joined) we have shown that age-related changes in the systemic environment can induce aging phenotypes in the brain; furthermore, we have identified β2-microglobulin (B2M), a component of the major histocompatibility class 1 (MHCI) molecules, as a pro-aging factor affecting the hippocampal neurogenic niche. Here, we implicate a specific subset of classical MHCI molecules, H2-Kb and H2-Db, in negatively regulating adult neurogenesis and associated hippocampal-dependent functions. Absence of H2-Kb and H2-Db in young adult MHCI knockout mice resulted in a decreased pool of Tbr2-positive intermediate progenitors while increasing neurogenesis and neuronal survival in the hippocampus *in vivo*. This robust increase in neurogenesis at young age ultimately led to stem cell exhaustion and decreased neurogenic output in aged MHCI knockout mice. At a cognitive level, adult MHCI knockout animals also exhibited differences in their performance in the radial arm water maze and contextual fear conditioning paradigms, indicating changes in hippocampal-dependent learning and memory. Collectively, this work reveals a novel role for classical MHCI molecules in regulating the age-related decline in adult neurogenesis and cognitive function. Given MHCI expression increases in the aging hippocampus, it now becomes an exciting prospect to examine whether temporally controlled abrogation of MHCI in the aged brain can enhance neurogenesis and cognitive functions at old age.

Disclosures: **K. Lin:** None. **L.K. Smith:** None. **S.A. Villeda:** None.

Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 220.25/E16

Topic: C.05. Aging

Support: Arizona Biomedical Research Comission Grant: ADHS14-082982

University of Arizona Intramural Funds

Title: A role for Nrf2 in neural stem cell function during aging

Authors: ***L. MADHAVAN**[1], M. J. CORENBLUM[1], S. RAY[2], M. LONG[3], B. HARDER[3], D. ZHANG[3], C. BARNES[4];
[1]Neurol., [2]Neurosci. and Cognitive Sci. Program, [3]Pharmacol. and Toxicology, [4]Psychology, Univ. of Arizona, Tucson, AZ

Abstract: Although it is known that the regenerative function of neural stem/progenitor cells (NSPCs) declines with age, causal mechanisms underlying this phenomenon are not understood. In this context, we have systematically analyzed subventricular zone NSPCs in various groups of rats across the aging spectrum, using *in vitro*, *in vivo*, and behavioral techniques. These studies indicate that although NSC function continuously declines with advancing age, there is a critical time period during middle-age (13-15 months) when a striking reduction in NSPC survival and regeneration (proliferation and neuronal differentiation) occurs. We also find that this specific temporal pattern of NSPC deterioration correlates with the decreasing expression of the redox-sensitive transcription factor nuclear erythroid factor 2 like 2, or Nrf2, in the NSPCs. When Nrf2 expression was suppressed in 'young' NSPCs, using short interfering RNAs, the survival and regeneration of the NSPCs was significantly compromised and their behavior mirrored 'old' NSPCs. Conversely, Nrf2 overexpression in 'old' NSPCs rendered their behavior similar to `young' NSPCs and they showed increased survival and regeneration. Furthermore, examination of NSPCs in young Nrf2 knock-out (Nrf2 -/-) mice revealed a lower number of NSPCs in the subventricular zones of these animals, when compared to age-matched wild type controls. In addition, the proliferative potential of the NSPCs, and their ability to produce new neurons, was also notably compromised in the Nrf2 -/- mice. These results identify a novel regulatory role for Nrf2 in NSPC function during aging, and have important implications towards developing NSPC based strategies to support healthy aging and to treat age-related neurodegenerative disorders.

Disclosures: L. Madhavan: None. **M.J. Corenblum:** None. **S. Ray:** None. **M. Long:** None. **B. Harder:** None. **D. Zhang:** None. **C. Barnes:** None.

Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

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Topic: C.05. Aging

Support: Swedish Research Council

Swedish Brain Foundation

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Swedish Society for Medical Research

Foundation for Geriatric Diseases at Karolinska Institutet

Karolinska Institutet Research Foundations

Title: Mitochondrial dysfunction leads to altered lipid homeostasis in prematurely aging mtDNA mutator (PolgAmut/mut) mice

Authors: *J. M. ROSS[1], G. COPPOTELLI[1], M. SHAFAATI[1], M. OLIN[1], B. HOFFER[2], I. BJÖRKHEM[1], L. OLSON[1];
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Abstract: Accumulation of mitochondrial DNA (mtDNA) mutations resulting in mitochondrial dysfunction has been heavily implicated in mitochondrial diseases as well as aging and age-related diseases, such as Alzheimer's and Parkinson's disease. To study the effects of progressive mitochondrial dysfunction, homozygous knock-in mice expressing a proof-reading deficient version of the nucleus-encoded catalytic subunit (*PolgA*) of mtDNA polymerase-γ has been developed (Trifunovic *et al., Nature,* 2004). The mtDNA mutator mice ($PolgA^{mut/mut}$) have very high levels of point mutations (20-30 mutations per mtDNA molecule) and linear deletions (~25% of total mtDNA), and show many signs of premature aging such as reduced life span (42-43 wks), alopecia, weight loss, anemia, sarcopenia, loss of subcutaneous fat, reduced fertility, impaired hearing, osteoporosis, and organ enlargement. Elevated lactate levels in brain and other tissues have been described as a hallmark and a pre-symptomatic marker of aging in both prematurely aging mtDNA mutator and normally aging mice (Ross *et al., PNAS*, 2010). Recently, it has also been shown that germline mtDNA mutations cause anticipation of impaired fecundity, produce stochastic brain malformations when maternal mtDNA mutations are combined with homozygosity for the *PolgA* mutation, and aggravate aging phenotypes and lifespan in homozygous mtDNA mutator mice as well as in mice with a wild-type nuclear DNA background (Ross *et al., Nature,* 2013; *Sci Rep,* 2014). Investigating lipid metabolism, we found increased serum and hepatic cholesterol levels, but no change in triglycerides in >40 wk-old mtDNA mutator mice. Liver size and weight were increased. Cytochrome *c* oxidase (COX) enzyme histochemistry showed deficiency in COX (Complex IV) activity and Oil Red O staining indicated increased lipid droplets in liver sections. We are now investigating key genes involved in the formation and breakdown of cholesterol, such as HMG CoA Synthase, HMG CoA Reductase, LDL-Receptor, and CYP7A1, in order to explain the increased cholesterol levels. Additionally, we found an overall decrease in mtDNA mutator brain size with minimal difference in weight. We are now using histological and biochemical approaches to determine if there is a loss in both grey and white matter and if there are brain-specific changes in lipid metabolism. These findings could provide insights into dyslipidemia and disorders of lipoprotein metabolism, both commonly described among elderly patients.

Disclosures: **J.M. Ross:** None. **G. Coppotelli:** None. **M. Shafaati:** None. **M. Olin:** None. **B. Hoffer:** None. **I. Björkhem:** None. **L. Olson:** None.

Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

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Topic: C.05. Aging

Support: NIH/NIEHS ES-008146

Title: Age-dependent high copper contents and expressions of copper regulatory proteins in the subventricular zone and choroid plexus

Authors: *X. FU, W. JIANG, W. ZHENG;
Purdue Univ. Sch. of Hlth. Sci., West Lafayette, IN

Abstract: Copper (Cu) is an essential element for normal brain development and function. Our recent data by X-ray fluorescent microscopy suggest that Cu is highly concentrated along the lateral walls of brain lateral ventricles where subventricular zone (SVZ) is located. Anatomically, SVZ is in direct contact with cerebrospinal fluid (CSF), which is secreted by a neighboring tissue choroid plexus. Changes in Cu regulatory gene expressions in the SVZ and choroid plexus as the function of aging may determine Cu levels in the CSF and SVZ. This study was designed to investigate the associations between age, Cu levels, and Cu regulatory genes in SVZ and plexus. The SVZ and choroid plexus were dissected from brains of 3-week, 10-week or 9-month old male rats. Analyses by atomic absorption spectroscopy revealed that the SVZ of adult and old animals contained the highest Cu level compared with other tested brain regions. Significant positive correlations between age and Cu levels in SVZ and plexus were observed; the SVZ Cu level of old animals was 7.5- and 5.8-fold higher than those of young and adult rats ($p<0.01$), respectively. Quantitation by qPCR of the transcriptional expressions of Cu regulatory proteins showed that the SVZ expressed the highest level of Cu storage protein MTs, while the choroid plexus expressed the high level of Cu transporter protein Ctr1. Noticeably, Cu levels in the SVZ were positively associated with type B slow proliferating cell marker Gfap ($p<0.05$), but inversely associated with type A proliferating neuroblast marker Dcx ($p<0.05$) and type C transit amplifying progenitor marker Nestin ($p<0.01$). Dmt1 had significant positive correlations with age and Cu levels in the plexus ($p<0.01$). These findings suggest that Cu levels in all tested brain regions are increased as the function of age. The SVZ shows a different expression pattern of Cu-regulatory genes from the choroid plexus. The age-related increase of MTs and Gfap, and decrease of Ctr1 in the SVZ may contribute to the high Cu level in this neurogenesis active brain region. In addition, the inverse correlations between SVZ Cu content and Nestin expression, as well as Dcx, may imply a potential role of Cu in regulating the adult neurogenesis activity in this region.

Disclosures: **X. Fu:** A. Employment/Salary (full or part-time):; Purdue University. **W. Jiang:** None. **W. Zheng:** None.

Poster

220. Aging: Metabolism, Diet, and Oxidative Stress

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Topic: C.05. Aging

Support: NIH Grant AG047612

NIH Grant GM088804

NIH Grant NS082001

Title: Aging-vulnerable and -resilient activity patterns in an identified *Drosophila* motor neuron: Acceleration by high temperature and distortion by oxidative stress

Authors: *A. IYENGAR[1], H. RUAN[2], C.-F. WU[2];
[2]Biol., [1]Univ. of Iowa, Iowa City, IA

Abstract: In *Drosophila*, molecular pathways influencing longevity have been extensively studied. However, corresponding neurophysiological changes underlying aging-related behavioral deterioration remains to be explored. To elucidate salient features in functional decline of neural circuit function, we examined the activity of an identified motor neuron (DLMn5), which drives DLM flight muscle, but also actuates additional motor circuit outputs including giant-fiber (GF) mediated jump-and-escape reflex and electroconvulsion seizure activity in the CNS. The convergence of these motor outputs on DLMn5 enables dissections of age- and stress-vulnerable circuit components and their physiological parameters within the same individual. We studied the extent of decline for a number of motor functions progressing across the lifespan and found both age-sensitive and age-resilient trajectories. For example, we observed relatively little deterioration in GF components responsible for the escape reflex across the lifespan (latency changes less than 15 %, threshold changes less 5%, between 1 and 95% mortality). Conversely, markers of age-dependent plasticity were observed in central inputs to the GF pathway (doubling of refractory period, and a 2 - 4 fold increase in habituation rate), flight motor circuits (doubling of firing rate), and seizure discharges (30 % reduction in threshold) over the same period. Manipulations of specific environmental or mutational stressors could result in drastically different effects on the progression of specific motor patterns and physiological parameters. Interestingly, the age-resilient properties identified above remain intact where as the age-vulnerable counterparts revealed stress-specific responses. Importantly, we found a relatively straightforward compression of the characteristic age-progression of ageing-vulnerable circuit properties when individuals were reared at increased temperature (29°C). This finding provides experimental evidence justifying the common practice in *Drosophila* aging studies conducted at 29°C for expedited experiments. Another important factor recognized in aging processes is oxidative stress. We examined the effects of increased reactive oxygen species (ROS) in Sod1 mutants due to reduced Cu/Zn superoxide dismutase activity. We found distinct effects on age-trajectories when compared with the responses to high temperature rearing. Our study juxtaposes the relative age-resilience of the GF circuit (including the GF, PSI interneuron,

and DLMn5) with the age-vulnerability in other central circuits driving motor activities through DLMn5 directly or mediated by GF input.

Disclosures: **A. Iyengar:** None. **H. Ruan:** None. **C. Wu:** None.

Poster

221. Genetic Models of Autism Spectrum Disorder

Location: Hall A

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Topic: C.06. Developmental Disorders

Support: Global PhD Fellowship, National Research Foundation of Korea (NRF)

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Brain Research Program, National Research Foundation of Korea (NRF)

Institute for Basic Science (IBS) IBS-R002-D1

Title: Shank3-mutant mice lacking exon 9 show altered excitation/inhibition balance, enhanced rearing, and spatial memory deficit

Authors: ***J. LEE**[1], C. CHUNG[1], S. HA[1], D. LEE[2], D.-Y. KIM[1], H. KIM[2], E. KIM[1,3]; [1]Dept. of Biol. Sci., KAIST, Daejeon, Korea, Republic of; [2]Korea Univ., Seoul, Korea, Republic of; [3]Inst. for Basic Sci. (IBS), Daejeon, Korea, Republic of

Abstract: Shank3 is a scaffolding protein important for organizing the postsynaptic signaling complex at excitatory synapses. Mutations in *SHANK3* gene are implicated in autism spectrum disorders (ASDs) in humans. Several *Shank3* knockout mouse models have been generated in order to study the mechanisms underlying *SHANK3* mutation-induced ASDs. In this study, we generated $Shank3^{\Delta 9}$ mice, in which exon 9 of *Shank3* is deleted to induce gene knockout. Among the major *Shank3* transcript variants, $Shank3^{\Delta 9}$ mice lacked the longest variant (Shank3a), which contains the ankyrin-repeat domain, but other major variants were intact. We thus analyzed the temporal and spatial expression patterns of Shank3a variant. Behaviorally, $Shank3^{\Delta 9}$ mice showed increased rearing in a novel environment, and mild deficit in Morris water maze task. However, they did not show autistic-like phenotypes. Interestingly, we found increased inhibitory synaptic transmission in the hippocampus, but decreased inhibitory transmission in the medial prefrontal cortex of $Shank3^{\Delta 9}$ mice. These results suggest that ankyrin repeat-containing Shank3 variants are important for E/I (excitation/inhibition) balance, rearing behavior, and spatial memory.

Disclosures: **J. Lee:** None. **C. Chung:** None. **S. Ha:** None. **D. Lee:** None. **D. Kim:** None. **H. Kim:** None. **E. Kim:** None.

Poster

221. Genetic Models of Autism Spectrum Disorder

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 221.02/E21

Topic: C.06. Developmental Disorders

Support: Seaver Foundation Postdoctoral Fellowship

Title: Behavioral characterization of SHANK3-deficient rats on an 8-arm radial maze

Authors: *M. YOUNG[1,2], M. L. SHAPIRO[3];
[1]Neurosci., Icahn Sch. of Med. At Mount Sinai, New York, NY; [2]The Seaver Autism Ctr. for Res. and Treatment, New York, NY; [3]Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Phelan-McDermid syndrome is a developmental disorder caused by deletions or mutations in chromosome 22q13.3 that lead to the loss of one functional copy of the gene for SHANK3, a postsynaptic scaffolding protein. This single mutation is a highly penetrant cause of autism, with ~70% of affected individuals meeting the criteria for autism and ~80% meeting criteria for autism spectrum disorders. To investigate how SHANK3 mutations lead to behavioral impairments in autism, we tested SHANK3 heterozygotes and knockouts on an 8-arm radial maze. Initial acquisition, reversal and strategy switching tasks were evaluated. Additionally, we investigated whether rats lacking SHANK3 are capable of performing slow, medium, and fast reversals of the spatial goal arm. Preliminary results suggest that SHANK3 heterozygosity has no effect on the ability of rats to acquire an initial spatial goal arm, to reverse to a different goal arm, or to switch to a body turn strategy, or to remember any of these tasks after 24 hours. Further analysis will determine the effect of full SHANK3 knockout on these measures.

Disclosures: **M. Young:** None. **M.L. Shapiro:** None.

Poster

221. Genetic Models of Autism Spectrum Disorder

Location: Hall A

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Topic: C.06. Developmental Disorders

Support: Autism Speaks Pilot Grant

NIMH R01 MH081880

NIMH R37 MH049428

NIH DP2 MH100011

NIMH R01 MH100292

Simons Foundation Pilot Grant

Title: Pten is necessary to establish normal numbers of GABAergic cortical interneurons

Authors: *D. VOGT, K. K. A. CHO, A. T. LEE, V. S. SOHAL, J. L. R. RUBENSTEIN; Dept. of Psychiatry, Univ. of California San Francisco, San Francisco, CA

Abstract: Cortical GABAergic interneurons, primarily derived from the medial and caudal ganglionic eminences (MGE and CGE), are highly diverse in morphology, and function. Their dysfunction has been implicated in neuropsychiatric disorders, including schizophrenia, epilepsy, and autism spectrum disorder (ASD). One hypothesis of ASD is that an imbalance in excitation/inhibition in the brain underlies some ASD phenotypes and a functional understanding of candidate genes associated with ASD biology is needed. MGE and CGE-derived interneurons migrate long distances and distinct subgroups will express parvalbumin and somatostatin (MGE-derived) as well as by vasoactive intestinal peptide and reelin, lacking somatostain, (CGE-derived) as they integrate into the cortex. We examined the function of the ASD candidate gene Pten in cortical GABAergic interneuron development using different spatial and temporal Cre-driver mouse lines. Pten inhibits PI3K/Akt/mTor signaling and interacts with other ASD candidate genes through this pathway. We found that Pten inhibits Akt signaling in MGE cells and is necessary for interneuron survival, with somatostatin+ interneurons showing the greatest loss. Interestingly, this only occurs if Pten is deleted in progenitor cells. In addition, Pten differentially regulates parvalbumin+ interneurons, and its loss results in ectopic projections in the cortex, altered behavior and increased cortical inhibition. To understand the impact of human ASD mutations, we developed an *in vivo* complementation assay to determine the function of PTEN ASD mutant alleles in cortical interneuron development. We found that these human ASD alleles were hypofunctional compared to wild type PTEN and did not act in a dominant-interfering manner. Together, these results suggest an important role for Pten and PI3K/Akt/mTor signaling in cortical interneuron development.

Disclosures: D. Vogt: None. **K.K.A. Cho:** None. **A.T. Lee:** None. **V.S. Sohal:** None. **J.L.R. Rubenstein:** F. Consulting Fees (e.g., advisory boards); Neurona Therapeutics.

Poster

221. Genetic Models of Autism Spectrum Disorder

Location: Hall A

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Topic: C.06. Developmental Disorders

Support: EU-AIMS, Innovative Medicines Initiative Joint Undertaking (115300)

Title: Conditional deletion of autism risk gene PTEN in the dopaminergic system leads to morphological, neurochemical, and behavioral abnormalities; can it be rescued?

Authors: K. T. E. KLEIJER, A. HOYER, M. J. H. KAS, *J. H. BURBACH;
Brain Ctr. Rudolf Magnus, UMC Utrecht, Utrecht, Netherlands

Abstract: Autism spectrum disorder (ASD) is a severe neurodevelopmental disorder, which presents with impairments in social behavior, communication and increased repetitive and restrictive behavior as core symptoms. In the last decade, hundreds of genes have been associated with ASD. Although the field has made much progress in delineating functions of ASD risk-genes, the origins of ASD symptomatology remains elusive. One major ASD risk-gene is phosphatase and tensin homolog (PTEN), which is found to be mutated in circa 10% of ASD-patients with macrocephaly. PTEN is hierarchically on top of signal transduction pathways, which regulate other genes that are strongly connected to ASD dysfunctions, such as FMR1, TSC1/2. To examine the role of PTEN in a specific brain system and its association with neurodevelopmental symptoms, a conditional knockout (cKO) mouse was generated, in which Pten was deleted in the midbrain dopaminergic (mDA) system during development (E11.5) using a Pitx3-Cre driver. The morphological, behavioral and neurochemical character of the cKO mouse are being analyzed to gain insight into the neuronal functions of PTEN and to provide robust mouse phenotypes allowing to test novel therapeutics for their ability to ameliorate the phenotypes. Morphologically, the fields of mDA neurons in the Substantia Nigra pars compacta (SNc) and Ventral Tegmenal Area (VTA) were enlarged at three stages; embryonic day (E) 18.5, postnatal day (P) 8 and adulthood. At all three ages, cell size was enlarged and thickened dendrites and enhanced axonal projections towards the striatum and prefrontal cortex were found. Behaviorally, the cKO mouse showed increased baseline locomotor activity (at adult age), which suggests enhanced DA signaling. To analyze the effect of PTEN on neurotransmission and organization of the DA system, neurochemical studies are currently in progress. The data show developmental phenotypes in the midbrain DA system which will be used to test compounds interfering with downstream pathways of PTEN for their ability to ameliorate or reverse ASD-related symptoms.

Disclosures: K.T.E. Kleijer: None. **A. Hoyer:** None. **M.J.H. Kas:** None. **J.H. Burbach:** None.

Poster

221. Genetic Models of Autism Spectrum Disorder

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 221.05/E24

Topic: C.06. Developmental Disorders

Support: Simons Foundation

Title: Treatment with novel erk inhibitor rescues pathophysiology associated with 16p11.2 chromosomal deletion in mice

Authors: *J. PUCILOWSKA[1], J. VITHAYATHIL[2], C. KELLY[2], J. C. KARLO[2], R. BRAMBILLA[3], G. E. LANDRETH[2];
[1]Neurosci., [2]Case Western Reserve Univ., Cleveland, OH; [3]San Raffaele Scientific Inst., Milan, Italy

Abstract: Autism Spectrum Disorders (ASDs) are complex, highly heritable neurodevelopmental disorders affecting approximately 1 in 100 children. Copy number variations (CNVs) are associated with 5-10% of children with ASDs and the 16p11.2 variation is genetically linked to approximately 1% of all ASDs. Even though the human 16p11.2 deletion is one of the most common CNVs in autism, the causative pathophysiology associated with this chromosomal abnormality is largely unknown. The 593-kb deletion contains the ERK1 gene as well as other genes that regulate the ERK/MAPK pathway. It has recently been appreciated that perturbations in signaling through the ERK MAP kinase pathway are a significant genetic cause of a range of neurodevelopmental disorders including autism. In fact, data from preclinical and clinical studies strongly suggests that changes in ERK signaling are central to the pathophysiology associated with ASDs. We have previously shown that a murine model of 16p11.2 deletion exhibits a reduction in brain size and perturbations in cortical cytoarchitecture. These changes are largely due to altered progenitor proliferation dynamics and premature cell cycle exit, which resulted in premature depletion of progenitor pools and deficits in the number and birth frequency of neurons populating cortical lamina. Importantly, the 16p11.2del mice exhibit a paradoxical increase in ERK signaling during neurogenesis, which is coincident with the development of aberrant cortical cytoarchitecture. Therefore, we postulated that treatment with ERK inhibitors may correct the pathophysiology observed in these mice. We treated the 16p11.2del mice with ERK inhibitors during a critical window at the start of neurogenesis. We administered a BBB permeant ERK inhibitor for 5 consecutive days starting on embryonic day 10 and evaluated the ERK inhibitor and vehicle treated 16p11.2del and control mice at E14.5 and P2. We observed a rescue of the developmental deficits associated with the 16p11.2 deletion at E14.5 by IHC and western blot analysis. Furthermore, we verified the embryonic rescue by counting the number of lamina-specific neurons at P2, showing restoration of neuronal numbers and cortical cytoarchitecture in the inhibitor treated deletion mice. Importantly, we tested the mice in a battery of behavioral tests including elevated plus maze, open field, NOR and maternal behavior and report rescue of the behavioral deficits associated with the 16p11.2 chromosomal deletion. In conclusion, our data suggests that ERKs may represent a therapeutic target since

MAPK inhibitors have been previously investigated for treatment of other developmental syndromes.

Disclosures: **J. Pucilowska:** None. **J. Vithayathil:** None. **C. Kelly:** None. **J.C. Karlo:** None. **R. Brambilla:** None. **G.E. Landreth:** None.

Poster

221. Genetic Models of Autism Spectrum Disorder

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Topic: C.06. Developmental Disorders

Support: R01HD069560

R01MH093697

Title: Autism-related shank3 exon 13 stop mice exhibit altered behavior and synaptic dysfunction in striatum and hippocampus

Authors: ***T. C. JARAMILLO**, H. SPEED, Z. XUAN, S. LIU, C. POWELL;
Neurol. & Neurotherapeutics, UT Southwestern Med. Ctr., Dallas, TX

Abstract: Mutations/deletions in the Shank3 gene are associated with autism spectrum disorders (ASD) and intellectual disability (ID). Here we present electrophysiological and behavioral consequences in heterozygous and homozygous mice with targeted disruption of the PDZ domain in Shank3 (exon 13 STOP mutant mice, Shank3E13). Insertion of a transcriptional stop cassette prior to exon 13 leads to a loss of the two highest molecular weight isoforms of Shank3. Behaviorally, both Shank3E13 heterozygous (HET) and knockout (KO) mice display increased repetitive grooming, deficits in social interaction tasks, and decreased rearing. Shank3E13 KO mice also display deficits in spatial memory in the Morris water maze. Baseline hippocampal synaptic transmission and short-term plasticity are preserved in Shank3E13 HET and KO mice, while both HET and KO mice exhibit impaired hippocampal long-term plasticity. Additionally, Shank3E13 HET and KO mice display impaired striatal glutamatergic synaptic transmission. These results demonstrate that this novel Shank3 mutant model leads to ASD-associated behavioral abnormalities along with widespread synaptic dysfunction. The conditionally reversible nature of this model allows for future studies to examine the temporal and regional nature of Shank3's involvement in these phenotypes.

Disclosures: **T.C. Jaramillo:** None. **H. Speed:** None. **Z. Xuan:** None. **S. Liu:** None. **C. Powell:** None.

Poster

221. Genetic Models of Autism Spectrum Disorder

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Topic: C.06. Developmental Disorders

Support: IRACDA Grant K12GM093854

Title: Autism related stress circuits exhibit enhanced norepinephrine system activity in the Engrailed-2 (En2) knockout model of neurodevelopmental disorders

Authors: J. W. LUNDEN[1], M. GENESTINE[1], C. C. PENG[1], V. MIRABELLA[1], S. PREM[1], J. H. MILLONIG[1], *E. M. DICICCO-BLOOM[2];
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Abstract: Autism spectrum disorder (ASD) is a pervasive neurodevelopmental disorder characterized by impairments in social interactions and the presence of repetitive/restricted behaviors. The neural patterning transcription factor En2 is involved in development of the embryonic mid-hindbrain region, where monoamine neurons emerge, and has been associated with ASD. Our previous studies indicate that En2 knockout (KO) mice exhibit deficits in social interactions, depression related tasks (forced swim, tail suspension), fear conditioning and spatial learning, all associated with diminished hippocampal neurogenesis and norepinephrine (NE) fiber innervation. While these deficits in hippocampal neurogenesis and NE systems suggest likely abnormalities in stress system structures and function, nothing is known about NE fiber innervation and neural activity in limbic regions such as amygdala and paraventricular nucleus of the hypothalamus (PVN). To examine this issue, postnatal day 60-70 wild type (WT) and KO mice (N=4-6/genotype) were assessed immunochemically for protein levels of NE transporter (NET) and tyrosine hydroxylase (TH, the rate-limiting enzyme for NE biosynthesis) in brain regions using western blotting and numbers of fibers expressing NET on tissue sections. Functional activity in the amygdala and PVN is being assessed using c-Fos immunostaining. En2 KO mice exhibited 1.7-fold ($p<0.02$) and 1.5-fold ($p<0.002$) increases in NET and TH protein levels respectively in amygdala compared to WT controls. NET fiber counts were increased 2.6-fold ($p<0.004$) in the basolateral amygdala and 1.7-fold ($p<0.016$) in the PVN. Preliminary nuclear c-Fos staining suggests that the increased NET innervation in the En2 KO PVN is associated with increased neural activation. These observations indicate that NE fiber innervation is increased in some En2-KO limbic system regions, a result that contrasts with the reduced fibers in the dorsally localized hippocampus. This region-specific dysregulation of fiber growth may contribute to abnormalities in depression-related tasks, fear conditioning and social interactions. More broadly, these studies of a neurodevelopmental animal model are defining a surprising array of monoamine system abnormalities in the forebrain that may be a consequence of disordered early development of hindbrain regulatory pathways.

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Poster

221. Genetic Models of Autism Spectrum Disorder

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 221.08/E27

Topic: C.06. Developmental Disorders

Support: DFG - WO 1732/1-1

Title: Autism-relevant social communication deficits and aberrant cognitive phenotypes in mice lacking the post-synaptic scaffolding protein SHANK1: a developmental perspective

Authors: ***A. Ö. SUNGUR**, M. C. E. JOCHNER, T. M. REDECKER, R. K. W. SCHWARTING, M. WÖHR;
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Abstract: Autism spectrum disorders (ASD) are a class of neurodevelopmental disorders characterized by persistent social communication deficits across multiple contexts, together with repetitive patterns of behavior. Among the most promising ASD candidate genes is the *SHANK* gene family, including *SHANK1*. To study the contribution of *SHANK1* mutations to ASD symptoms throughout development, *Shank1+/+, Shank1+/-,* and *Shank1-/-* mice were compared in behavioral assays developed to detect social communication deficits and aberrant cognitive phenotypes as pups, juveniles, and adults. When assessing isolation-induced ultrasonic vocalizations as a measure for communication during early development, call rate exhibited the typical inverted U-shaped developmental pattern in all genotypes. However, *Shank1-/-* pups were found to be developmentally delayed and characterized by a less prominent inverted U-shaped call emission pattern, reflecting an overall reduction in ultrasonic calling. Furthermore, testing under social conditions revealed genotype-dependent deficits even more prominently, regardless of the familiarity of the social context. As juveniles, social approach and recognition were evident irrespective of genotype. In contrast, object recognition was affected by the *Shank1* deletion, with *Shank1-/-* mice being severely impaired, not showing a preference for the novel object. In adulthood, *Shank1-/-* males and controls displayed normal social approach, but impaired social recognition. Object recognition was additionally impaired in adult *Shank1-/-* males. Conversely, adult *Shank1-/-* females exhibited deficits in social recognition only. At the neuroanatomical level, reductions in brain volume of adult *Shank1-/-* mice were detected by means of magnetic resonance imaging (MRI), consistent with findings obtained in other *Shank* mouse models and some individuals with ASD. In summary, the present findings indicate that *Shank1* deletions lead to communication deficits and an aberrant cognitive phenotype, together with age- and sex-dependent effects on social behavior.

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Poster

221. Genetic Models of Autism Spectrum Disorder

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Title: FoxP1 orchestration of ASD-relevant signaling pathways in the striatum

Authors: *D. ARAUJO[1], A. G. ANDERSON[1], S. BERTO[1], W. RUNNELS[1], M. HARPER[1], S. AMMANUEL[1], M. A. RIEGER[2], H.-C. HUANG[1,3], K. RAJKOVICH[1], K. LOERWALD[1], J. D. DEKKER[4], H. O. TUCKER[4], J. D. DOUGHERTY[2], J. R. GIBSON[1], G. KONOPKA[1];
[1]UT Southwestern Med. Ctr., Dallas, TX; [2]Washington Univ. Sch. of Med., St. Louis, MO; [3]Jackson State Univ., Jackson, MS; [4]UT Austin, Austin, TX

Abstract: Mutations in the transcription factor *FOXP1* are causative for neurodevelopmental disorders such as autism. However, the function of FOXP1 within the brain remains largely uncharacterized. Here, we identify the gene expression program regulated by FoxP1 in both human neural cells and patient-relevant heterozygous *Foxp1* (*Foxp1*$^{+/-}$) mouse brain. We demonstrate a role for Foxp1 in the transcriptional regulation of autism- and Fragile X-related pathways as well as genes involved in neuronal activity. We show that Foxp1 regulates the excitability of medium spiny neurons in the striatum and that reduction of Foxp1 correlates with defects in ultrasonic vocalizations. Finally, we demonstrate that FoxP1 has an evolutionarily conserved role in regulating pathways involved in striatal neuron identity through manipulation of FOXP1 levels followed by genome wide expression studies in human neural progenitors. These data support an integral role for FoxP1 in regulating signaling pathways vulnerable in autism and the specific regulation of striatal pathways important for vocal communication.

Disclosures: **D. Araujo:** None. **A.G. Anderson:** None. **S. Berto:** None. **W. Runnels:** None. **M. Harper:** None. **S. Ammanuel:** None. **M.A. Rieger:** None. **H. Huang:** None. **K. Rajkovich:** None. **K. Loerwald:** None. **J.D. Dekker:** None. **H.O. Tucker:** None. **J.D. Dougherty:** None. **J.R. Gibson:** None. **G. Konopka:** None.

Poster

221. Genetic Models of Autism Spectrum Disorder

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Topic: C.06. Developmental Disorders

Support: Gift Funds from Ms. Nancy Lurie Marks

Gift Funds from Fraternal Order of Eagles

Title: Neurodevelopmental mTORC1 activity predicts cell size and the effect of mutations in autism risk genes

Authors: *W.-C. HUANG, D. PAGE;
The Scripps Res. Inst., Jupiter, FL

Abstract: PTEN and FMR1 are two susceptibility genes for autism spectrum disorder (ASD) that encode regulators of the PI3K-mTOR pathway. Phosphorylation of ribosomal protein S6 (p-S6) is a downstream readout of mTOR activity. Altered levels of p-S6 have been reported in the postmortem cerebral cortex of individuals with autism and in mouse models of autism risk genes. However, it is not known when during development and in which cell types dysregulation of p-S6 signaling occurs and whether this contributes to the symptoms of ASD. Our goal is to identify common cell types and time windows in which p-S6 is dysregulated across two mouse models of autism risk genes, Pten and Fmr1, and to study the relationship between p-S6 dysregulation and social behavioral deficits. We report that in the developing brain of wild type mice, p-S6 immunoreactivity is differentially enriched in large versus small cell types during the first two postnatal weeks, and that developmental levels of p-S6 predict cell size across cortical layers. We find that p-S6 levels are particularly enriched in layer V neurons in the cerebral cortex, and levels of p-S6 are transiently elevated during early postnatal life in both Pten+/- and Fmr1-/y mice. Consistent with p-S6 being an indicator of cell growth, we find developmental overgrowth of layer V neuronal soma and dendrites in these models. Strikingly, we do not observe increased cell soma size in neurons of layers II/III or VI (which have relatively low levels of p-S6), indicating that cell types that have high level of p-S6 in the developing brain display the greatest magnitude of overgrowth in response to Pten or Fmr1 mutations. We predict that this non-uniform overgrowth will have considerable consequences on circuit assembly and synchronization, particularly given that cortical layer V neurons project to numerous subcortical

areas important for social behavior. Consistent with this, we find that selectively suppressing mTORC1 signaling in the cerebral cortex is sufficient to rescue both overgrowth of layer V neurons and social behavioral deficits in Pten+/- mice. Moreover, S6K1 inhibitor treatment during early postnatal life results in lasting rescue of these phenotypes in Pten+/- mice. Taken together, our study suggests that p-S6 enriched cells (e.g. layer V neurons) are particularly vulnerable to alterations in growth caused by mutations in ASD risk genes that regulate mTOR signaling, and that inhibition of S6K1 during early postnatal life is a therapeutic strategy relevant for individuals with ASD who have been exposed to risk factors associated with dysregulated mTOR signaling.

Disclosures: W. Huang: None. **D. Page:** None.

Poster

221. Genetic Models of Autism Spectrum Disorder

Location: Hall A

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Topic: C.06. Developmental Disorders

Support: The Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan

Title: Association of single-nucleotide polymorphisms of the CD157/BST1 gene with autism spectrum disorders in a Japanese population

Authors: *S. YOKOYAMA, N. A. MAHMUDA, T. MUNESUE, H. HIGASHIDA; Kanazawa Univ., Kanazawa, Japan

Abstract: CD157, also referred to as bone marrow stromal cell antigen-1 (BST-1), is a glycosylphosphatidylinositol-anchored protein that facilitates pre-B-lymphocyte growth. Previous studies have reported associations between single-nucleotide polymorphisms (SNPs) in the CD157/BST1 gene with Parkinson's disease. In addition, we have recently demonstrated that mice deficient in the CD157/BST1 gene exhibited anxiety-related and depression-like behaviors (Lopatina, O. et al., Front. Neurosci. 8, 133, 2014). It is unclear, however, whether SNPs of the CD157/BST1 gene is related to other brain disorders. We therefore carried out a case-control study to test polymorphisms of the CD157/BST1 gene for association with autism spectrum disorders (ASDs). DNA samples obtained from 147 ASD patients at Kanazawa University Hospital in Japan and 150 unselected Japanese volunteers were examined by the sequence-specific primer-polymerase chain reaction method combined with fluorescence correlation spectroscopy. This study was approved by the ethics committees of Kanazawa University School of Medicine. Of 121 SNPs, two SNPs exhibited significantly higher allele frequencies in the ASD cases than in the unselected controls [rs4301112, odds ratio (OR) = 6.4, 95% CI = 1.9 to

22, p = 0.0007; and rs28532698, OR = 6.2, 95% CI = 1.8 to 21, p = 0.0012; Fisher's exact test; p < 0.002 was considered significant after multiple testing correction]. In addition, CT genotype in rs10001565 was more frequently observed in the ASD group than in the control group (OR = 15, 95% CI = 2.0 to 117, p = 0.0007; Fisher's exact test). Haplotype analysis revealed that 13 cases (9.0%, n = 145) carried all the minor alleles of the three SNPs (AG/AG/CT for rs4301112-rs28532698-rs10001565), whereas only one (0.7%, n = 141) did in the control group (OR = 14.2, 95% CI = 1.4 to 110). In LD analysis of these SNPs, two haplotype blocks were identified: a 5-kb one comprising the ASD-associated rs4301112, rs28532698 and rs10001565, and a 12-kb one including the SNPs associated with Parkinson's disease. These data suggest that genetic variations of the CD157/BST1 gene might serve as a risk factor for ASDs.

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Poster

221. Genetic Models of Autism Spectrum Disorder

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Topic: C.06. Developmental Disorders

Support: Max Planck Society

DFG (Ra 380/18-1)

Title: Functional characterization of coding FOXP1 variants found in individuals with intellectual disability, autism and language impairment

Authors: *P. DERIZIOTI[1], E. SOLLIS[1], A. VINO[1], C. GILLISEN[2], H. FROHLICH[3], S. GRAHAM[1], R. PFUNDT[2], D. DIMITROPOULOU[1], H. BRUNNER[2,4], G. RAPPOLD[3], S. FISHER[1,5];
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Abstract: In recent years, next-generation DNA sequencing approaches have uncovered heterozygous variants disrupting the FOXP1 transcription factor in a number of patients with sporadic autism spectrum disorder (ASD), intellectual disability (ID), global developmental delay, and moderate to severe speech delay. The presence of speech and language deficits in this emerging FOXP1-deficiency syndrome is of particular interest because FOXP1 is the closest paralogous gene to FOXP2, which is disrupted in a rare form of speech and language disorder.

To date, FOXP1 variants include whole gene deletions, translocations, nonsense variants, missense variants and frameshift variants. The detection of whole gene deletions suggests that haploinsufficiency is the main pathogenic mechanism. Furthermore, the probands carrying FOXP1 variants are often severely affected, suggesting that these mutations are not inherited and in all cases where parental DNA has been tested the variants have been found to occur de novo. We have previously performed functional analyses on de novo frameshifting variants identified in cases of ASD/ID to assess the biological significance of these variants and gained significant insights into the molecular pathways underlying this syndrome. In this study, we report novel de novo FOXP1 variants detected using clinical whole-exome sequencing in three unrelated individuals with ASD/ID and language impairment. To understand their role in disease etiology, we performed extensive functional analyses in human cellular models, testing the effects of de novo FOXP1 variants on several aspects of protein function, including subcellular localization and transcriptional repression properties. In addition, because FOXP1 and FOXP2 directly interact with each other in several brain regions, with the potential to co-regulate downstream targets, including those involved in language development (such as CNTNAP2), we tested the effect of the FOXP1 variants on protein-protein interactions with FOXP2. We then compared the functional effects of the variants detected here to those conferred by FOXP1 variants previously detected in cases of ASD, ID and/or language impairment. Our findings support the hypothesis that de novo variants represent significant causal factors in severe sporadic disorders. Moreover, our data highlight the importance of performing functional characterisation to help uncover the biological significance of variants identified by genomics approaches, thereby providing insight into pathways underlying complex neurodevelopmental disorders.

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Poster

221. Genetic Models of Autism Spectrum Disorder

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Asan Institute for life sciences Grant 2015-624

Title: Autism phenotype and megalencephaly in zinc transporter 3 (ZnT3) null mice: Possible involvement of MMP activation and BDNF upregulation

Authors: *M. YOO, T.-Y. KIM, J.-Y. KOH;
Asan Life Sci. Res. Inst., Seoul, Korea, Republic of

Abstract: Synaptic zinc in the forebrain plays diverse roles in synaptic transmission and plasticity. For instance, it may contribute to polymerization of Shank3, a postsynaptic density protein, mutations of which gene have been linked to autism spectrum disorder (ASD). Hence, synaptic zinc in brain may play certain roles in ASD. In the present study, using ZnT3 null mice that are devoid of synaptic zinc, we examined social behaviors, brain sizes and cytoarchitectures, MMP activities, BDNF expression, and possible relationships among these. At 4-5 weeks of age, male ZnT3 null mice, but not female ones, exhibited reduced scores on 3-chamber sociability and social novelty tests as compared with wild-type control mice. In the reciprocal social interaction test, open field test, and marble-burying test, male ZnT3 null mice exhibited autistic behaviors. At 1-5 weeks of age, the size of frontoparietal cortex and the density of neurites were significantly greater in male ZnT3 null mice than in WT mice. Consistent with enhanced neurotrophic stimuli in ZnT3 null mice, levels of BDNF and TrkB in neurons and astrocytes were upregulated in male ZnT3 null mouse brains. Moreover, activities of MMP2 and MMP9 were also increased. Although it is unclear how the ZnT3 null state caused increases in MMP activities, BDNF levels, and megalencephaly in male mice, the absence of synaptic zinc or ZnT3 mRNA/protein during the critical period of brain development may contribute in a gender-dependent fashion to changes associated with ASD.

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Poster

221. Genetic Models of Autism Spectrum Disorder

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Topic: C.06. Developmental Disorders

Support: NIH Grant 1F05MH097457-01

Title: Purkinje cells-restricted expression of mutant DISC1 in anterior cerebellum produces neurobehavioral abnormalities relevant to schizophrenia and autism spectrum disorders

Authors: *A. V. SHEVELKIN[1,2], B. N. ABAZYAN[2], C. YANG[2], O. A. MYCHKO[2], G. L. RUDOW[2], J. C. TRONCOSO[2], M. V. PLETNIKOV[2];
[1]P.K.Anokhin Inst. Norm Physiol, Moscow, Russian Federation; [2]Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Disrupted-In-Schizophrenia-1(DISC1) and its variants have been associated with neurodevelopmental disorders, including schizophrenia and autism spectrum disorders (ASD). Purkinje cells (PC) express DISC1. We generated a mouse model of inducible and selective expression of mutant DISC1 in PC of anterior lobuli of the cerebellum (II-V and internal side of VI). We sought to analyze the brain and behavioral alterations in this mouse model. We evaluated volume of the cerebellum and PC in mice at postnatal (P) day 21 and 150 after assessing behavioral phenotypes in male and female mice in novelty-induced activity, elevated plus maze, Y maze, object and place recognition, fear conditioning and rotarod. Conventional western blotting and electrophysiological technics were used in the experiments. All protocols were approved by the Animal Care and Use Committee at Johns Hopkins University. We found a significant decrease in PC soma size at P21 but not at P150. Analysis of soma PC size showed presence of small and big soma PC size in the anterior cerebellum of control mice, but we observed only small soma PC size in mutant DISC1 mice at P21. Neither total number of PC nor volume of the cerebellum were significantly altered in mutant DISC1 mice. No up-regulation of cellular markers of inflammation was observed in mutant mice. Mutant male but not female mice demonstrated abnormal social interaction, hyperactivity and deficient novel object recognition. We observed no group differences in elevated plus maze, spontaneous alteration or spatial recognition in Y maze. Preliminary electrophysiological experiments found no changes in excitability and Rinput of PC in mutant DISC1 mice. Evaluation of potential synaptic alterations is in progress. Mutant DISC1 mice had comparable expression of NR1 and NR2A but significantly more expression of SNAP-25 and PSD-95 in the cerebellum but not in the cortex. Our findings indicate that mutant DISC1 affects PC morphology at P21 and produces cognitive and social abnormalities in adult mice. This may have the potential to advance our knowledge of the role of DISC1 in maturation and function of the cerebellum related to neurodevelopmental disorders.

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Poster

221. Genetic Models of Autism Spectrum Disorder

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McMorris Autism Training Program

NIMH T32NS007413 Training Program in Neurodevelopmental Disabilities

Title: Male-specific amygdala dendritic spine abnormalities in mice haploinsufficient for Protocadherin 10, an autism-associated gene

Authors: *H. SCHOCH[1], A. BANERJEE[2], S. FERRI[1], A. S. KREIBICH[2], H. DOW[2], S. HIRANO[4], R. T. SCHULTZ[3], C.-G. HAHN[2], T. ABEL[1], E. BRODKIN[2], D. FELDMEYER[5,6]; [1]Biol., Univ. of Pennsylvania, Philadelphia, PA; [2]Psychiatry, [3]Ctr. for Autism Research, Children's Hosp. of Philadelphia, Pediatrics, Perelman Sch. of Medicine, Univ. of Pennsylvania, Philadelphia, PA; [4]Dept. of Cell Biol., Kansai Med. Univ., Hirakata City, Osaka, Japan; [5]Function of Neuronal Microcircuits Group, Inst. of Neurosciences and Med. (INM-2), Res. Ctr. Jülich, Jülich, Germany; [6]Psychiatry, Psychotherapy and Psychosomatics, RWTH Aachen Univ., Aachen, Germany

Abstract: Behavioral and cognitive impairments observed in autism spectrum disorder (ASD) are thought to arise from abnormal neuronal connectivity in the developing brain, but the molecular basis of these deficits is largely unknown. Protocadherin 10 (Pcdh10) is a protocadherin superfamily neural cell adhesion molecule that has been associated with ASD in human genetic studies. Mouse Pcdh10 is expressed highly in olfactory, limbic, and striatal regions, including the basolateral amygdala, and plays an important role in activity-dependent synaptic pruning. Juvenile male mice lacking a single allele of Pcdh10 display reduced social approach behavior, but no difference in social behavior was observed in female Pcdh10+/- mice. Social approach behavior strongly activates neurons in the basolateral amygdala, a brain region that expresses high levels of Pcdh10. To determine whether abnormal neural connectivity may underlie the behavioral deficits we observed, we measured dendritic length and spine density in Golgi stained lateral and basolateral amygdala neurons from Pcdh10+/- males. We found that neurons of male but not female Pcdh10+/- mice have increased spine density, with increases specifically observed in thin elongated filopodia-type spines. In the amygdala of Pcdh10+/- male mice, we found reduced levels of NMDAR subunit NR1 in the post-synaptic density. These data suggest that abnormal regulation of synapse formation and NMDAR function in the lateral and basolateral amygdala may underlie the social approach deficits observed in male Pcdh10+/- mice.

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Poster

221. Genetic Models of Autism Spectrum Disorder

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Topic: C.06. Developmental Disorders

Support: NIH Grant 5T32MH073124-10

MIND Institute, UC Davis

Title: Evaluation of r-baclofen in novel object recognition in the Fmr1 knockout mouse model of Fragile X syndrome

Authors: *T. M. KAZDOBA[1], P. T. LEACH[2], J. N. CRAWLEY[2];
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Abstract: Fragile X syndrome (FXS) is one of the most commonly inherited forms of intellectual disability. Autism spectrum disorder (ASD) is comorbid in approximately 25% of diagnosed FXS cases. Clinically, FXS symptoms can include cognitive impairments, hyperactivity, seizures, social phobia, anxiety and repetitive behaviors. FXS is caused by a CGG repeat mutation on the X chromosome, which expands a region containing the FMR1 gene, which encodes FMRP, an RNA-binding protein that regulates protein translation. The Fmr1 knockout (KO) mouse is a well-characterized rodent model of FXS that has been used as a tool to further understand the consequences of FMRP loss, as well as evaluate putative therapeutic compounds to reverse FXS-relevant symptoms, including modulators of excitatory and inhibitory neurotransmission. Early clinical trials with STX209 (Arbaclofen), a selective GABAB receptor agonist, showed promise on measures of social avoidance and social responsiveness in FXS and autism (Berry-Kravis et al., 2012, Erickson et al., 2014). In Fmr1 KO mice, r-baclofen administration restored abnormal protein synthesis, improved dendritic spine abnormalities and reduced audiogenic seizures (Henderson et al., 2012). Here, we evaluated the efficacy of r-baclofen in Fmr1 KO male mice on the FVB genetic background in two behavioral phenotypes, novel object recognition and hyperactivity in the open field. Normal novel object recognition was confirmed in Fmr1 wildtype littermates (WT), and the absence of significant novel object recognition was confirmed in Fmr1 null mutants, after administration of a saline vehicle. Acute treatment with 1 and 3 mg/kg of r-baclofen, administered intraperitoneally 60 minutes before testing, restored novel object recognition in Fmr1 null mutants, while having no detrimental effects in WT littermates. During the initial habituation phase, neither dose of r-baclofen significantly affected locomotor activity in either Fmr1 WT or KO mice. Our findings suggest that in addition to having beneficial effects on protein translation, dendritic morphology and seizure activity, r-baclofen may improve cognitive deficits associated with FXS.

Disclosures: **T.M. Kazdoba:** A. Employment/Salary (full or part-time):; UC Davis/MIND Institute. **P.T. Leach:** A. Employment/Salary (full or part-time):; UC Davis/MIND Institute. **J.N. Crawley:** A. Employment/Salary (full or part-time):; UC Davis/MIND Institute.

Poster

221. Genetic Models of Autism Spectrum Disorder

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: C.06. Developmental Disorders

Support: Autism Speaks Grant 8703

Title: Shank3B knockout mice display unusual adult reciprocal social interactions, ultrasonic vocalizations, repetitive self-grooming, seizure activity and EEG gamma-power

Authors: *J. L. SILVERMAN[1], M. C. PRIDE[1], N. A. COPPING[1], J. E. HAYES[1], S. H. T. LAMMERS[2], S. C. DHAMNE[2], A. ROTENBERG[2], E. CHADWICK[2], D. G. SMITH[3], M. SAHIN[2], J. N. CRAWLEY[1];
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Abstract: Translocation and breakpoint mutations in *SHANK3* have been implicated in autism spectrum disorder (ASD). Mutant mouse models have been generated to evaluate the biological and behavioral consequences of *Shank3* gene mutations, including targeted mutations in the ankyrin (*Shank3A*), PDZ (*Shank3B*) or Homer binding domains within the *Shank3* gene (Bozdagi et al., 2010; Peca et al., 2011; Bangash et al., 2011; Wang et al., 2011; Kouser et al., 2013). The present experiments in *Shank3B* mice were designed to (a) evaluate replicability of published phenotypes and (b) evaluate novel behavioral and physiological phenotypes of high relevance to ASD. Social approach and repetitive behavior assays were conducted as previously described (Silverman et al., 2010, 2012). In addition, an analysis of male-female reciprocal social interactions and ultrasonic vocalizations was conducted in male subjects paired with freely moving unfamiliar estrus C57BL/6J females. By continuous wireless video telemetry, we also recorded EEG seizures, gamma oscillations and circadian rhythms in independent cohorts. Separate cohorts were also challenged with pentylenetetrazole (PTZ), a GABA-A antagonist and potent convulsant. Adult male-female social interactions were lower in *Shank3B* null mutants than wildtype littermates on several key social parameters. Fewer ultrasonic vocalizations were emitted during the male-female interaction session. Self-grooming was higher in the null mutants than in their wildtype controls, in both sexes, replicating and extending earlier findings. Relative to wildtype controls, *Shank3B* null mutant males exhibited more frequent and longer spontaneous electrographic seizures at night. In contrast, during daytime, these mice did not have seizures and had increased power in the gamma (30-80 Hz) frequency band on EEG. Upon PTZ-challenge, in daytime, the *Shank3B* null mutants showed a significantly reduced number of myoclonic seizures. These results confirm the robustness of elevated self-grooming in *Shank3B* null mutant mice, and confirm social deficits using sensitive assays, supporting the use of this line as a mouse model of autism. Moreover, our EEG and video seizure analyses point to a possible discrepancy in seizure vulnerability such that these mice are more prone to seizures at night, and yet are seizure-resistant during the day. The increase in gamma power on EEG

suggests a plausible increase in GABAergic reserve in this phenotype that may account for the seizure resistance. These EEG and behavioral abnormalities may represent quantitative, translational biological markers of pathophysiology associated with mutations in the *Shank3* gene.

Disclosures: **J.L. Silverman:** None. **M.C. Pride:** None. **N.A. Copping:** None. **J.E. Hayes:** None. **S.H.T. Lammers:** None. **S.C. Dhamne:** None. **A. Rotenberg:** None. **E. Chadwick:** None. **D.G. Smith:** None. **M. Sahin:** None. **J.N. Crawley:** None.

Poster

221. Genetic Models of Autism Spectrum Disorder

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 221.18/E37

Topic: C.06. Developmental Disorders

Support: SFARI 248429

NIH T32NS007413

McMorris Autism Training Grant

Title: 16p11.2 mouse model of autism demonstrates impaired novel object recognition with intact spatial object recognition and contextual fear conditioning

Authors: ***S. L. FERRI**[1], W. O'BRIEN[2], J. WALSH[3], N. BOWMAN[2], R. HAVEKES[1], T. ABEL[1];
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Abstract: The 16p11.2 deletion is the most common copy number variant (CNV) associated with autism spectrum disorders (ASD). Humans with ASD and some mouse models of ASD exhibit social and cognitive deficits, including impairments in object categorization and identification tasks. Using mice with a 0.39 Mb deletion on chromosome 7, a region that shares conserved synteny with the critical interval on chromosome 16 associated with ASD, we tested males and females in novel object recognition (NOR), spatial object recognition (SOR) and contextual fear conditioning (CFC) tasks. Mice with the 16p11.2 deletion exhibited a deficit in a long-term memory paradigm of novel object recognition, suggesting impaired perirhinal cortex function. However, no deficits were found in spatial object recognition or contextual fear conditioning, suggesting intact hippocampal function. Interestingly, deficits in novel object recognition are age- and sex-dependent, showing greatest impairment in young males. Thus, our findings provide insight into the neural circuitry most severely affected by 16p11.2 deletion and may serve to guide future investigations of molecular and cellular mechanisms that underlie ASD-associated cognitive deficits.

Disclosures: **S.L. Ferri:** None. **W. O'Brien:** None. **J. Walsh:** None. **N. Bowman:** None. **R. Havekes:** None. **T. Abel:** None.

Poster

221. Genetic Models of Autism Spectrum Disorder

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Topic: C.06. Developmental Disorders

Support: NIH Grant NS085709

NIH Training Grant MH073124

Title: Touchscreen visual discrimination learning deficits in the Ube3a mouse model of Angelman syndrome

Authors: *P. T. LEACH[1], T. M. KAZDOBA[2], K. SISON[2], C. M. GALL[3], G. S. LYNCH[4], J. N. CRAWLEY[2];
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Abstract: Angelman syndrome is a monogenic neurodevelopmental disorder with intellectual impairments. Angelman syndrome is caused by mutations within the maternally-derived UBE3A gene, which is a paternally imprinted region of chromosome 15. The Ube3a mouse model of Angelman syndrome, harboring the heterozygous maternal deletion, m-/p+, has been previously reported to show significant cognitive deficits in tasks including Morris water maze reversal and fear conditioning. Here we investigate cortically dependent learning in Ube3a mice and their wildtype (WT) littermates, employing a forefront touchscreen-based operant task with high face validity to human cognitive testing paradigms. We trained WT and Ube3a m-/p+ male and female mice in a pairwise visual discrimination task. Subject mice were trained with images presented randomly at the left or right locations and touches to the image location were rewarded while touches to the blank location were punished with a 20 sec timeout period. Training commenced for each mouse until individual performance was ≥ 80% correct for 2 days. Pairwise visual discrimination training trials consisted of two images, an X symbol and an = symbol, of matched illumination. Images were simultaneously presented on the front panel, randomly alternating at the left and right image locations. Mice were randomly assigned to be rewarded for pressing the X or the = image. Pressing the incorrect image led to a 20 sec timeout period. The primary dependent measure was the number of days required for each subject to reach the criterion of ≥ 80% correct for 2 days. Data were analyzed using a parametric unpaired t-test and

a nonparametric survival/completion curve analysis, as well as trials to criterion and daily % correct performance. WT completed acquisition in 14 ± 3 days, while heterozygous m-/p+ Ube3a mice completed acquisition in 27 ± 4 days. Both parametric and nonparametric statistics revealed that Ube3a subjects took significantly longer to reach criterion as compared to WTs. These results demonstrate that the Ube3a mutation results in cognitive deficits observable in an operant task that is dependent on intact cortical function. In addition, rotarod motor learning deficits were detected in Ube3a mice, providing a confirmation of consistency with previous reports of rotarod deficits in Ube3a mutant lines of mice. Our findings lend support to using touchscreen assays to evaluate cognitive phenotypes in mouse models of neurodevelopmental disorders, and for preclinical evaluation of pharmacological agents, in a task with high face validity to human cognitive testing methods.

Disclosures: P.T. Leach: None. **T.M. Kazdoba:** None. **K. Sison:** None. **C.M. Gall:** None. **G.S. Lynch:** None. **J.N. Crawley:** None.

Poster

221. Genetic Models of Autism Spectrum Disorder

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Topic: C.06. Developmental Disorders

Support: Simons Foundation Autism Research Initiative (SFARI)

National Defense Science and Engineering Graduate (NDSEG) Fellowship, 32 CFR 168a

Title: Hyperactivity and male-specific sleep deficits in the 16p11.2 deletion mouse model of autism

Authors: *C. C. ANGELAKOS[1], A. J. WATSON[2], K. S. KRAINOCK[2], W. T. O'BRIEN[1], T. ABEL[2];
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Abstract: Sleep disturbances are prevalent in several neurodevelopmental disorders, including autism spectrum disorders (ASDs) and attention deficit-hyperactivity disorder (ADHD). Evidence from genome-wide association studies indicates that chromosomal copy number variations (CNVs) are significantly associated with increased prevalence of these neurodevelopmental disorders. In particular, CNVs in chromosomal region 16p11.2 profoundly increase the risk for ASD and ADHD, disorders which are more common in males than females. We hypothesized that mice hemizygous for the 16p11.2 deletion (16p11.2 del/+) would exhibit sex-specific sleep and activity deficits. To test his hypothesis, we recorded activity patterns using infrared beam breaks in the home cages of male and female 16p11.2 del/+ and wildtype littermates. Activity was monitored under standard conditions (12:12 light-dark cycle) as well as

under continuous darkness in order to assess circadian rhythms. We found that both male and female 16p11.2 del/+ mice exhibit robust home cage hyperactivity in comparison to controls, which persisted in 24-hour darkness. In additional experiments, male and female mice were implanted with electroencephalography (EEG) and electromyography (EMG) electrodes, and sleep was assessed during a 24-hour period. 16p11.2 del/+ male, but not female mice, exhibited significantly more time awake and significantly less time in non-rapid-eye-movement (NREM) sleep during the 24-hour period. Analysis of bouts of sleep and wakefulness revealed that 16p11.2 del/+ males, but not females, showed significant changes in the distribution of wake time. Specifically, in the males a greater proportion of wake time was spent in long bouts (greater than 42 minutes in duration) compared to controls, and a significantly lower proportion of wake time was spent in shorter bouts. These changes in hyperactivity, wake time, and wake time distribution in the males are similar to the sleep disturbances observed in human ADHD and ASD patients, suggesting that the 16p11.2 del/+ mouse model may be a useful genetic model for studying human neurodevelopmental disorders.

Disclosures: C.C. Angelakos: None. **A.J. Watson:** None. **K.S. Krainock:** None. **W.T. O'Brien:** None. **T. Abel:** None.

Poster

221. Genetic Models of Autism Spectrum Disorder

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Topic: C.06. Developmental Disorders

Support: Brain and Behavior Foundation (NARSAD)

Brain Canada

NSERC

CIHR

Title: Characterization of neuronal phenotypes in the 15q13.3 microdeletion mouse model

Authors: *B. K. UNDA[1], M. UDDIN[2], S. WHITE[1], A. FORSINGDAL[3], J. NIELSEN[3], S. W. SCHERER[2], K. K. SINGH[1];
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Abstract: Copy number variations (CNVs) are chromosomal deletions or duplications that confer high risk for many neuropsychiatric conditions. The 15q13.3 CNV microdeletion has been associated with high risk for epilepsy, intellectual disability, schizophrenia and autism spectrum

disorder (ASD). However, it remains unknown what neurodevelopmental abnormalities underlie the clinical phenotypes. To study this, we are utilizing a 15q13.3 microdeletion mouse model that displays characteristic behavioural features of 15q13.3 syndrome, including epilepsy, increased stereotyped behaviour and deficits in spatial learning and memory, supporting its validity as a translational model. To better understand how these behavioural abnormalities arise, we are performing morphological analysis of different brain regions. Preliminary analysis of postnatal neuronal morphology revealed alterations in dendritic morphology in excitatory cortical pyramidal neurons. We are following up with electrophysiological experiments to determine if the morphological abnormalities lead to functional deficits in neural connectivity. To understand the pathophysiology of 15q13.3 syndrome, we are dissecting candidate disease-causing genes using patient exome and transcriptome data to determine if there are discrete mutations in specific genes within this CNV, or genes that have a higher burden of missense variants. Preliminary exome sequencing of ASD probands revealed inherited and de novo variants in specific genes within the 15q13.3 region. Future work will focus on determining whether loss of these genes can account for the observed morphological defects in the mouse model and elucidating the role of these genes and associated signaling networks in neurodevelopment.

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Poster

221. Genetic Models of Autism Spectrum Disorder

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Topic: C.06. Developmental Disorders

Support: Simons Foundation Autism Research Initiative

McMorris Program for Autism Research

NARSAD

Title: Deficits in learning operant associations, but not motivation, in CNTNAP2 and Shank3b mouse models of autism

Authors: *N. M. GRISSOM[1], S. E. MCKEE[1], L. WALSH[1], M. R. MARINI[1], T. M. REYES[1,2], T. ABEL[1];
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Abstract: Autism spectrum disorders (ASD) are characterized by disruptions in behavior including restricted interests, repetitive behaviors, and difficulties performing appropriate social

interactions. Several theories have been put forward to reconcile how these disparate symptoms might be related. The Social Motivation Hypothesis suggests that ASD symptoms are related to insufficient motivation to pursue reinforcers. In contrast, the Predictive Impairment in Autism Hypothesis suggests that ASD symptoms are related to difficulties in being able to predict the outcomes of events, including ones own actions, that might lead to reinforcement. A number of mouse models of genetic lesions associated with autism spectrum disorders (ASD) have been developed, making it possible to directly test whether loss of function at these ASD-associated genes impact the ability to form action-outcome predictions or to maintain motivation. We have recently tested knockouts of contactin-associated protein-like 2 (CNTNAP2) and Shank3b, both of which are important to synaptic function. Both mouse lines have been demonstrated to have cognitive deficits including elevated repetitive behaviors and problems with reversal learning. However, whether there are specific deficits in motivation or outcome prediction have not been tested. Male and female adult wildtypes and knockouts were tested in 9-hole mouse operant chambers for both the acquisition of a simple Fixed Ratio task, and following acquisition, for motivation using a Progressive Ratio task. CNTNAP2 null males were significantly delayed in learning the association between the operant response and reinforcement in the Fixed Ratio task, while CNTNAP2 null females were unaffected. In Shank3b null mice, both sexes were impaired in acquiring the action-outcome association in the Fixed Ratio task. However, neither genotype showed any impairment in motivation as assessed by Progressive Ratio following training in the Fixed Ratio task. Therefore, the deficits CNTNAP2-/- males and Shank3b -/- animals of both sexes displayed in learning the Fixed Ratio task are unlikely to be attributable to impairments in motivation. Instead, operant deficits in mouse models of ASD may be due to difficulty predicting reinforcement as an outcome of the operant response. Deficits in outcome prediction may be a common phenotype related to genetic lesions associated with ASD.

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Poster

221. Genetic Models of Autism Spectrum Disorder

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 221.23/E42

Topic: C.06. Developmental Disorders

Title: Characterization of prenatal zinc deficient animals regarding possible autism like behavior

Authors: *A. M. GRABRUCKER[1], G. EHRET[2], T. BOECKERS[3], S. GRABRUCKER[3]; [1]Neurocenter of Ulm Univ., Ulm, Germany; [2]Dept. of Neurobio., [3]Inst. for Anat. and Cell Biol., Univ. Ulm, Ulm, Germany

Abstract: Autism is a neurodevelopmental disorders characterized by impairments in communication and social behavior, and by repetitive behaviors. The idea that many genes implicated in Autism might converge on a single pathway present at excitatory glutamatergic synapses has recently been raised by multiple studies. For instance, our recent data provide evidence that a common synaptic pathway (Nrxn-Nlgn-Shank) found at excitatory synapses is disrupted in ASD. However, although genetic factors might be largely responsible for the occurrence of autism they cannot fully account for all cases and it is likely that in addition to a certain combination of autism-related genes, specific environmental factors might act as risk factors triggering the development of autism. On such environmental risk factor might be zinc deficiency. Zinc is one of the most prevalent metal ions in the brain and participates in processes such as neurogenesis, neuronal migration and differentiation, thereby shaping brain development and function. Here, we show the impact of maternal zinc deficiency on the behavior of the offspring later in life regarding a possible ASD phenotype. To that end, we generated a mouse model for prenatal zinc deficiency and examined the mice for their general health and neurological reflexes, muscle strength and motor coordination, juvenile play, anxiety, hyperactivity, sociability and social novelty with and without object and in male-male and female-female reciprocal social interactions, olfactory habituation, ultrasonic vocalizations, and memory and learning. Our results show that prenatal zinc deficient mice later in life display deficits in vocalization, increased aggression and slight abnormalities towards social stimuli and in social situations. Thus, given that maternal zinc deficiency alone is sufficient to alter the behavior of the adult offspring towards a more “autistic-like” behavior, it is likely that prenatal zinc deficiency together with a specific genetic predisposition might act as important risk factor for the development of Autism.

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Poster

221. Genetic Models of Autism Spectrum Disorder

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Topic: C.06. Developmental Disorders

Support: FRQS Research Scholar Grant

NSERC grant 8245

FRQS Doctoral Award

Title: Local information influences visual shape perception in autism spectrum across different developmental periods

Authors: *A. PERREAULT[1,2], C. HABAK[4], L. MOTTRON[3], F. LEPORE[5], A. BERTONE[2,6,3]; [1]Psychology Dept., Univ. De Montréal, Outremont, QC, Canada; [2]Perceptual Neurosci. Lab. for Autism and Develop. (PNLab), [3]Ctr. d'excellence en Troubles envahissants du développement de l'Université de Montréal (CETEDUM), Hôpital Rivière-des-Prairies, Montreal, QC, Canada; [4]Educational Neurosci. Res. Centre, Hlth. and Special Educ., Emirates Col. for Advanced Educ., Abu Dhabi, United Arab Emirates; [5]CERNEC, Dept. of Psychology, Univ. de Montréal, Montreal, QC, Canada; [6]Dept. of Educ. and Counselling Psychology, McGill Univ., Montreal, QC, Canada

Abstract: Background. Although studies have identified altered visual perception in autism at early and higher levels of visual analysis, much less is known about whether alterations at each level are related at different periods of development. Objectives. To assess whether the types of local information, mediated by early (local) level perception, differentially affect intermediate level (global) visual perception in autism at different periods of development. Methods. 40 autistic and 44 non-autistic participants, matched for full-scale IQ and age, were placed into school-aged (7-12 years), adolescent (13-17 years) and adult (18-27) age groups. All participants were asked to discriminate between perfect circles and RFP, whose contours (a) contained either 3, 5, or 10 bumps, and (b) were either luminance- or texture-defined. Depending on the number of bumps surrounding the contour, both local and global visual analysis can be targeted. The size (or amplitude) of the bumps was varied: the larger the amplitude, the easier it was to discriminate a RFP from a perfect circle. RFP discrimination thresholds were measured using a method of constant stimuli and a 2-ATFC procedure. Results. Separate 2 (group) X 3 (age group) X 3 (# of RF) mixed factorial ANOVAs were conducted for luminance- and texture-defined RFP conditions. For luminance-defined RFP, no group-differences were identified at any RFP condition for any of the developmental periods assessed. For texture-defined RFP, group-differences were identified for adolescent and adult groups, with decreased performance across both global (3 and 5 bumps) and local (10 bumps) RFP conditions in autism. Conclusions. The differential effect of type of local attribute (luminance vs texture) on global shape discrimination supports the hypothesis that decreased global perception in autism, when present, may have early (local) visual origins. Specifically, manipulating the complexity (from luminance to texture) of local contour elements affected global shape perception to a greater extent in autism. Based on the present results, it can be argued that alterations at early levels of analysis can in part contribute to the atypical perception of objects in autism, and this is especially evident later in development.

Disclosures: A. Perreault: None. **C. Habak:** None. **L. Mottron:** None. **F. Lepore:** None. **A. Bertone:** None.

Poster

221. Genetic Models of Autism Spectrum Disorder

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Topic: C.06. Developmental Disorders

Support: CIHR operating grant

Title: Sensory filtering and cognitive impairments in a VPA rat model of autism

Authors: A. DE SILVA[1], *S. SCHMID[2];
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Abstract: Autism is a highly prevalent neurodevelopmental disorder with approximately 70-90% of individuals displaying an atypical response to sensory stimulation. Studies have shown that patterns of abnormal sensory filtering in autistic children have been associated with poor attention, impaired academic performance and high anxiety levels. This emphasizes a correlation between sensory filtering mechanisms and cognitive function. We tested sensory filtering and related cognitive impairments in an established valproic acid (VPA) rat model of autism. 600 mg VPA (or saline) was injected in a pregnant rat at gestation day 12.5. We hypothesized that sensory filtering impairments in the offspring is correlated with disruptions in cognition. We used startle boxes to assess sensory filtering processes and locomotor boxes to measure exploration behavior, anxiety, and habituation during adolescence and adulthood. We then assessed learning and attention in these animals through the 5-choice-serial-reaction-time-task (5-CSRTT) using Bussey touch screen boxes. Preliminary results reveal a lower startle response amplitude in VPA animals of both sexes compared to control animals. VPA animals showed less short-term and long-term habituation of startle, which was more prominent in female animals. In contrast, male VPA animals showed less prepulse inhibition of startle at different prepulse levels and interstimulus intervals between prepulse and pulse. VPA animals also showed a trend to lower exploratory behavior in the locomotor boxes, and both male and female animals spent significantly less time in the centre of the boxes than their control counterparts, indicating a higher level of anxiety in VPA animals. Interestingly, testing in the 5-CSRTT showed both extremely low performers and some rare extremely high performers in the VPA group in both task learning during training and in attention testing. Systemic injections of the acetylcholine esterase inhibitor galantamine showed no effect on performance in the 5-CSRTT. We conclude that the VPA rat model is a valid model for autism spectrum disorders and that there is a correlation of sensory filtering disruption and low cognitive performance. Future studies will also involve injections of Riluzole and Ritalin which are currently used for treatment in autism spectrum disorders in clinical trials, and of an allosteric BK channel opener, in order to determine its effectiveness in enhancing attention.

Disclosures: A. De Silva: None. **S. Schmid:** None.

Poster

221. Genetic Models of Autism Spectrum Disorder

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Topic: C.06. Developmental Disorders

Support: RO1 NS048455/Nancy Lurie Marks Family Foundation and the Jane Botsford Johnson Foundation

Title: Lower anisotropy and higher diffusivity in limbic system white matter in the autism spectrum

Authors: *M. J. ALSHIKHO[1,3,4], N. SHETTY[1,3,4], S. GHOSH[1,3,4], E. M. RATAI[2,3,4], M. R. HERBERT[1,3,4];
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Abstract: Objective: To evaluate the relationship between white matter volume (WMV) and the physical patterns of diffusion tensor imaging (DTI) in an effort to better characterize white matter (WM) microstructure in autism spectrum disorders (ASDs). Background: ASDs are biologically based and behaviorally defined neurodevelopmental disorders characterized by impairments in social communication, and social interaction, as well as by restricted and repetitive patterns of behavior, interests, and activities. Children with ASD often find it hard to recognize facial expressions, interpret the emotions behind them or manage their own emotions. We hypothesized that increased white matter volume in the limbic system, a critical mediator of emotion, could be a result of abnormalities in the extracellular space and not just in the cells themselves. The neuroinflammation identified in ASDs can not only enlarge glial cell size and alter glial cell function but also increase the space outside the cells, leading structurally to increased WMV and functionally to disturbances in limbic system activity. Methods and Materials: Magnetic resonance volumetric and diffusion tensor imaging were performed on 50 males, 23 ASD (10.43y± 1.77, NVIQ 99.77±17.11), 27 typically developing (TD; 9.94y± 2.49, NVIQ 113.34±16.59). Subjects were ASD diagnosed using ADOS and clinical assessment. WMV and DTI metrics (FA, MD, AD, RD) were generated by Freesurfer. General linear model analysis was carried out to study the relationship between WMV, subject age and DTI metrics in the amygdala, hippocampus, hypothalamus, putamen and cingulate gyrus. Results: WMV was significantly different between the groups in the right and left thalamus, caudate, putamen, amygdala, accumbens-areas and the cingulate gyrus. We found positive and significant correlation between WMV and diffusivity and negative correlation between WMV and anisotropy in ASD group in the right and left amygdala, right thalamus, right and left putamen and in the right anterior cingulate. The correlation between WMV and age in the right and left thalamus, caudate, putamen and amygdala was significantly positive in the ASD group. Conclusions: The positive correlation of WMV with diffusivity and the negative correlation between WMV and anisotropy in ASD invite a more serious investigation of the possible contribution of an expansion of the fluid space between the cells to the increase in WMV. Such

microstructural changes in the limbic system would likely arise from neuroinflammation and could help mediate the social and behavioral aspects of ASD.

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Poster

221. Genetic Models of Autism Spectrum Disorder

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Topic: C.06. Developmental Disorders

Support: Telethon Grant

ERC DISEASEAVATARS

Title: Novel approaches to the reconstruction of transcriptomic and epigenomic networks underlying dysfunction in CNV-dependent neurodevelopmental disorders

Authors: *G. TESTA[1], P.-L. GERMAIN[2], A. ADAMO[2], M. ZANELLA[2], G. D'AGOSTINO[2], A. VITRIOLO[2], V. ALBERTIN[2], C. FASANO[3], S. TEMPLE[3], K. BENNETT[4];
[1]Univ. of Milan and European Inst. of Onco, Milan, Italy; [2]European Inst. of Oncology, Milan, Italy; [3]The Neural Stem Cell Inst., Rensselaer, NY; [4]Rensselaer Polytechnic Inst., Rensselaer, NY

Abstract: The critical challenge for induced Pluripotent Stem Cell (iPSC)-based disease modeling is to trace the effect of molecular dysregulations through successive layers of biological complexity to ultimately map them up onto clinical phenotypes. We recently described the thus far largest cohort of disease-specific, transgene-free iPSC, focusing on two neurodevelopmental disorders caused by symmetrical copy number variations (CNV) of 7q11.23, Williams syndrome and 7q-microduplication syndrome, that display a striking combination of shared and symmetrically opposite neurodevelopmental phenotypes (Adamo et al. *Nature Genetics* 2015). We found that already in iPSC 7q11.23 CNV disrupt transcriptional circuits across disease-relevant pathways, a dysregulation that is selectively retained upon differentiation. Importantly, we further uncovered that transcriptomic dysregulation in the pluripotent stage was enriched for genes that peak in the proneural differentiation stages or in the deep-layer formation stage of cortical development, further supporting the idea that the malleable chromatin of iPSCs offers also an experimental window of opportunity to gauge disease-relevant neurodevelopmental trajectories. We now present the dissection of transcriptomic and epigenomic profiles observed *in vitro* into distinct regulatory pathways, thereby offering mechanistic insights and targets of intervention. To this end, we introduce a novel methodology to the reconstruction of gene networks, which overcomes the key limitations of current reverse

engineering-based methods, namely the difficulty of their interpretation and their poor reproducibility, especially across small sample sizes. Our novel approach leverages the power of larger, external datasets to yield more robust networks. On the basis of several hundreds of ChIP-seq experiments, predicted and curated transcription factor binding sites, and relevant transcriptomic datasets, we assembled a cell-type-specific probabilistic model of regulator-target interaction. By calculating the shortest probabilistic path that connects differentially-expressed genes to genes of the CNVs underlying the diseases under study, we obtained robust and highly actionable networks that revealed important dysregulations that had escaped differential expression analysis and offer compelling mechanistic hypotheses explaining the transcriptional effect of these CNVs, with far-reaching implications for the field of iPSC-based neurodevelopmental disease modeling.

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Poster

222. Therapeutic Strategies for Remyelination

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Topic: C.09. Demyelinating Disorders

Support: Department of Defense Multiple Sclerosis Concept Award MS100350

Title: Neuregulin1 modulation of experimental autoimmune encephalomyelitis

Authors: ***F. SONG**[1], H. DEOL[1], J. A. LOEB[1], E. ALLENDER[1], E. H. SIMPSON[2]; [1]Dept. of Neurol. and Rehabil., Univ. of Illinois at Chicago, Chicago, IL; [2]Columbia Univ., New York, NY

Abstract: Background: The mechanisms that modulate disease severity and progression in multiple sclerosis (MS) are poorly understood. One potential mediator is neuregulin1 (NRG1) which is a membrane bound and secreted growth and differentiation factor that regulates glial development, survival, synaptogenesis, axoglial interactions, and microglial activation. We developed a targeted NRG1 antagonist called HBD-S-H4 that given intrathecally reduces microglial activation in a rat spinal cord pain model, suggesting that it may have similar effects in other diseases. Here we test the hypothesis that blocking NRG1 in the central nervous system (CNS) could be a therapeutic approach to reduce neuroinflammation and demyelination in MS. Objective: To determine the effects of a NRG1 antagonist (HBD-S-H4) on myelin oligodendrocyte glycoprotein (MOG)-induced experimental autoimmune encephalomyelitis (EAE). Design/Methods: HBD-S-H4 is a novel humanized fusion protein consisting of a decoy

Her4 receptor fused to NRG1's heparin-binding, targeting domain. MOG-induced EAE was induced in transgenic mice that were developed to express this fusion protein in the CNS, and compared to control mice. More and less potent EAE disease induction models were compared as well as gender-specific differences. Results: Transgenic expression of HBD-S-H4 in the CNS did not result in any significant neurological or other overt phenotypes. With induction of less potent EAE disease, female HBD-S-H4 mice were less severely affected compared to their male HBD-S-H4 littermates. However, with more potent disease induction, female HBD-S-H4 mice were affected more than the males of the same genotype, suggesting complex interaction between NRG1 signaling and gender. Measurements of the gene and cellular pathology of microglia in male and female HBD-S-H4 Tg mice with EAE are currently underway. Conclusions: Blocking NRG1 is protective in female mice in mild disease, but this effect was not seen in more severe disease, suggesting a complex interplay between NRG1 and sex hormones in EAE that is dependent on disease severity. One possible explanation for this may be from combined effects of NRG1 and estrogens on microglia. Future studies of gender and hormonal influences on NRG1 signaling in microglia will be important to understand the heterogeneity of disease pathology and the therapeutic potential of targeting microglial activation in human MS in men and women.

Disclosures: F. Song: None. **H. Deol:** None. **J.A. Loeb:** None. **E. Allender:** None. **E.H. Simpson:** None.

Poster

222. Therapeutic Strategies for Remyelination

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Capital Medical Development Special Program (No. 2011-1001-06)

Title: Epimedium flavonoids ameliorate neuroinflammatory and neuropathological changes in rodents

Authors: *L. YIN[1], Z. QU[3], M. LIANG[3], L. ZHANG[2], L. LI[2];
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Abstract: The present study was designed to determine whether epimedium flavonoids (EF) had effect on the development of experimental autoimmune encephalomyelitis (EAE) in rats and cuprizone (CPZ)-induced neuropathological changes in the corpus callosum of C57BL/6 mice. EAE was induced by immunization of adult female Lewis rats with partially purified myelin basic protein (MBP) prepared from guinea-pig spinal cord homogenate. EF was administrated intragastrically once a day after immunization until day 14 post immunization (p.i.). Administration of EF (20 and 60 mg/kg) significantly reduced clinical score of neurological deficit in EAE rats; alleviated demyelination and inflammatory infiltration; and inhibited astrocytes activation, production of proinflammatory molecules such as interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α), nitric oxide (NO) and nuclear transcription factor (NF-κB) in the spinal cord of EAE rats. Treatment with EF also enhanced the expression of 2', 3'-cyclic nucleotide 3'-phosphohydrolase (CNPase) and nerve growth factor (NGF), increased the number of oligodendrocytes and protected the ultrastructure of myelin sheaths and axons in the spinal cord of EAE rats. To induce demyelination, 8 week old mice were fed with 0.2% CPZ for a maximum period of 6 weeks. EF treatment for a period of 3 weeks effectively decreased the breakdown of myelin, OL loss, and oligodendrocyte precursor cell (OPC) accumulation in CPZ-fed mice. In addition, EF administration significantly increased the cortical expression level of insulin-like growth factor 1 (IGF-1). Our results showed that EF inhibited the development of partial MBP-induced EAE in rats and protected against CPZ-induced neuropathological changes. These effects involved reducing neuroinflammation and enhancing myelination and neurotrophins and our findings suggest that EF may be useful for the treatment of multiple sclerosis.

Disclosures: **L. Yin:** None. **Z. Qu:** None. **M. Liang:** None. **L. Zhang:** None. **L. Li:** None.

Poster

222. Therapeutic Strategies for Remyelination

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HD 5PO1 HD 09402

University of Chicago Innovation Fund

Title: Phasic treatment with IFNγ stimulates release of CNS exosomes that protect against spreading depression

Authors: *A. D. PUSIC, K. M. PUSIC, R. P. KRAIG;
Univ. of Chicago, Chicago, IL

Abstract: Migraine is a common headache disorder characterized by unilateral, intense headaches that are preceded by an aura in one-third of patients. Spreading depression (SD), the most likely cause of migraine aura and perhaps migraine pain, is a self-propagating wave of transient depolarization accompanied by a transient negative shift of the interstitial direct current potential.1,2 In prior work, we found that SD elevates IFNγ production, and increases production of pro-inflammatory cytokine and reactive oxygen species production in the CNS. Conditions of inflammation have deleterious effects on myelin integrity, including decompaction of myelin sheaths. This decompaction of myelin is relevant to SD, wherein increased production of reactive oxygen species and inflammatory cytokines causes transient demyelination that increases susceptibility to subsequent SD perhaps via ephaptic transmission.3 Though existing therapies offer only modest benefits, environmental enrichment (EE; volitionally increased physical, social, and intellectual activity) has been clinically shown to reduce migraine frequency and SD.4 This effect may involve adaptive responses initiated by phasic production of IFNγ during EE. Accordingly, we focused on the potential for physiological levels of IFNγ to protect against SD. In contrast to the detrimental effects of elevated IFNγ from SD3, IFNγ applied phasically or as a 12-hour pulse (equivalent to a single cycle of phasic treatment) to mimic EE produced opposite effects - myelin basic protein (MBP) significantly increased, SD threshold significantly increased, and OS was significantly reduced. IFNγ-stimulated slice cultures produced exosomes that conferred adaptive changes comparable to those evoked by phasic or pulsed IFNγ treatment. Furthermore, we found that exosomes produced by IFNγ-stimulated primary microglia produced the same effect, suggesting that these cells are the source of nutritive exosomes harvested from intact slice cultures. microRNA profiling of exosomal contents revealed the presence of a number of miRNA species involved in myelination and anti-inflammatory pathways, that may account for these effects. Thus, our results support further research on exosomes produced by IFNγ-stimulated immune cells (including microglia) as a possible therapeutic target for prevention of SD, and by extension, migraine.

Disclosures: **A.D. Pusic:** None. **K.M. Pusic:** None. **R.P. Kraig:** None.

Poster

222. Therapeutic Strategies for Remyelination

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Support: Novartis

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Title: Siponimod - direct CNS effects of a 2nd generation immunomodulatory drug?

Authors: *P. EHLING, M. CERINA, V. NARAYANAN, A. M. HERRMANN, S. PANKRATZ, S. BOCK, K. GOEBEL, S. G. MEUTH;
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Abstract: Introduction: Siponimod (BAF312) is a selective sphingosine 1-phosphate1,5 receptor modulator currently under clinical development for the treatment of secondary progressive multiple sclerosis. Its precursor drug fingolimod is approved for the treatment of relapsing-remitting multiple sclerosis, an autoimmune, neurodegenerative disease. Both drugs are shown to inhibit peripheral lymphocyte egress thereby reducing pathological immune cell invasion into the brain. However, non-immunological central nervous system (CNS) effects of S1PR modulatory drugs are still a matter of debate. Here, we test the hypothesis that beside its immunomodulatory effect siponimod exerts a direct effect in the CNS. Methods: We stereotactically induce lesions targeted to either white or grey matter regions by injection of proinflammatory cytokines in mice that were immunized with myelin oligodendrocyte glycoprotein 10 days earlier (focal experimental autoimmune encephalomyelitis, EAE). To allow distinction between peripheral and central drug effects siponimod was administered either systemically (3 mg/kg) or directly to the brain lesion site via implantable osmotic pumps and brain infusion cannulae (1 µg/day). Behavioural, histological, flow cytometric analyses were performed both 2 and 5 days after focal EAE induction. Results: Siponimod induced lymphopenia and attenuated EAE symptoms in systemically treated mice. Flow cytometry revealed reduced immune cell infiltrates at both grey and white matter lesions compared to sham-treated controls. After systemic siponimod treatment locomotor activity was slightly increased in the open field arena 2 days and 5 days after focal EAE induction. In the rotarod setup, treated mice with white matter lesions revealed less locomotor deficits 5 days after focal EAE induction. In first experiments with direct drug application to the brain lesion via osmotic pumps, lymphocyte counts in peripheral blood and lymph nodes were indifferent between treated and non-treated mice. Conclusion: Systemic siponimod ameliorates the EAE disease course by inhibiting lymphocyte egress from lymphoid organs. However, drug application directly to the brain lesion site does not affect peripheral immune cell counts. Investigations concerning central drug effects are still ongoing work.

Disclosures: **P. Ehling:** 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.; Novartis. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Novartis. F. Consulting Fees (e.g., advisory boards); Novartis. **M. Cerina:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and

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Poster

222. Therapeutic Strategies for Remyelination

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Support: Irish Research Council Grant GOIPG/2013/921

Muscular Dystrophy Ireland

University College Dublin

Title: Evaluation of nefiracetam as a regenerative therapeutic in an animal model of demyelinating Charcot Marie Tooth disease

Authors: *L. ALVEY[1], R. P. MURPHY[2], K. J. MURPHY[2], K. H. KEANE[1], J. F. X. JONES[1], M. PICKERING[1];
[1]Sch. of Med. and Med. Science, Univ. Col. Dublin, Belfield, Ireland; [2]Sch. of Biomolecular and Biomed. Science, Univ. Col. Dublin, Belfield, Ireland

Abstract: Demyelinating Charcot Marie Tooth disease (CMT) is an inherited progressive peripheral neuropathy characterised by degeneration of the myelin sheath that surrounds the axons of peripheral nerves, and to date there are no pharmacological interventions to treat them. The trembler-J mouse is used as a model for investigating peripheral nerve demyelination. As previous studies indicated that nefiracetam was capable of accelerating remyelination after injury in the central nervous system, we set out to evaluate the efficacy of the drug to induce repair in a model of peripheral demyelination. Adult trembler-J mice were treated with nefiracetam (30mg/kg ip) for 30 days, and motor function was assessed with rotarod testing. While no differences in motor phenotype were detected between control and treatment groups, we examined nerve structural pathology for evidence of any impact of nefiracetam treatment. Myelin thickness and fibre calibre were significantly reduced in trembler-J mice relative to wild type, and no difference was seen between nefiracetam and vehicle treated animals. We have recently described previously unreported feature of nerve pathology in these animals (Power et al, Muscle & Nerve 51: pg246-522, 2015), where axon length relative to nerve length is increased in the trembler-J mouse. This pathology was also unchanged by nefiracetam treatment. Nodal density was also assessed as an indicator of intermodal distance. While the trembler-J mice exhibited a higher nodal density (suggesting shorter intermodal distance) as expected, nefiracetam treatment significantly reduced this to the same level as seen in the wild type nerves. While the drug appeared to be ineffective on the disease phenotype, we noted a loss of large calibre fibres in the trembler-J mice, suggesting a loss of motor neurons, which would imply our intervention at the late stage of disease was inappropriate. Because there seemed to be a normalisation of internodal distance, we then investigated whether there was evidence for a direct effect of the drug on Schwann cells. Using a novel aligned dorsal root ganglion explant culture system, we found treatment with 1 μM nefiracetam significantly increased Schwann cell density and migration away from the ganglion, and also increased axon growth at the same time. We conclude that, while nefiracetam is unlikely to be an effective intervention in advanced CMT, we cannot rule out that it may act directly on Schwann cells and be an effective therapeutic for earlier intervention.

Disclosures: L. Alvey: None. **R.P. Murphy:** None. **K.J. Murphy:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Holder of patent relating to compound investigated in this study. **K.H. Keane:** None. **J.F.X. Jones:** None. **M. Pickering:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Holder of patent relating to compound investigated in this study.

Poster

222. Therapeutic Strategies for Remyelination

Location: Hall A

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Program#/Poster#: 222.06/F4

Topic: C.09. Demyelinating Disorders

Title: Amantadine ameliorates gait deficits and disease severity in an animal model of multiple sclerosis

Authors: J. NGUYEN[1], *B. BRIGHAM[1], J. OKSMAN[2], T. AHTONIEMI[2], B. SAVA[3], B. BUISSON[3];
[1]Adamas Pharmaceuticals, Emeryville, CA; [2]Charles River Discovery Res. Services, Kuopio, Finland; [3]Neuroservice, Aix en Provence, France

Abstract: Multiple Sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS). MS is characterized by the impairment of axonal transmission and the reduction in nerve conduction velocity, resulting in neurological and motor deficits. Dalfampridine is approved as a treatment to improve walking in MS. It is thought that dalfampridine improves walking in MS patients by blocking potassium channels, thereby improving nerve impulses across regions of demyelination in axons. In a mouse model of MS, dalfampridine has been shown to ameliorate gait deficits but did not alter the disease course (Gobel et al 2013). Amantadine HCl is an NMDA receptor (NMDAr) antagonist approved for the treatment of influenza infection and Parkinson's disease. In addition to its glutaminergic activity, amantadine has also been shown to modulate other neurotransmitter systems, inhibit microglial activation, and elevate levels of brain-derived neurotrophic factor (BDNF). Amantadine has been shown in clinical trials to reduce fatigue in MS, but its effects on gait and walking have not been evaluated. Here, we characterize the *in vitro* effects of amantadine on potassium channel activity and the efficacy of amantadine in a mouse model of MS. First, we compared the effects of amantadine to dalfampridine on the blockade of potassium currents in rat coronal brain slices. Both amantadine and dalfampridine blocked potassium leak currents and delayed rectifying currents in a concentration-dependent fashion, but potassium current blockade occurred at 10-100 fold lower concentration with amantadine compared to dalfampridine. Next, we assessed the effects of amantadine in experimental autoimmune encephalomyelitis (EAE), a mouse chronic model of MS. EAE was induced in female C57BL/6 mice by subcutaneous injection of myelin oligodendrocyte glycoprotein (MOG35-55), and disease severity and gait parameters were measured over 28 days. We found that chronic administration of clinically relevant doses of amantadine reduced the severity of clinical disease, improved gross motor function and improved walking following EAE in mice. Together, the data suggest that amantadine may have clinical utility as treatment in MS, and provide a framework for further clinical evaluation. References: Gobel et al. (2013) 4-Aminopyridine ameliorates mobility but not disease course in an animal model of multiple sclerosis, Experimental Neurology, 248: 62.

Disclosures: **J. Nguyen:** A. Employment/Salary (full or part-time):; Adamas Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Adamas Pharmaceuticals. **B. Brigham:** A. Employment/Salary (full or part-time):; Adamas Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Adamas Pharmaceuticals. **J. Oksman:** None. **T. Ahtoniemi:** None. **B. Sava:** None. **B. Buisson:** None.

Poster

222. Therapeutic Strategies for Remyelination

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Topic: C.09. Demyelinating Disorders

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HD 09402

University of Chicago Innovation Fund

Title: Dendritic cell-derived exosomes containing miR-219 are effective after nasal administration to rat brain - implications for treatment of multiple sclerosis and migraine

Authors: ***J. SCHUMER**, A. D. PUSIC, R. P. KRAIG;
Neurobio., Univ. of Chicago, Chicago, IL

Abstract: Environmental enrichment (EE) consists of increased intellectual, physical and social activity, and is known to reduce brain disease. In previous work, we have shown that exosomes derived from the serum of EE animals increased myelination and reduced oxidative stress (OS). These effects are, in part, due to increased exosomal miR-219, which has been shown to be necessary and sufficient to promote differentiation of oligodendrocyte precursors into myelinating cells.1 We have also shown that the effects of EE can be mimicked ex-vivo by IFNγ stimulated dendritic cell-derived exosomes (IFNγ-DC-Exos). IFNγ-DC-Exos increase remyelination and reduces OS in hippocampal brain slice cultures.2 This led us to propose IFNγ-DC-Exos as a novel therapeutic for demyelinating diseases, which include multiple sclerosis and migraine [modeled here by spreading depression (SD)]. We are exploring the efficacy of nasally delivered1,2,6 IFNγ-DC-Exos on *in vivo* models of MS (using lysolecithin injections into corpus callosum) and migraine (using SD threshold, SDT). Nasal delivery of IFNγ-DC-Exos followed by lysolecithin injection significantly ($p < 0.019$; $n = 3$/group) improved recovery from

demyelination three days later (lysolecithin alone: 1.00 ± 0.14; lysolecithin+ IFNγ-DC-Exos: 0.4 ± 0.07). Also, nasal treatment IFNγ-DC-Exos significantly ($p < 0.001$) increased SDT [sham: 1.00 ± 0.14; unstim-DC-Exo: 1.10 ± 0.28; IFNγ-DC-Exos: 57.0 ± 4.27 (n = 3-5/group)] a day later. The nutritive exosomes were equivalently effective ($p < 0.001$) in brain slice cultures (control: 1.00 ± 0.45; IFNγ-DC-Exos: 12.5 ± 1.52; n = 8/group). One day after nasal treatment, CNS protein carbonylation (as an indicator of OS) was significantly ($p = 0.035$) reduced (sham: 7.25 ± 0.83, n = 7; IFNγ-DC-Exos: 4.58 ± 0.55, n = 5). This led us to ask if miR-219 plays a role in OS reduction. Transfection of exosomes with a miR-219 inhibitor significantly ($p < 0.0001$) abrogated the reduction in OS seen with IFNγ-DC-Exo-treated slice cultures. As a negative control, exosomes were transfected with a non-targeting miRNA inhibitor, which had no effect (control: 1.00 ± 0.1; IFNγ-DC-Exos: 0.28 ± 0.002; +inhibitor: 1.06 ± 0.17; +negative control: 0.13 ± 0.02; n = 9/group). Thus, we have shown that nasal administration sucessfully delivered IFNγ-DC-Exos to the CNS, and had a functional effect on both myelin content and oxidative status. Furthermore, we have identified miR-219 as an important mediator of these effects. [1] Kraig & Pusic, 2014 [2] Pusic et al., 2014 [3] Pusic et al., 2015 [4] Grinberg et al., 2012 [5] Grinberg et al., 2013 [6] Pusic et al., 2014

Disclosures: J. Schumer: None. **A.D. Pusic:** None. **R.P. Kraig:** None.

Poster

222. Therapeutic Strategies for Remyelination

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Topic: C.09. Demyelinating Disorders

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NMSS CA10441A1

Title: Combinatorial treatment strategies attenuate EAE

Authors: *K. P. KOENIG, J. C. NISSEN, S. E. TSIRKA;
Mol. and Cell. Pharmacol., Stony Brook Univ. Med. Sch., Stony Brook, NY

Abstract: Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system characterized by inflammation and neurodegenerative processes. Currently, MS therapy involves the long-term use of immunomodulators, which tend to have numerous side effects and varying efficacies among MS patients. Additionally, these immunomodulators do not aid in promoting remyelination, which is essential to the return of neuronal function. Benztropine, a FDA-approved drug used for treatment of Parkinson's disease, was recently identified as an effective inducer of oligodendrocyte precursor cell differentiation *in vitro* and in experimental autoimmune encephalomyelitis (EAE), the most common animal model used to study MS. Thus,

we sought to study the effects of a combinatorial therapy of benztropine and tuftsin, an immunomodulatory tetrapeptide shown to polarize microglia to the M2 phenotype, on the MOG-induced EAE disease course, demyelination, and microglial infiltration and polarization. Here we show that combinatorial treatment with both benztropine and tuftsin seem to markedly ameliorate the EAE disease course. Further, this treatment strategy reduces the pathological hallmarks of MS as well, as these animals have reduced demyelination and decreased microglial activation, with an overall anti-inflammatory immune phenotype.

Disclosures: **K.P. Koenig:** None. **J.C. Nissen:** None. **S.E. Tsirka:** None.

Poster

222. Therapeutic Strategies for Remyelination

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Topic: C.09. Demyelinating Disorders

Support: UND SMHS Seed Grant

Title: Triacetin therapy alters spinal cord lipid metabolism in mice subjected to experimental autoimmune encephalomyelitis (EAE)

Authors: *A. C. CHEVALIER, T. A. ROSENBERGER;
Univ. of North Dakota, Grand Forks, ND

Abstract: Triacetin therapy increases brain acetyl-CoA metabolism, increases phosphocreatine levels, alters the expression of adenosinergic enzymes and receptors, increases histone and non-histone protein acetylation, and inhibits neuroglia activation. However, it is unknown if triacetin therapy alters lipid metabolism within the central nervous system. To begin to address this question, we quantified the effect treatment had on spinal cord lipid content and phospholipase levels in mice subjected to experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis. EAE was induced with myelin oligodendrocyte glycoprotein peptide (MOG 35-55, 50μg per mouse), and prophylactic triacetin therapy was maintained for 40 days (glycerol triacetate, 4g/kg via oral gavage daily). We found that treatment attenuated the onset of clinical symptoms in EAE mice treated with GTA compared to control-treated mice. Further, triacetin therapy prevented the loss of ethanolamine and choline glycerophospholipid, phosphatidylserine, esterified fatty acids, and cholesterol in these mice. Triacetin therapy did not alter cholesteryl esters, but EAE injury did result in a significant decrease in these molecules within the spinal cord. With regard to enzymes involved in the metabolism of membrane phospholipids, treatment increased phosphorylated cytosolic phospholipase A2 (cPLA2) and non-phosphorylated cPLA2 levels but did not alter the levels of phospholipase C (PLC) β and PLC δ. Both phosphorylated and non-phosphorylated cPLA2 levels increased significantly with injury but were returned to

baseline levels following treatment. In addition, β-actin was significantly increased in mice subjected to EAE compared to control animals suggesting that EAE disrupts actin polymerization and/or metabolism. In conclusion, these data suggests that triacetin therapy may alter spinal cord lipid metabolism in mice subjected to EAE.

Disclosures: A.C. Chevalier: None. **T.A. Rosenberger:** None.

Poster

222. Therapeutic Strategies for Remyelination

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HD 5PO1 HD 09402

University of Chicago Innovation Fund

Title: Environmental enrichment stimulates immune cell secretion of exosomes that promote CNS myelination and reduce inflammation

Authors: *K. M. PUSIC, A. D. PUSIC, R. P. KRAIG;
Dept. of Neurol., Univ. of Chicago, Chicago, IL

Abstract: Grey matter demyelination is an important component of multiple sclerosis (MS) pathogenesis, particularly in the secondary progressive disease phase. Extent of damage is strongly correlated with neurological dysfunction. Aging likewise occurs with cognitive decline from myelin loss, and age-associated failure to remyelinate significantly contributes to MS progression. In prior work, we show that serum exosomes from young animals can increase oligodendrocyte precursor cell (OPC) differentiation into mature myelinating oligodendrocytes - both under normal conditions and after acute demyelination.1 Environmental enrichment (EE) of aging animals produced exosomes that mimicked this effect. We found that both young and EE serum-derived exosomes were enriched in miR-219, which is necessary and sufficient for OPC differentiation.2 Exosomes found in the blood can originate from a multitude of sources, and it is precisely this attribute that makes them ideal candidates as biomarkers of disease. For example, in a variety of solid organ cancers, tumor-derived exosomes can be isolated from blood.3 However, based on work showing that the properties of serum-derived EE exosomes could be reproduced ex-vivo using primary dendritic cell cultures4, we focused on immune cells. Given

the broad beneficial effects of EE on immune function5, it is likely the pro-myelinating effect is not due to a specific cell type, but a common property of many immune cells. In support of this, we found that exosomes produced by all EE immune cell types significantly increased MBP in slice cultures, whereas those from immune cells of non-enriched animals had no effect. This suggests that EE globally alters immune function in a way that supports brain health. However, circulating blood cell exosomes produced a more robust effect than those derived from cells of the spleen and lymph nodes. miRNA expression profiling showed that all circulating blood cell exosomes examined contained miR-219, but it is interesting to note other miRNA species present at high levels in a subset of EE exosome types: miR-9 and miR-17, which are involved in OPC proliferation; miR181a, which has an anti-inflammatory function; and miR-665, which may affect brain immune function in a manner that dysregulates neuronal excitability. Thus, we have begun to explore the impact of these exosome on oxidative status and resolution of inflammation. This may help us determine the role of additional enriched miRNAs; information that could be useful in engineering more effective and specific exosomes as therapeutics against brain demyelination and oxidative stress.

Disclosures: **K.M. Pusic:** None. **A.D. Pusic:** None. **R.P. Kraig:** None.

Poster

222. Therapeutic Strategies for Remyelination

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The Miami Project To Cure Paralysis (RB, IH)

The State of Florida (IH)

Title: Prolonged electrical stimulation of a hindbrain raphe region attenuates experimental autoimmune encephalomyelitis in mice

Authors: S. S. SLOLEY[1], P. M. MADSEN[1,2], M. M. CARBALLOSA[1], A. VITORES[1], R. BRAMBILLA[1], *I. D. HENTALL[1];
[1]Miami Project Cure Paral, Univ. Miami Schl Med., Miami, FL; [2]Dept. of Neurobio. Res., Inst. of Mol. Med., Odense, Denmark

Abstract: Interactions between the nervous and immune systems are critical in various chronic neurological diseases, such as multiple sclerosis (MS). MS is an immune-mediated inflammatory disease of the central nervous system characterized by widespread demyelination and axonal loss

in the brain and spinal cord, which ultimately account for disease severity and irreversible disability. Strategies aimed at reducing inflammation and promoting remyelination are the two most promising interventions to improve function and slow disease progression in MS therapy. Since electrical stimulation targeting a raphe nucleus has proved efficacious in promoting myelin sparing in models of CNS trauma, we explored whether this treatment may also be beneficial in experimental autoimmune encephalomyelitis (EAE), a model of MS. EAE was induced in mice by injection of MOG35-55 peptide. Eight days after EAE symptoms emerged (mean onset 17 days after induction), a self-powered encapsulated stimulator, controlled and read wirelessly, was implanted on the mouse's skull, and an integral microelectrode stereotaxically positioned in the hindbrain's nucleus raphe magnus (NRM). Stimulation (-30 μA, 1 ms pulses, 8 Hz) was given during 12 daylight hours, alternating 5 minutes on and off, over 5-29 days. Controls had inactive stimulators. EAE severity, scored daily on a 0 to 6 scale by a blinded observer, was reduced by about 0.5 points in stimulated mice compared to controls, starting around 20 days after EAE onset. The number of days of stimulation was linearly correlated with later EAE improvement. Mice were sacrificed and histological analyses performed 4-5 weeks after EAE onset. The numbers of degenerated collapsed axons and infiltrating immune cells in the thoracic spinal cord were significantly reduced in stimulated mice, while myelinated axons showed a trend toward increase. In conclusion, NRM activity improves EAE clinical symptoms and reduces axonal pathology in spinal cord, suggesting it may have neuroprotective effects in neuroinflammation. Ascending midbrain raphe nuclei may have similar effects, given the disseminated nature of MS. Hence activation of neurons in raphe areas or their input regions, such as by deep brain stimulation (DBS) or drugs, could offer a potential MS therapy.

Disclosures: S.S. Sloley: None. **P.M. Madsen:** None. **M.M. Carballosa:** None. **A. Vitores:** None. **R. Brambilla:** None. **I.D. Hentall:** None.

Poster

222. Therapeutic Strategies for Remyelination

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 222.12/F10

Topic: C.09. Demyelinating Disorders

Support: NIH Grant D43TW008333

TTUHSC Seed Grant, 2

Title: FTY720 (Gilenya) stimulates neurotrophic factor expression in oligodendroglial cells

Authors: I. SEGURA, *R. G. PEREZ;
Biomed. Sci., Texas Tech. Univ. HSC El Paso, El Paso, TX

Abstract: Multiple system atrophy (MSA) is a rare and rapidly progressing demyelinating neurodegenerative disorder with no effective treatments. Most MSA patients progress from diagnosis to death in 10 years. Postmortem analyses of MSA brains reveal accumulation of the protein α-synuclein (aSyn) in glial cytoplasmic inclusions (GCI) of oligodendroglia cells (OLGs), the myelinating cells of the brain. MSA brains show reduced levels of glial cell line derived neurotrophic factor (GDNF) and brain derived neurotrophic factor (BDNF). Supplementation of MSA cell and mouse models with these factors decreases MSA-related dysfunction. Nerve growth factor (NGF) is another protective molecule for OLGs and neurons. It protects against neuroinflammation, promotes axon extension and neuronal survival, induces differentiation of oligodendrocyte precursor cells (OPCs) and stimulates myelination by OLGs. Hence, therapies that increase expression of NGF, GDNF or BDNF should be beneficial for MSA. Research shows that histone deacetylase (HDAC) inhibitors increase the levels of acetylated histone 3 (AcH3) and acetylated histone 4 (AcH4), which up-regulates GDNF, BDNF and/or NGF expression in neuronal and/or glial cells. FTY720 (Gilenya), an FDA-approved oral drug for multiple sclerosis, stimulates BDNF and GDNF expression in neurons and microglia. Recently, phosphorylated FTY720 (FTY720-P) was shown to inhibit HDACs; however, HDAC inhibition and effects on neurotrophic factor expression are mostly unexplored and have never been assessed in OLGs. To assess this we treated an OLG cell line, OLN-93, with FTY720 or vehicle for 6 - 24 hr, then measured levels of AcH3 and AcH4 using immunoblotting. We also measured NGF, GDNF and BDNF expression by quantitative real time PCR. FTY720 treatment caused a significant increase in the levels of AcH3 and AcH4 by 6 hr, but by 24 hr only AcH3 was significantly increased. OLN-93 NGF expression was significantly increased at 6 and 24 hr after FTY720, and neither GDNF nor BDNF were significantly changed in OLGs. This increased NGF expression in OLGs is another protective mechanism elicited by FTY720 that may prevent demyelination and associated neurodegeneration in MSA. Furthermore, FTY720-induced changes in NGF expression seem to mirror those of global histone acetylation. Further exploring HDAC inhibition by FTY720 and the role of aSyn in MSA will help determine if changes in histone acetylation are responsible for altered expression of neurotrophic factors in MSA.

Disclosures: I. Segura: None. **R.G. Perez:** None.

Poster

222. Therapeutic Strategies for Remyelination

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Program#/Poster#: 222.13/F11

Topic: C.09. Demyelinating Disorders

Support: AUB medical practice plan

Lebanese National Center for Scientific Research

Title: Effect of Vitamin D replacement on cognition in Multiple Sclerosis patients

Authors: *H. J. DARWISH, R. HADDAD, S. OSMAN, S. GHASSAN, B. YAMOUT, S. KHOURY;
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Abstract: Multiple Sclerosis (MS) is a chronic inflammatory disease of the central nervous system with genetic and environmental risk factors including low serum vitamin D. Recent studies correlated low serum 25(OH)D levels with cognitive dysfunction in adults. In this study we evaluated the change in cognitive function after vitamin D supplementation in MS patients with low serum 25(OH)D (<25μg/ml) compared to subjects with normal 25(OH)D levels (serum level >35 μg/ml). A total of 113 adult MS patients with relapsing-remitting disease stable on interferon-beta therapy were recruited. Demographic and health behavior information were collected, depression and anxiety were assessed using the Arabic- Hopkins Symptoms Checklist (HSCL-25), cognitive performance was measured, at baseline and 3 months after vitamin D supplementation (10,000 IU daily for 3 months or 50,000 IU weekly for 3 months), using the Arabic-Montreal Cognitive Assessment (MoCA), Stroop Test, Symbol Digit Modalities Test (SDMT) and Brief Visual Memory Test delayed recall (BVMT-DR). Of the recruited subjects, 89 were found eligible with low (<25, n=41) or normal (>35, n=48) 25(OH)D . After 3 months, anxiety scores decreased significantly in those with low baseline 25(OH)D and showed a significant improvement on the BVMT immediate (10 and 30 sec), DR (20 min) and MoCA. Yet, those with normal 25(OH)D level improved on Stroop test and BVMT (10 and 20 sec). At baseline, the low 25(OH)D group scored less on all cognitive tests except Stroop; this difference was significant for SDMT and BVMT-DR. Serum 25(OH)D level correlated positively and significantly with BVMT-DR. Exercise was positively associated with cognitive performance in all tests except the Stroop[BVMT, 10 sec r=. 61, 20 sec r= .64, 30 sec, r=.70, DR, r= .54, SDMT, r= .56, MoCA= .46; $p < 0.05$], and correlated strongly with cognitive performance in those with low 25(OH)D [BVMT, 10 sec r=.946, 20 sec r= .80, 30 sec, r=.891, DR, r= .81, SDMT, r= .747, MoCA= .614; $p < 0.01$]. The normal 25(OH)D group had lower anxiety scores at baseline. Alcohol consumption correlated positively with SDMT, BVMT and MoCA in the group with low 25(OH)D and BVMT and SDMT in the normal group. Age and years of education correlated positively with all cognitive tests regardless of 25(OH)D level. Cognitive performance and anxiety in MS seem to be affected by low 25(OH)D level. There is a positive correlation between exercise and cognitive performance in all subjects; but is stronger in the group with low 25(OH)D suggesting compensatory exercise role in this group. Of interest is the positive alcohol effect on cognitive performance that needs further exploration.

Disclosures: H.J. Darwish: None. **R. Haddad:** None. **S. Osman:** None. **S. Ghassan:** None. **B. Yamout:** None. **S. Khoury:** None.

Poster

222. Therapeutic Strategies for Remyelination

Location: Hall A

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Program#/Poster#: 222.14/F12

Topic: C.09. Demyelinating Disorders

Support: MS Society UK Research Grant 964/12

Title: Recognising the role of hypoxia in neuroinflammatory disease, and the effects of oxygen as a therapy

Authors: *M. AMATRUDA[1], A. MATIS[1], A. DAVIES[1], R. DESAI[1], M. LINDNER[2], C. LININGTON[2], K. J. SMITH[1];
[1]Inst. of Neurology, Univ. Col. London, London, United Kingdom; [2]Univ. of Glasgow, Inst. of Infection, Immunity and Inflammation, Glasgow, United Kingdom

Abstract: The mechanisms responsible for the neurological deficits and demyelination in relapsing-remitting multiple sclerosis (MS) are complex, and remain unclear. Here we focus on the potential role of tissue hypoxia in contributing to the loss of neurological function, and the possibility that oxygen therapy may ameliorate the deficits. We examine the presence of tissue hypoxia in passive transfer EAE, where paralysis occurs in absence of demyelination, and the role of oxygen therapy in active EAE, a model in which we have previously described a correlation between hypoxia in the spinal cord and the severity of the neurological deficits. In passive EAE, an oxygen-sensitive probe inserted into the spinal cord revealed tissue hypoxia at the time of expression of neurological deficit. Indeed, the oxygen partial pressure (PO2) was comparable with the PO2 values we have observed in rats with active EAE and the same disease severity. Histologically there was a significantly higher expression of hypoxia-related markers. The findings suggest that hypoxia contributes to the neurological deficits in passive EAE, as we have found in active EAE. In active EAE we have now extended our observations to examine the consequences of oxygen therapy. We report that treatment with 95-100% oxygen improved neurological function in 50% of the treated rats within only one hour of exposure, and the improvement reversed upon return to room air for the following hour. To explore the time-dependence of oxygen therapy, oxygen (75%) was applied for 24 hours either at the first, second or third day of disease expression. Oxygen significantly reduced the neurological deficit; notably the greatest improvement occurred when administered on the first day. We then tested the optimal duration for oxygen treatment (24, 48 and 72 hours at 75% oxygen) from onset of disease, and found the greatest benefit was achieved with 48 hours exposure ($p < 0.05$). Interestingly, when administered at the onset of deficit, oxygen reduced the severity of the disease progression for up to two additional days once returned to room air. We conclude that tissue hypoxia is an important but currently overlooked cause of neurological deficits in neuroinflammatory disease, and that oxygen administration can promptly ameliorate the deficits.

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Poster

222. Therapeutic Strategies for Remyelination

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 222.15/F13

Topic: C.09. Demyelinating Disorders

Support: Employee of AbbVie

Title: Fingolimod sustains neuronal and electrophysiological function in a mouse model of myelin oligodendrocyte glycoprotein (MOG) induced experimental autoimmune encephalomyelitis (EAE)

Authors: *S. S. POPP, P. SCHLEESE, B. K. MÜLLER, K. WICKE;
Pharmacol., AbbVie Deutschland GmbH & Co. KG, Ludwigshafen, Germany

Abstract: Fingolimod is a sphingosine-1-phosphate receptor modulator and is approved for treating multiple sclerosis. It prevents an autoimmune reaction in MS by sequestering lymphocytes in lymph nodes. Here, we demonstrate that prophylactic therapy protected against the emergence of experimental autoimmune encephalomyelitis EAE symptoms in a rotarod performance test and against abnormalities to the H-reflex, a marker for spasticity since spasticity is a common manifestation in multiple sclerosis. Fourty female C57BL/6 mice were randomly assigned to three treatment groups (sham (n=10), vehicle (n=15) and fingolimod (n=15)). Vehicle and fingolimod mice received MOG emulsified in complete Freund's adjuvant containing heat-inactivated Myobacterium tuberculosis and pertussis toxin injections. The sham mice did not receive any treatment. Three days after MOG injection all groups received daily peroral treatment injection for three weeks (sham and vehicle mice: water and fingolimod mice: 1mg/kg fingolimod in water). Eight days after MOG treatment the mice were scored daily for neurological signs by a person blinded to the treatment. Twenty-one and twenty-two days after MOG treatment the H-reflex was measured; 22 days after the MOG injection the rotarod test was performed, as well. We observed a significant difference between the fingolimod treated animals and the vehicle treated animals in the H/M wave ratio as well as in the rotarod performance test. The sham animals were also significantly different from vehicle treated animals in both tests. Interestingly, we did not find a significant difference in the EAE scores between the fingolimod and vehicle group. This may be due for being a subjective test whereas rotarod and H-reflex are two objective tests. The two objective tests seem to be more sensitive than the subjective one. Our results clearly demonstrate that prophylactic treatment with fingolimod at a clinically relevant concentration prevents the development of neurological disability. Here we show for the first time that the H-reflex can be used as measurement for the assessment of the efficacy of substances used in an animal model of disseminated EAE. This result correlates with the behavioral finding. Disclosures: All authors are employees of AbbVie. The design, study conduct, and financial support for this research was provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication.

Disclosures: **S.S. Popp:** A. Employment/Salary (full or part-time):; AbbVie. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AbbVie. **P. Schleese:** A. Employment/Salary (full or part-time):; AbbVie. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AbbVie. **B.K. Müller:** A. Employment/Salary (full or part-time):; AbbVie. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AbbVie. **K. Wicke:** A. Employment/Salary (full or part-time):; AbbVie. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); AbbVie.

Poster

222. Therapeutic Strategies for Remyelination

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 222.16/F14

Topic: C.09. Demyelinating Disorders

Support: Quinnipiac University

Title: Chronic oral riluzole or caloric restriction but not acute riluzole ameliorates symptoms of experimental autoimmune encephalomyelitis

Authors: ***A. J. BETZ**[1], R. ROTOLO[2], J. DEMURO[3], G. DRUMMOND[4], C. LITTLE[1], L. TELISKA[5], B. DALENA[1], S. CASTILLO[5], L. FRUEHAUF[5], T. STRANGE[5], T. MEDWID[2], A. BARBER[6], L. JOHNS[4];
[1]Psychology, [2]Med. Lab. Sci., [3]Mol. and Cell Biol., [4]Hlth. Sci., [5]Psychology, Behavioral Neurosci., Quinnipiac Univ., Hamden, CT; [6]Hopkins Sch., New Haven, CT

Abstract: Experimental Autoimmune Encephalomyelitis (EAE) is an animal model of multiple sclerosis (MS). We characterized the impairments associated with EAE in female C57BL/6 mice. Mice were immunized subcutaneously with 100 μg of myelin oligodendrocyte glycoprotein emulsified in incomplete Freund's adjuvant supplemented with 500ug mycobacterium tuberculosis H37RA and 200 ng of intraperitoneal pertussis toxin on days 0 and 2. Tail paralysis was observed daily. In Experiment 1, we found that caloric restriction (CR) and chronic oral administration of riluzole, a glutamate release inhibitor, delayed the onset and severity of EAE. CR and riluzole both reduced nociceptive behavior. Array and protein analysis revealed alterations in inflammatory and regulatory cytokines. Moreover, we found altered expression of STAT3, GFAP, and Foxp3 which are notable determinants involved in the immune response. Altered immunological function was indicated by reduced inflammation in the spinal cords of mice treated with chronic oral riluzole. In Experiment 2, we found that acute oral administration did not delay the onset or severity of EAE. These findings indicate a compelling need to delineate the roles of glutamate, the immune response, and CR in EAE.

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Poster

222. Therapeutic Strategies for Remyelination

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Topic: C.09. Demyelinating Disorders

Support: NIH Grant NS073132

NMSS Grant RG4813-A-2

NMSS Grant RG 5239-A-3

Title: Activation of NF-κB protects oligodendrocytes against inflammation

Authors: ***W. LIN**[1], W. WISESSMITH[1], S. JAMISON[1], R. SCHMIDT-ULLRICH[2]; [1]Neurosci., Univ. of Minnesota, Minneapolis, MN; [2]Max-Delbrueck-Center for Mol. Med., Berlin, Germany

Abstract: Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), characterized by inflammation, demyelination, and oligodendrocyte loss. Transcription factor Nuclear Factor κ B (NF-κB) plays a critical role in inflammatory diseases by regulating inflammation and cell viability. Activation of NF-κB has been observed in oligodendrocytes in MS lesions. Although *in vitro* studies suggest that NF-κB activation promotes oligodendrocyte survival in response to inflammatory mediators, the effects of NF-κB activation on oligodendrocytes in MS and its animal models remain unknown. Interferon-γ (IFN-γ) is regarded as a key proinflammatory cytokine in MS. The presence of IFN-γ in the CNS during development results in inflammation, oligodendrocyte death, and myelin loss. Interestingly, our previous study shows that IFN-γ activates NF-κB in oligodendrocytes *in vitro* and *in vivo*. In this study, using a mouse model that expresses IκBαΔN, a super-suppressor of NF-κB, specifically in oligodendrocytes, we found that NF-κB inactivation in oligodendrocytes exacerbated IFN-γ-induced cell death and myelin loss in young, developing mice, but did not alter inflammation elicited by this cytokine in the CNS. Thus, this finding implies the cytoprotective effects of NF-κB activation on oligodendrocytes in neuroinflammatory diseases such as MS.

Disclosures: **W. Lin:** None. **W. Wisessmith:** None. **S. Jamison:** None. **R. Schmidt-Ullrich:** None.

Poster

222. Therapeutic Strategies for Remyelination

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Topic: C.09. Demyelinating Disorders

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US National Multiple Sclerosis Society (FG 2067-A-1)

Title: The muscarinic acetylcholine receptor as a therapeutic target for remyelination and axonal integrity in multiple sclerosis

Authors: *F. MEI[1,4], K. LEHMANN-HORN[2], C. TEUSCHER[5], A. GREEN[1], J. WESS[6], J. LAWRENCE[7], S. TONEGAWA[8], S. ZAMVIL[2], S. P. J. FANCY[1,3], J. R. CHAN[1]; [1]Dept. of Neurol. and Program in Neurosci., [2]Dept. of Neurol. and Program in Immunology,, [3]Dept. of Pediatrics, Univ. of California San Francisco, San Francisco, CA; [4]Histology and Embryology, Third Military Med. Univ., Chongqing, China; [5]Dept. of Medicine, Immunobiology Program, C331 Given Med. Building, Univ. of Vermont, Burlington, VT; [6]Mol. Signaling Section, Lab. of Bioorganic Chem., US Natl. Inst. of Hlth., Bethesda, MD; [7]COBRE Ctr. for Structural and Functional Neurosci., The Univ. of Montana, Missoula, MT; [8]Howard Hughes Med. Institute, RIKEN–MIT Neurosci. Res. Ctr., MIT, Cambridge, MA

Abstract: Neuronal degeneration underlies the progressive and chronic disability in MS and is currently untreatable. Emerging evidence indicates that myelin is important not only for neuronal function but for the survival of axons, suggesting that remyelination therapies may represent a promising approach to preserve axonal integrity in MS. Muscarinic receptor (MR) antagonists significantly promote oligodendrocyte differentiation and remyelination, however the receptor target(s) of these compounds is currently unknown. The non-selective antagonism of all MR subtypes (M1R-M5R), as well as other off-target receptors, limits the utility of these compounds for MS therapy. As oligodendrocyte precursor cells (OPCs) express all five MR subtypes, we used MR knockout mice to identify the receptor subtype responsible for the beneficial actions of MR antagonists in MS. Here we identify one MR subtype as the specific receptor that inhibits oligodendrocyte differentiation and myelination and mediates the effects of MR antagonists. Deletion of the MR phenocopies the effects of the anti-muscarinic compounds resulting in

enhanced differentiation and myelination. More importantly, disruption of the MR function reduces the severity and enhances recovery from experimental autoimmune encephalomyelitis (EAE) with robust remyelination and subsequent preservation of axonal integrity. Additionally, enhanced remyelination in the lysolecithin-induced focal demyelination model suggests that the MR acts to inhibit the kinetics of remyelination of endogenous oligodendroglia. To determine enhanced remyelination is sufficient to protect axons against degeneration, the MR in oligodendroglia is conditionally deleted under the control of CNPase promoter. We will assess remyelination and axonal integrity in the MR conditional knockout EAE mice. Together, our findings indicate that the MR mediates potent inhibition of oligodendrocyte differentiation and myelination and antagonizing the MR may represent a promising therapeutic approach to stimulate remyelination and preserve axonal integrity in MS.

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Poster

222. Therapeutic Strategies for Remyelination

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Program#/Poster#: 222.19/F17

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The Miami Project To Cure Paralysis (RB, JRB)

Danish MS Society (PMM)

Title: Oligodendroglial TNFR2 mediates membrane TNF-dependent repair in neuroimmune disease by promoting oligodendrocyte differentiation and remyelination

Authors: *P. MADSEN[1,2], D. MOTTI[3], D. E. SZYMKOWSKI[4], J. R. BETHEA[5], R. BRAMBILLA[1,6];
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Abstract: Tumor necrosis factor (TNF) has been associated with the pathophysiology of multiple sclerosis (MS) as patients have elevated concentration of TNF in serum, CSF and within active lesions. TNF exists in two forms, transmembrane (tmTNF) and soluble (solTNF), whose

functions are mediated by TNFR1 and TNFR2. Due to differential binding affinities of the ligands solTNF signals only via TNFR1 and tmTNF through both TNFR1 and TNFR2. The cellular processes activated by the two receptors are often opposite: TNFR1 mediates apoptosis and inflammation, TNFR2 mediates cell survival, resolution of inflammation and myelination. Numerous studies have underscored the importance of distinguishing between the functions of solTNF and tmTNF and have associated MS and its animal model experimental autoimmune encephalomyelitis (EAE) to the detrimental effects of solTNF via TNFR1, while tmTNF is important for repair and remyelination. Here we demonstrate that TNFR2 expressed in the oligodendrocyte lineage is a key mediator of the protective functions of tmTNF in EAE. CNP-cre:TNFR2fl/fl mice with conditional ablation of TNFR2 in oligodendrocytes showed exacerbation of EAE with increased axonal damage and myelin pathology, as well as reduced remyelination. The EAE clinical profile was not improved by treatment with the solTNF inhibitor XPro1595, which suppresses EAE in TNFR2fl/fl control mice, indicating that for tmTNF to be beneficial a functional TNFR2 in oligodendrocytes is required. Lack of oligodendroglial TNFR2 also resulted in increased loss of oligodendrocyte precursor cells and mature oligodendrocytes after EAE. In oligodendrocyte cultures from WT and TNFR2-/- mice we demonstrated that TNFR2 promotes differentiation, but is not required for proliferation or survival. Finally, in oligodendrocyte-enriched spinal cord suspensions from naïve and acute EAE TNFR2fl/fl and CNP-cre:TNFR2fl/fl mice differential expression of microRNAs that are known regulators of oligodendrocyte differentiation and inflammation, such as members of the miR-219, miR-138 and miR-338 clusters. Taken together our data provide the first direct evidence that TNFR2 in oligodendrocytes is important in driving differentiation, thereby sustaining the tmTNF-dependent repair process in neuro-immune disease. Our studies identify TNFR2 in the CNS as a viable molecular target for the development of remyelinating agents for progressive MS, addressing the most pressing need in MS therapy today.

Disclosures: **P. Madsen:** None. **D. Motti:** None. **D.E. Szymkowski:** None. **J.R. Bethea:** None. **R. Brambilla:** None.

Poster

223. Demyelinating Disorders: Human and Animal Studies

Location: Hall A

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Program#/Poster#: 223.01/F18

Topic: C.09. Demyelinating Disorders

Title: Immunophenotyping and cytokine expression in MOG_{35-55} induced experimental autoimmune encephalomyelitis mouse model

Authors: M. VIHMA, M. TAAVITSAINEN, J. OKSMAN, *S. KIM, R. HODGSON, P. J. SWEENEY, A. NURMI;
Charles River Discovery Services, Kuopio, Finland

Abstract: Multiple sclerosis (MS) is an autoimmune disease causing a wide range of symptoms by demyelination in central nervous system (CNS). To study mechanisms of MS by using preclinical *in vivo* models, there are several of options but one of the most common models is an MOG_{35-55} induced experimental autoimmune encephalomyelitis (EAE) in mice. In addition to using behavioral, histological and immunohistochemical read outs to assess disease burden and tissue pathology, evaluation of molecular mechanisms and relationship between peripheral and central resident immune cells in the EAE is important. In this study our aim was to characterize MOG_{35-55} mouse model immunological profile by immunophenotyping different subsets of cells in CNS and in peripheral lymph nodes during the course of EAE phenotype. Mice were inoculated with MOG_{35-55} containing adjuvant and disease progression was followed up until day 14 or 21 after which mice were terminated. Cells were harvested and processed from CNS and lymph nodes after which immunophenotyping of subsets of CD45+ cells (CD4+, CD8+, CD11b+ and B-cells) were evaluated by flow cytometry . In addition, cytokine expression of inflammatory cytokines, such as TNF-a, in plasma and CSF were evaluated by Luminex multiplexing assay. Taken together, this study describes immunophenotypic characteristics of resident and infiltrated subsets of CD45+ cells in the CNS as well as profile for cells extracted from lymph nodes during different stages of the disease (EAE) and in relation to naïve mice. Immunophenotypic profile of CNS and lymph nodes is supplemented with cytokine expression levels in plasma and CSF extracted from EAE mice at corresponding time-points. Data will provide insight to this model characteristics through understanding the dynamics of infiltrated and resident immune cells and inflammatory cytokine signals in circulation during the disease progression.

Disclosures: M. Vihma: None. **M. Taavitsainen:** None. **J. Oksman:** None. **S. Kim:** None. **R. Hodgson:** None. **P.J. Sweeney:** None. **A. Nurmi:** None.

Poster

223. Demyelinating Disorders: Human and Animal Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 223.02/F19

Topic: C.09. Demyelinating Disorders

Title: Characterization of the MOG_{35-55} induced experimental autoimmune encephalomyelitis mouse model for multiple sclerosis by behavior and immunohistochemistry

Authors: *J. OKSMAN, S. KIM, L. TÄHTIVAARA, A. NURMI;
Charles River Discovery Services, Kuopio, Finland

Abstract: One of the most common preclinical *in vivo* models used in the study of mechanisms of multiple sclerosis (MS) is the MOG_{35-55} induced experimental autoimmune encephalomyelitis (EAE) in mice. This technique involves inoculation with MOG_{35-55} protein, which is emulsified to complete Freud's adjuvant, to evoke inflammatory responses against proteins. Further, an injection of pertusis toxin (PTx) allows immune cells access to the CNS trough the blood brain barrier (BBB) causing T cell related demyelination. Fingolimod (FTY-720), an immune modulating drug approved by FDA for treatment of MS, is commonly used as a positive control in MOG_{35-55} induced EAE in mice. FTY-720 is a sphingosine-1-phosphate receptor modulator, which can isolate the lymphocytes in lymph nodes to prohibit immune cells from reacting against antibodies. In the current study, mice were inoculated with MOG_{35-55} on Day 0 and followed until Day 35. FTY-720 served as a positive control compound with therapeutic and prophylactic therapy. The clinical status of the mice was scored and body weights were measured daily. At the study end-point, the mice were terminated for tissue sample collection. Cervical segments of spinal cord were collected and fixed for immunohistochemical (IHC) analyses. Demyelination was analyzed by myelin basic protein staining; microglia activation was analyzed by Iba-1 staining; and inflammation was analyzed with CD68 staining. MOG_{33-55} induced mice start to develop disease symptoms at around two weeks after immunization. After which the disease progression is either primarily dominant or relapsing and remitting type. Mice can reach complete or partial paralysis which also causes muscle atrophy and loss of body weight. After the period of 35 days, IHC analyses of spinal cord cervical segment reveals demyelination, microglia activation and macrophage expression in MOG_{35-55} inoculated mice. In the light of the model phenotype, mouse MOG_{35-55} EAE model is a useful model to study novel compounds against MS. In addition to models disease related behavioral and tissue pathological outcomes and their relationships, model is also useful to examine molecular mechanisms and therapeutic targets in detail to understand human disease better and to develop more effective therapies against MS.

Disclosures: **J. Oksman:** None. **S. Kim:** None. **L. Tähtivaara:** None. **A. Nurmi:** None.

Poster

223. Demyelinating Disorders: Human and Animal Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 223.03/F20

Topic: C.09. Demyelinating Disorders

Title: Cuprizone model in mice: characterization of white matter, tissue pathology and inflammation by mri and spect

Authors: K. LEHTIMÄKI, T. HUHTALA, A.-M. ZAINANA, R. HODGSON, *A. J. NURMI; Charles River Discovery Res. Services, Kuopio, Finland

Abstract: Understanding the pathology and processes that are involved in animal models of demyelinating diseases such as multiple sclerosis is a critical component of assessing the utility of the model as a tool to develop novel treatments or biomarkers. . The mouse cuprizone model was developed to produce a wide range of pathological and symptomatic endpoints that recapitulate clinical aspects of MS.To understand better changes that are underlying the phenotype observed in cuprizone model mice, we sought to apply noninvasive imaging techniques to understand the magnitude of demyelination and inflammation over time in the model. Given that many of the symptomatic endpoints demonstrate recovery following discontinuation of cuprizone treatment, we also examined tissue pathology that coincides with reversal of behavioral deficits. To address these questions we used magnetic resonance imaging (MRI), and single photon emission computer tomography (SPECT) and analysis of brain and spinal cord to determine if mice exhibit pathology that could be linked to the observed phenotype of the model both during and following cuprizone exposure. C57Bl/6 female mice were subjected to diet induced cuprizone (0.3% w/w) exposure or regular powdered diet in their home cage. Exposure was 6 weeks on cuprizone following which the cuprizone diet was discontinued and switched to normal diet for 3 weeks. Diffusion tensor magnetic resonance imaging (DTI-MRI) was performed at 3 and 6 weeks after the onset of cuprizone diet. [123I]-TSPO SPECT for inflammation was examined after 6 weeks of exposure with cuprizone. In this presentation we describe tissue pathology end-points (white matter changes, inflammation) in mouse cuprizone model that dynamically evolves during and after cuprizone challenge. Observed tissue changes are compared with behavioral data to identify which imaging markers correlate with phenotypic changes in the model. Taken together, data presented in this presentation provide further insight to the relationship between tissue pathology and the observed behavioral phenotype of cuprizone-exposed mice.

Disclosures: K. Lehtimäki: None. **T. Huhtala:** None. **A. Zainana:** None. **R. Hodgson:** None. **A.J. Nurmi:** None.

Poster

223. Demyelinating Disorders: Human and Animal Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 223.04/F21

Topic: C.09. Demyelinating Disorders

Title: Cuprizone model in mice: characterization of tissue pathology by immunohistochemistry and flow cytometry

Authors: M. VIHMA, *L. TAHTIVAARA, A.-M. ZAINANA, R. HODGSON, A. NURMI; Charles River Discovery Res. Services Finland Ltd, Kuopio, Finland

Abstract: Chronic cuprizone treatment produces a CNS-wide demyelination in mice that is used to model MS and other demyelinating diseases. The construct validity of this model is a function on the extent to which the pathology in the animal recapitulates the pathology in MS. Here we use immunohistochemical and immunophenotyping methods to characterize the effects of cuprizone on demyelination and inflammation and to compare those results to symptomatic endpoints measured using behavioral methods. C57Bl/6 female mice were subjected to diet induced cuprizone (0.3% w/w) exposure or regular powdered diet in their home cage. Exposure was 6 weeks on cuprizone following which the cuprizone diet was discontinued and switched to normal diet for 3 weeks. Brains were harvested from mice at 3, 6 and 9 week time-points for histological/immunohistochemical analysis of brain and spinal cord, with emphasis on demyelination (anti-myelin basic protein (anti-MBP) staining) and inflammatory markers (Iba-1, CD4+, CD8+ CD68+). Finally, we characterized key cell surface markers from cells extracted from CNS and lymph nodes with flow cytometry. In this presentation we describe tissue pathology end-points (white matter changes, inflammation) in mouse cuprizone model that dynamically evolves during and after cuprizone challenge. Observed tissue changes are linked with behavioral data to identify which histological, immunohistochemical and immunophenotype markers correlate with behavioral phenotype of the model. Taken together, data presented in this presentation provide further insight to the relationship between tissue pathology and the observed behavioral phenotype of cuprizone-exposed mice.

Disclosures: M. Vihma: None. **L. Tahtivaara:** None. **A. Zainana:** None. **R. Hodgson:** None. **A. Nurmi:** None.

Poster

223. Demyelinating Disorders: Human and Animal Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 223.05/F22

Topic: C.09. Demyelinating Disorders

Title: Behavioral Characterization of the Cuprizone Model of demyelination in mice

Authors: A.-M. ZAINANA, *S. HÄTINEN, J. PUOLIVÄLI, T. HEIKKINEN, R. HODGSON, A. NURMI;
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Abstract: Demyelination is a key factor involved in many neurological diseases including multiple sclerosis (MS). There have been multiple attempts to create novel models that will broaden our understanding of demyelinating disease such as MS and allow for the development of novel treatments. One such model involves the delivery of cuprizone in the diet, which produces a clear demyelination across the CNS in mice. Much has been done to describe the pathology in the brain following cuprizone treatment, but there has been relatively little to

determine the clinical relevance of the model using behavioral endpoints. In this presentation we describe the characterization of the cuprizone model in mice, which shows clear and highly reproducible behavioral changes during and after the cuprizone exposure. C57Bl/6 female mice were given cuprizone (0.3% w/w) in their diet or regular powdered diet. Exposure lasted 6 weeks after which the cuprizone supplementation of the diet was discontinued and the animals were switched to normal diet for 3 weeks. The mice were assessed usinga neurological index, locomotor activity (LMA), and rotarod (RR) during week 3, 6 and 9 as well as elevated plus maze (EPM) and contextual fear conditioning (CFC) test on week 6. In addition, the mice were subjected fine motor gait analysis by MotoRater (MR) assay to evaluate in depth motor performance of the mice on week 6. When LMA was evaluated after 3 weeks of cuprizone exposure, locomotor speed was reduced, but there was no significant difference in distance traveled when compared to vehicle. At 6 weeks the mice clearly showed differences in LMA (distance, rearings, time in center, speed and inactivity time) when compared to controls. However, at 3 weeks after discontinuation of cuprizone , the cuprizone-treated mice were not different from controls in LMA measures with exception of locomotive speed. Mice that had been 6 (but not 3) weeks on cuprizone diet mice showed significant decrease in fall latency in RR. MR analysis showed clear gait impairment in mice that had been 6 weeks on the cuprizone diet. In the EPM, the mice showed anxiety-like behavior with decreased time spent in open arms when compared to control mice at 6 weeks. In CFC, the mice showed freezing behavior for context and cue similar to controls, but also significant freezing behavior in an altered context. These data describe multiple motor and cognitive behavioral deficits while mice were on cuprizone diet during the 3-week follow-up period. Taken together the results described herein provide a better understanding of how to use the cuprizone model for research and drug development.

Disclosures: **A. Zainana:** None. **S. Hätinen:** None. **J. Puoliväli:** None. **T. Heikkinen:** None. **R. Hodgson:** None. **A. Nurmi:** None.

Poster

223. Demyelinating Disorders: Human and Animal Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 223.06/F23

Topic: B.11. Glial Mechanisms

Support: AIHS Postgraduate Fellowship CA#3723

Title: Mechanisms of myelin loss in copper depleted mice: implications for Progressive MS

Authors: ***A. V. CAPRARIELLO**, K. W. C. POON, J. R. PLEMEL, L. LU, J. F. DUNN, P. K. STYS;
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Abstract: Copper is an essential trace element and co-factor for myriad enzymes involved in fundamental biological processes ranging from cellular respiration to anti-oxidation. That copper is also important for myelin physiology is suggested by reduced myelination in genetic diseases of copper transport, and strengthened by decades of rodent studies in which stereotyped demyelination follows ingestion of the copper chelator cuprizone (CPZ). Despite its widespread use as a rodent model of demyelinating diseases such as multiple sclerosis (MS), the precise mechanistic connections between CPZ and demyelination remain unexplained, as does the extrapolation of findings from CPZ-fed mice to relevant neurological diseases. The current study seeks to elucidate mechanistic links between copper deficiency and demyelination. Building on the lab's findings in cultured neurons that copper is an important regulator of NMDA receptor (NMDAR) activity, the hypothesis is that copper chelation causes demyelination through an indirect mechanism involving excitotoxic injury to the oligodendrocyte and myelin directly, both of which express glutamate receptors. Given our recent findings that copper also controls desensitization of the AMPA receptor (AMPAR), the demyelinating action of CPZ may also reflect AMPAR-dependent excitotoxicity. To test these possibilities, various strains of wild-type and NMDAR knockout mice were CPZ-treated and the effects of copper chelation on subcellular as well as systems-level processes were analyzed by a combination of NMR, laser-scanning fluorescence, and CARS imaging modalities in both *in vivo* and *in vitro* preparations. *In vivo*, the absence of GluR3A conferred partial protection from CPZ-mediated demyelination, while GluR3A/2D double knockout mice were fully protected. Current studies seek to clarify the *in situ* expression of glutamate receptors within affected white matter regions and to determine the relative susceptibilities of the oligodendrocyte soma versus its myelin processes to glutamate excitotoxicity. Understanding the link between copper chelation and demyelination carries important implications not only for evaluating the relevance of the CPZ mouse model to MS but for treating the white matter damage that occurs in copper diseases such as Menkes or Wilson's Disease.

Disclosures: A.V. Caprariello: None. **K.W.C. Poon:** None. **J.R. Plemel:** None. **L. Lu:** None. **J.F. Dunn:** None. **P.K. Stys:** None.

Poster

223. Demyelinating Disorders: Human and Animal Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 223.07/F24

Topic: C.09. Demyelinating Disorders

Support: California Institute of Regenerative Medicine (CIRM) - New Faculty Physician Scientist Translational Research Award

Title: Cell intrinsic and microenvironmental etiologies of chemotherapy-induced white matter injury

Authors: *E. M. GIBSON, L. S. WOOD, S. CARTMELL, A. K. GOLDSTEIN, J. LENNON, S. NAGARAJA, S. E. MILLER, P. J. WOO, A. OCAMPO, M. MONJE;
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Abstract: An unintended consequence of cancer chemotherapy is thought to be depletion or dysfunction of the oligodendroglial precursor cells (OPCs) present throughout the central nervous system that give rise to the myelinating oligodendrocytes necessary for fast saltatory conduction of neural impulses. The resultant injury to the myelinated infrastructure of the brain may underlie chemotherapy-associated symptoms of slowed information processing, deficits in attention, concentration, fine motor skills, working memory and learning. OPCs are necessary for developmental myelination, as well as for ongoing myelin remodeling throughout life. Cancer therapy-induced damage to the OPC pool is thus particularly devastating for children who have not yet completed developmental myelination. To confirm the extent of OPC population depletion in children receiving chemotherapy, we examined post-mortem brain samples from the frontal lobes of children treated with multi-agent traditional chemotherapy. We found that OPCs are depleted specifically in subcortical white matter. In contrast, grey matter OPCs are preserved in comparison to age-matched control subjects. Methotrexate, an antimetabolite chemotherapeutic, is a commonly used agent in pediatric cancer therapy and is particularly associated with white matter injury and cognitive dysfunction. We have developed a mouse model of juvenile methotrexate (MTX) chemotherapy exposure in which mice are treated with MTX or PBS (one dose each week from P21-35 for a total of three doses). One month following the last dose of MTX, mice exhibit behavioral deficits in motor speed as measured by the CatWalk gait system, as well as depletion of deep cortical grey matter and subcortical white matter OPCs. As in human subjects, superficial grey matter OPC density was preserved. Concomitant with deep cortical and subcortical OPC depletion, we observed an increase in immature oligodendrocytes, suggesting increased but incomplete differentiation of OPCs. Experiments allografting healthy, GFP-labeled OPCs into the environment of the previously chemotherapy-treated brain to probe the gliogenic microenvironment are ongoing. OPCs isolated from whole brain exhibit an IC_{50} less than the measured MTX concentration achieved in the brain with this paradigm, suggesting that a direct cytotoxic effect on OPCs plays a role in depletion of the subcortical OPC population. Collectively, these data suggest both direct and microenvironmental MTX toxicity on oligodendroglial lineage cells.

Disclosures: **E.M. Gibson:** None. **L.S. Wood:** None. **S. Cartmell:** None. **A.K. Goldstein:** None. **J. Lennon:** None. **S. Nagaraja:** None. **S.E. Miller:** None. **P.J. Woo:** None. **A. Ocampo:** None. **M. Monje:** None.

Poster

223. Demyelinating Disorders: Human and Animal Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: C.09. Demyelinating Disorders

Support: Support from Don and Fran Herdrich.

Title: Understanding neuronal gene expression changes induced by distal demyelination or inflammatory insult

Authors: *B. CLARKSON, K. MIRCHIA, H. KIM, B. SAUER, R. LAFRANCE-COREY, C. HOWE;
Mayo Clinic, Rochester, MN

Abstract: MR imaging suggests an anatomic correspondence between WM and GM lesions in progressing MS patients, suggesting an underlying neuronal-signaling or neurodegenerative element to the progressive disease state. While retrograde signals evolving from stressed axons that alter soma gene expression have been previously described in models of axonal transection or crush injury, those resulting from inflammation and demyelination are unknown. Using microfluidic chambers that segregate neuronal soma and axons, we have previously demonstrated increased axonal MHC I following treatment with IFNg, a cytokine prominent in demyelinating MS lesions. Furthermore, expression of antigen-loaded MHC I predisposed axons to injury by antigen-specific CD8+ T cells *in vitro*. Here we report that neurons also upregulate expression of MHC class Ib mRNAs in response to axonal stimulation with IFNg. Genes most highly upregulated following cytokine treatment included H2-M3, H2-Q5, H2-Q6, H2-Q7, H2-Q8, H2-T10 , and H2-T23. We further report the development of an *in vivo* model system for measuring axonal vulnerability to antigen-specific immune attack following demyelination using an AAV vector to induce neuronspecific expression of axonally-targeted neoantigen (ovalbumin) tagged with cleavable eGFP. We also present optimized methods using AAV vectors in mice for infecting retinal, motor cortex, and hippocampal neurons to induce target gene expression in axons projecting to optic nerve and corpus collosum.

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Poster

223. Demyelinating Disorders: Human and Animal Studies

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: C.09. Demyelinating Disorders

Title: Multiple sclerosis patients have increased levels of the uremic toxin 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF)

Authors: *F. MIR, D. BLEMUR, S. A. SADIQ;
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Abstract: We found the uremic toxin 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF) to be significantly elevated in the cerebrospinal fluid (CSF) of untreated progressive multiple sclerosis (MS) patients in comparison to normal healthy controls, using global metabolomics profiling. CMPF is an endogenous metabolite of furan fatty acids in humans. CMPF is excreted into the urine via organic anion transporters but accumulates at very high concentrations in the serum of uremic patients. CMPF is mostly albumin bound and has previously been reported to inhibit cellular transport, erythropoiesis and mitochondrial respiration. It has also been implicated in the induction of beta cell dysfunction in diabetes; thyroid irregularities and neurologic dysfunction. We next sought to validate these results in a larger independent cohort of MS patients. CSF and blood was obtained from healthy controls and MS patients with informed consent under an IRB-approved protocol. Samples were immediately processed and stored at -80 degree until use. Data obtained was analyzed using Graphpad Prism. CMPF levels were quantitated using a competitive ELISA from NovaTein Biologicals, USA as per the manufacturer's instructions. Indeed serum CMPF levels were found to be significantly elevated in the MS patients (0.857±0.044μM) (n = 183) as compared to the normal healthy controls (0.0178±0.02 μM) (n = 38) with a p value of < 0.0001. Furthermore, the results show a more pronounced increase in CMPF levels in progressive MS patients as compared to the relapsing remitting patient group. On a cellular level, we have localized the CMPF transporter - solute carrier family 22 member 8/organic anion transporter 3 (SLC22A8), to the glia and neurons in the central nervous system. Functionally, high doses of CMPF were found to be toxic to neuroblastoma cells in culture, in part through an increase in reactive oxygen species generation. The results are the first report of increased CMPF levels in MS and clearly warrant further investigation of its role in the pathogenesis of MS.

Disclosures: F. Mir: None. **D. blemur:** None. **S.A. sadiq:** None.

Poster

223. Demyelinating Disorders: Human and Animal Studies

Location: Hall A

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Topic: C.09. Demyelinating Disorders

Title: Cortical spreading depression induces hemodynamic mini-strokes: a potential cause of white matter leisons in migraine

Authors: *B. DONMEZ-DEMIR[1], M. YEMISCI[1,2], K. KILIC[1], Y. GURSOY-OZDEMIR[1,2], M. MOSKOWITZ[3], T. DALKARA[1,2];
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Abstract: White matter lesions (WMLs) are prevalent in patients with migraine with aura (MA). The mechanisms underlying these putatively ischemic lesions are unknown. As suggested by observations in CADASIL patients, brief hypoperfusion attacks in cortical/subcortical areas supplied by penetrating arteries (PAs) may initiate a cortical spreading depression (CSD) wave and, hence, MA attack and; when hypoperfusion is prolonged, an ischemic lesion may emerge at subcortical areas supplied by long PAs that are particularly vulnerable to perfusion deficits. Alternatively, the source of PA hypoperfusion in MA patients could be the prolonged oligemia accompanying CSDs aggravated by unfavorable hemodynamic conditions. We have tested these possibilities in the intact mouse brain. Mice were anesthetized with ketamine/xylazine or isoflurane. The body temperature, pulse rate and tissue oxygen saturation were monitored and kept within physiological limits. A glass pipette filled with FeCl3 was gently touched the dura over a PA for 3 minutes to induce thrombosis. DC potentials were recorded with Ag-AgCl pellet electrodes placed over the thinned skull. We also monitored the blood flow changes with laser speckle contrast imaging. After 1 or 2 weeks, mice were perfused transcardially with 4% PFA and 5 μm-thick paraffin sections were stained with luxol-fast blue or hematoxylene and eosin staining or labeled with anti-myelin basic protein (to detect demyelination) or anti-CD68 antibodies (to detect activated microglia). We found that occlusion of a single cortical PA consistently (10 out of 10 mice) triggered a CSD originating from the tissue around the PA and, induced a slowly evolving ischemic injury. However, the ischemic lesion was generally confined to the cortex unlike the deep demyelinating WMLs seen in MA patients. In contrast, the cortical oligemia and PA constriction induced by a CSD caused ischemic histological changes in the deep paraventricular zone supplied by hemodynamically vulnerable long medullary arteries. When the unfavorable hemodynamic conditions were further compromised during recurrent CSDs, ischemic histological changes emerged in the cortical watershed areas between the middle-anterior and middle-posterior cerebral arteries. In conclusion, CSD has the potential to cause a delayed ischemic injury at hemodynamically vulnerable brain areas although majority of the cortex and subcortical areas remain histologically intact as generally accepted. This finding may account for the WMLs seen in MA.

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Poster

223. Demyelinating Disorders: Human and Animal Studies

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Topic: C.09. Demyelinating Disorders

Title: Clinical difference between idiopathic optic neuritis and viral optic neuritis

Authors: *S. KIM;
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Abstract: Introduction Optic neuritis (ON) is a demyelinating disease of the optic nerve. Most of the ON cases in the Asian population are classified as idiopathic. Idiopathic ON may be associated with a variety of systemic autoimmune disorders, such as multiple sclerosis (MS). But in some cases, especially ON in children, viral infection is known to be associated. A previous study by the optic neuritis study group (ONTT) showed that 26.1% of ON patients had a viral syndrome preceding visual loss, but the characteristics of the viral infection-associated group were not presented. In this context, our study was conducted to analyze the features of ON associated with viral infection. Method A retrospective chart review was performed on 124 patients with ON over 15 years old who visited a tertiary referral center in Korea from 2004 to 2014. Demographics, symptoms, signs and laboratory results were reviewed. Magnetic resonance images (MRI) and cerebrospinal fluid findings were also analyzed. Result Seventeen out of 124 ON patients had history of viral infection. Ten patients had history of upper respiratory infection symptoms, 2 patients had myalgia, 2 patients had fever, 2 patients had history of vaccination (for epidemic hemorrhagic fever and influenza) and one patient had history of herpes simplex infection on lips. There were 9 males (52.9%) and mean (±SD, range) age of onset was 36.0 7(±12.82, 18-64) years. Ten (76.9%) patients initially presented with poor visual acuity, equal or worse than counting finger. Ocular pain or headache was accompanied in 82.4% of cases. The optic disc swelling was observed in 41.2% of the patients. Good visual outcome, equal or better than 20/40, was observed in 84.6%. Bilateral optic neuritis was observed in 3 cases and recurrent optic neuritis was observed in 3 cases. CSF analysis was evaluated in 12 patients and none of them showed significant abnormal result. Antinuclear antibody was positive in 3 out of 12 patients. Central scotoma was observed in 7 patients, and other variable field defects were also observed. Optic nerve enhancement in MRI was observed in 8 out of 15 cases. Spine MRI was evaluated in 3 patients, and 2 patients showed increased signal intensity in T2 weighted image. Conclusion We focused on a group of viral infection-associated optic neuritis (VON) patients and compared it with idiopathic ON. VON showed distinct characteristics: (1) Lower female ratio (2) Poor initial visual acuity (3) Lower recurrence. Some similar features were also observed: (1) Headaches (2) Good visual prognosis (3) Optic disc swelling. These findings suggest that VON may differ in the cause itself but share similar pathophysiological pathways with other types of ON.

Disclosures: S. Kim: None.

Poster

223. Demyelinating Disorders: Human and Animal Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: C.09. Demyelinating Disorders

Title: Single cell analysis of multiple sclerosis cerebrospinal fluid B cells shows predominant IgM response in some MS patients

Authors: *J. LIN, P. H. AU, S. A. SADIQ;
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Abstract: Introduction: B cells are more and more recognized as an important immune component in the pathogenesis of MS. Traditionally CSF IgGs (oligoclonal bands) have always been a diagnostic biomarker of MS, and the subject of much research because the IgG is thought to represent an antigen driven response; CSF IgMs have not been as widely investigated. Recent publications indicate that the presence of CSF IgMs may be involved in more aggressive forms of MS, or may be a predictor of future conversion to clinical definite MS. In our study, we attempt to determine the isotype of the MS B cell response. Methods: CSF cells from MS patients were stained for CD19 and CD138. Single cells were sorted via FACS for CD19 or CD138 positivity. Gene specific primers for immunoglobulin gamma (IgG) and immunoglobulin mu (IgM) were used for reverse transcription. Nested PCRs were performed to amplify the variable region and a small portion of the constant region. Sequence analysis identified the immunoglobulin isotype. Results: Twenty of 47 patients who had single B-cell sequence analysis revealed a predominant IgM isotype (at least 50% or greater IgM cells). This predominant IgM response does not appear to correlate with disease type, disease activity, or even disease duration. In 3 patients with longitudinal samples, the relative amounts of IgM expressing B cells appear relatively consistent across samples: patient 1, 80% and 63%; patient 2, 40% and 38%; patient 3, 2% and 19%. Conclusion: IgM expressing B cells appear to be the main B cell repertoire in some MS patients. This result does not appear to correlate with disease type, disease phase and/or disease duration. Longitudinal data suggest that this IgM response is not a transient phenomenon and appears to be specific to that patient. Our results suggest different immune mechanisms in some MS patients affecting their B cells other than the classic antigen driven IgG response and should lead to further investigation.

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Poster

223. Demyelinating Disorders: Human and Animal Studies

Location: Hall A

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Program#/Poster#: 223.13/F30

Topic: C.09. Demyelinating Disorders

Title: Ultrastructure of the lower motor system in a mouse model of Krabbe disease (KD)

Authors: *V. CAPPELLO[1], P. PARLANTI[2,3], L. MARCHETTI[3], I. TONAZZINI[4], M. CECCHINI[4], V. PIAZZA[2], M. GEMMI[2];
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Abstract: Krabbe disease (KD) is a devastating pathology (Krabbe, 1916), caused by the deficiency of lysosomal enzyme GALC activity that induces the accumulation of the lipid-raft-associated neurotoxin psychosine. KD is characterized by the apoptosis of myelinating cells, axonopathy and activation of inflammatory processes. A treatment for KD is still missing; for this reason the identification of new hallmarks could be a key aspect for the development of therapies. In this study we used a transmission electron microscopy approach to study the lower motor system (LMS) of the Twitcher mouse (TWI), homozygous for the inactive form of GALC and adopted as KD experimental model, at a late pathological stage (P30). We collected information of pathological condition in the central and in the peripheral nervous system (CNS and PNS) and correlated, for the first time, those observations with the architecture of skeletal muscle. Our data clearly demonstrate that alterations, mild or absent in the bodies of motor neurons in the spinal cord (CNS), grow stronger moving towards the PNS and towards the gastrocnemius muscle (GM) which was found to suffer from denervation and showed evident signs of inflammation. These observations allow us to claim unambiguously that in TWI mice the pathology follows a dying-back progression. Furthermore, in TWI sciatic nerves we identified a peculiar sub-population of myelinating cells that wrap more than one axon contrarily to the usual behavior of SC. Multiple myelination processes were observed in the all the TWI sciatic nerves observes but the same phenomenology could not be found in any of the WT nerves. Due to their morphology and to the proximity to Remak bundles, it is possible to speculate that non-myelinating SCs implement a compensatory mechanism to restore, at least partially, the level of myelination in the sciatic nerve of TWI mice. In the sciatic nerve both motorneurons and the Schwann cells (SC) are affected and the number of myelinated axons is significantly reduced. The morphometric evaluation of those remaining showed larger axons with a disorganized cytoskeleton and thicker myelin sheaths. In the GM we identified a reduced percentage of innervation of the neuromuscular junctions accompanied to strong signs of tissue inflammation affecting both the mitochondrial pool and the sarcoplasmic reticulum. In conclusion, we performed a complete ultrastructural analysis of the LMS identifying the progression of the disease and observing for the first time other alterations that can be exploited as targets for novel therapies and for testing their effectiveness.

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Poster

223. Demyelinating Disorders: Human and Animal Studies

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Topic: C.09. Demyelinating Disorders

Title: Three cases of osmotic demyelination syndrome with an unusual onset and a favorable outcome

Authors: A. FUKUI, X. CAO, M. TAJITSU, J. NAGAI, T. YAMADA, Y. YAMBE, *T. MURASE;
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Abstract: [Background] Osmotic demyelination syndrome (ODS) is a demyelinating brain disease resulting from a rapid increase in plasma osmolality. We present three cases with an unusual onset and a favorable outcome. [Patients] Case 1: An HIV-infected 49-year-old man became infected with influenza virus. One week later, his serum Na+ was 106 mEq/L. Two weeks later, he was admitted to hospital because of malaise, gait disturbance, dysarthria and cognitive dysfunction, and his serum Na+ was 139 mEq/L. MRI revealed T2-hyperintense lesions in his central pons, bilateral basal ganglia, and caudate nuclei, leading to a diagnosis of extra and central pontine myelinolysis (CPM). Although subsequent MRI showed demyelinating lesions more clearly, his neurological deficits were gradually improved by rehabilitation, and recovered almost completely within 4 months of admission. Case 2: A 59-year-old man was admitted to hospital because of acute renal failure resulting from pneumonia and dehydration. His serum Na+ was 133 mEq/L, which increased to 161 mEq/L in 4 days. Dysarthria, disorientation, and orthostatic hypotension persisted. Three weeks after admission, MRI showed a T2-hyperintense lesion in his central pons, indicating CPM. His neurological symptoms were gradually improved by rehabilitation. Five months later, he almost completely recovered, but the MRI lesion in his pons remained. Case 3: A 60-year-old man was admitted to hospital because of malaise and impaired consciousness. His blood glucose was 667 mg/dL, HbA1c 16.6%, Na+ 132 mEq/L, plasma osmolality 318 mOsm/kg. CT showed a hypointense lesion in his central pons. After admission, disorientation persisted and he showed dysphagia. MRI 7 days after admission revealed a T2-hyperintense lesion in his pons, indicating CPM. Thereafter, his neurological symptoms gradually improved. MRI 5 months after hospitalization showed an improved hyperintense lesion, and his neurological symptoms had almost completely disappeared. [Discussion] These findings suggest that it remains difficult to prevent the onset of ODS. In Case 1, hyponatremia, possibly caused by the syndrome of inappropriate antidiuretic hormone secretion as a result of influenza virus infection, spontaneously resolved, resulting in ODS. In Case 2, it is remarkable that ODS occurred despite the not so low initial serum Na+ level. In Case 3, ODS appears to have occurred because of hyperglycemia before admission. An almost complete improvement of neurological symptoms occurred in all cases, consistent with reports

that a prognosis of ODS is not as dismal as previously thought. We note that neurological symptoms did not correlate with the changes in MRI lesions.

Disclosures: **A. Fukui:** None. **X. Cao:** None. **M. Tajitsu:** None. **J. Nagai:** None. **T. Yamada:** None. **Y. Yambe:** None. **T. Murase:** None.

Poster

223. Demyelinating Disorders: Human and Animal Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 223.15/F32

Topic: C.09. Demyelinating Disorders

Title: Neuroprotective effects of dihydrotestosterone in chronic experimental autoimmune encephalomyelitis

Authors: ***R. C. MELCANGI**[1], S. GIATTI[2], S. ROMANO[2], M. PESARESI[2], N. MITRO[2], B. VIVIANI[2], L. GARCIA-SEGURA[3], D. CARUSO[2];
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Abstract: Multiple sclerosis (MS) is a chronic inflammatory disease affecting the central nervous system. Clinical observations suggest that hormonal level alterations might modify disease progression and incidence. Specifically, decreased levels of testosterone are permissive for disease onset in male subjects. Accordingly, in the experimental autoimmune encephalomyelitis (EAE) model of MS, testosterone seems to exert protective effects. In this context, it is important to highlight that testosterone may be further metabolized into 17beta-estradiol or dihydrotestosterone (DHT). Based on this, the effects of DHT treatment in EAE Dark Agouti rats (i.e. an experimental model showing a protracted relapsing EAE) were analyzed. After 45 days from EAE induction, DHT treatment exerts beneficial effects on clinical score, on gliosis (i.e., decreased glial fibrillary acidic protein and major histocompatibility complex of class II staining) and on inflammation (i.e., decreased Translocator Protein 18kDa, interleukin-1beta, Toll Like Receptor 4 and nuclear factor kappa B expression) in spinal cord. Moreover, parameters linked to oxidative stress and tissue damage, like thiobarbituric acid-reactive substances levels and Bcl-2 associated X protein expression, and to mitochondrial activity (i.e., content of mitochondrial DNA and proteins), were improved after DHT administration. Furthermore, the metabolism of DHT into 3alpha- or 3beta-diol was analyzed. Indeed, assessment of the levels of these metabolites after DHT treatment seem to suggest that the protective effects here observed are due to DHT itself. Altogether, the present results indicate that DHT was effective in reducing the severity of chronic EAE and, consequently, may represent an interesting perspective for MS treatment.

Disclosures: **R.C. Melcangi:** None. **S. Giatti:** None. **S. Romano:** None. **M. Pesaresi:** None. **N. Mitro:** None. **B. Viviani:** None. **L. Garcia-Segura:** None. **D. Caruso:** None.

Poster

223. Demyelinating Disorders: Human and Animal Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 223.16/F33

Topic: C.09. Demyelinating Disorders

Support: Brian's Hope Foundation

Title: Blood plasma measurements of oxidative stress show phenotype specificity in x-linked adrenoleukodystrophy

Authors: B. R. TURK[1], C. TIFFANY[1], J. MARX[1], R. JONES[1], A. B. MOSER[1], *A. FATEMI[2]; [1]Kennedy Krieger Institute, Johns Hopkins Univ., Baltimore, MD; [2]Neurol., Johns Hopkins Univ., Baltimore, MD

Abstract: Background The disease process of X-linked adrenoleukodystrophy (ALD), a hereditary peroxisomal disorder is attributed to the mutation and dysfunction of the ALD protein, which leads to the accumulation of very long chain fatty acids (VLCFA), in turn leading to oxidative stress and cell death. Clinically, ALD is characterised by an unpredictable phenotypic variance and shift, even between monozygotic twins. Common presentation phenotypes are progressive spinal axonopathy, adrenomyeloneuropathy (AMN) or rapid cephalic demyelination, cerebral ALD (cALD). In this study, oxidative stress parameters were assessed in cALD, AMN, heterozygote female carriers. Methods Total antioxidant content (TRAP), glutathione (GLT), superoxide dismutase (SOD) and prostaglandin E2 (PGE2) were assessed in human plasma and fibroblast cell culture media. Results cALD patients (n=8) showed significantly lower ($p<0.0003$) plasma SOD levels than AMN patients (n=15), who in turn showed a lower tendency than both female heterozygote carriers (n=10) and age matched control subjects (n=9). No significant difference in glutathione was found in plasma. Cell culture media showed significantly higher PGE2 in cALD patients (n=6) compared to AMN patients (n=6), who in turn had lower levels than in controls and female heterozygotes. This differential in PGE2 content was mirrored by levels of phospholipase A2. Discussion SOD functions as an initial antioxidant barrier to the highly reactive radical superoxide anion in the brain. The decreased levels of SOD and increased proinflammatory cytokines in plasma and culture media of cALD patients may reflect higher levels of oxidative stress and/or inflammation, allowing insight into previously unreported phenotypic specific pathology as well as supporting biomarker development for future clinical trials.

Disclosures: **B.R. Turk:** None. **C. Tiffany:** None. **J. Marx:** None. **R. Jones:** None. **A.B. Moser:** None. **A. Fatemi:** F. Consulting Fees (e.g., advisory boards); BlueBirdBio, Inc..

Poster

223. Demyelinating Disorders: Human and Animal Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 223.17/F34

Topic: C.09. Demyelinating Disorders

Title: The effects of psychosocial stress during the remyelination phase in an animal model of multiple sclerosis

Authors: *M. MAKINODAN, K. YAMAMURO, D. IKAWA, Y. YAMASHITA, M. TORITSUKA, T. YAMAUCHI, S. FUKAMI, T. KISHIMOTO;
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Abstract: Recent findings have shown that psychosocial factors are implicated in the pathobiology of multiple sclerosis. Most of studies regarding psychosocial stress have focused on the onset or the relapse of the symptoms, that is, have aimed to examine the effects on the "demyelination". In this study, we sought to investigate whether psychosocial stress affects the biology in the remyelination phase since our previous study revealed that psychosocial stress dramatically changes myelination only during the developing phase of myelin (Makinodan et al., 2012). Myelin in the medial prefrontal cortex was depleted with cuprizone-treatment for four weeks and we observed whether the subsequent psychosocial differences for four weeks affect remyelination in the medial prefrontal cortex following the termination of cuprizone-treatment. We employed social isolation as a psychosocial stress and the four groups were compared; four mice in a cage (regular environment, RE) after no demyelination, one mouse in a cage (isolation, IS) after no demyelination, RE after demyelination, and IS after demyelination. Interestingly, remyelinatoin in the medial prefrontal cortex was severely impaired only in the group of IS after demyelination, and the medial prefrontal cortex-dependent behaviors were also altered only in the same group.

Disclosures: **M. Makinodan:** None. **K. Yamamuro:** None. **D. Ikawa:** None. **Y. Yamashita:** None. **M. Toritsuka:** None. **T. Yamauchi:** None. **S. Fukami:** None. **T. Kishimoto:** None.

Poster

223. Demyelinating Disorders: Human and Animal Studies

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Title: Relating white matter "potholes" to polygenic risk for Multiple Sclerosis

Authors: *D. RINKER[1], G. PRASAD[2], M. RENTERIA[3], N. JAHANSHAD[2], D. P. HIBAR[2], K. L. MCMAHON[4], G. I. DE ZUBICARAY[5], G. MONTGOMERY[3], N. G. MARTIN[3], M. J. WRIGHT[3], P. M. THOMPSON[2];
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Abstract: The pathogenesis of multiple sclerosis (MS) is currently unknown. Complex polygenic influence is thought to play a substantial role in disease risk. Previous analyses have looked at the relationship between polygenic risk scores and white-matter status as defined by voxel-wise fractional anisotropy. However, because MS lesions do not follow a predictable pattern in the white matter, we wanted to assess if there is any explanatory effect of polygenic risk scores on white matter status in healthy subjects. We analyzed data diffusion tensor imaging (DTI) and genotyping data from 528 healthy Australians (mean age 23.6 ± 2.2 years) scanned for the Queensland Twin Imaging Study. To calculate MS polygenic risk scores, the 102 most significant SNPs from the largest known MS GWAS [1] were LD pruned to leave 76 unique variants. Scores were then calculated from each subject's total number of risk alleles weighted by the GWAS-reported SNP effect, and controlled for missing SNPs. White-matter 'potholes' [2] were quantified for each subject by creating z-transformed FA maps, where a pothole was defined as any voxel with a preset number of standard deviations away from the subject's mean (.5-3). Data were split into two separate groups for analysis and replication; linear regression was used to relate potholes to polygenic risk scores, controlling for age and sex. In one subset, number of potholes (SD=.5) was significantly related to polygenic risk score (P=.03), but the replication set failed to reach significance (P=.3). With a more stringent definition of potholes (SD=2) the results failed to reach significance in each group (p=.08 and p=.1), but trended in the same positive direction relating polygenic risk scores to number of potholes. Similar results were found across thresholds. This preliminary analysis is an attempt to assess subtle genetic effects on white matter where consistent spatial differences between subjects are not hypothesized.
REFERENCES: [1] International Multiple Sclerosis Genetics Consortium & The Wellcome Trust Case Control Consortium 2, Nature, 2011. [2] White T, Schmidt M, Karatekin C. White

matter 'potholes' in early-onset schizophrenia: a new approach to evaluate white matter microstructure using diffusion tensor imaging. Psychiatry Res. 2009

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Poster

223. Demyelinating Disorders: Human and Animal Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 223.19/F36

Topic: C.09. Demyelinating Disorders

Support: State of Hessen (LOEWE)

Title: Alpha-methylacyl-CoA racemase regulates the T Cell response in experimental autoimmune encephalomyelitis

Authors: N. TAFFERNER[1], J. BARTHELMES[2], M. EBERLE[2], N. FERREIROS[2], T. ULSHÖFER[1], M. HENKE[1], A. WEIGERT[3], *N. DE BRUIN[1], G. GEISSLINGER[1], M. J. PARNHAM[1], S. SCHIFFMANN[1];
[1]Fraunhofer Inst. Mol Biol and Appl Ecol (IME), Frankfurt Am Main, Germany; [2]Inst. of Clin. Pharmacol., pharmazentrum frankfurt/ZAFES, Frankfurt, Germany; [3]Inst. of Biochem. I, Goethe-University, Frankfurt, Germany

Abstract: Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system and alteration of the metabolism of immune cells is an attractive strategy to modify their function during autoimmunity in MS. We investigated the effect of modulating fatty acid metabolism in an animal model of multiple sclerosis, myelin oligodendrocyte glycoprotein (MOG)35-55-induced, chronic progressive, experimental autoimmune encephalomyelitis (EAE) in C57/BL6 mice. Alpha-methylacyl-CoA racemase (AMACR) converts R-configurated branched fatty acids into the S-configuration, thereby preparing them for metabolic β-oxidation. We observed significant, selective, disease-dependent elevation of AMACR expression in various immune cells (monocytes, T Cells) isolated from blood, draining lymph nodes and spleen in EAE mice during the preclinical phase. *In vitro* studies revealed that genetic deletion of AMACR inhibits the proliferation of T Cells. Furthermore, activated T Cells isolated from AMACR KO mice are characterized by a higher production of IFN-γ, IL-17 and IL-10 and a lower production of IL-4 in comparison to T Cells with a wild type background. However, AMACR KO mice showed only a slight worsening of early clinical symptoms of EAE and no alteration in cognitive behavior in comparison to wild type mice. Interestingly, in the lymph nodes of AMACR KO EAE mice the cytokines are similar regulated as in *in vitro* activated T

Cells. AMACR was not regulated in white blood cells of MS patients. In conclusion, our data suggest that AMACR is regulated in immune cells during EAE but it is not essential for the development of EAE.

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Poster

223. Demyelinating Disorders: Human and Animal Studies

Location: Hall A

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Topic: C.09. Demyelinating Disorders

Support: This study was supported in part by a Grant from Teva Pharmaceutical Industries (Israel) (P.L.).

Title: Brain galanin is regulated in an animal model of multiple sclerosis

Authors: *P. LOPRESTI;
Dept. of Physiol. and Biophysics, Univ. of Illinois At Chicago, Chicago, IL

Abstract: The neuropeptide Galanin has been implicated in both neuroprotection and neurodegeneration. Galanin is overexpressed in the basal forebrain during Alzheimer's disease; an excess of Galanin has been implicated in cognitive impairment during the progression of this disease. Galanin is known to be expressed in inflammatory and non-inflammatory cells. Furthermore, Wraith et al., (2009) showed that Galanin expression is specifically and markedly up regulated in microglia in multiple sclerosis lesions and shadow plaques; whereas oligodendrocytes in the spinal cord upregulated Galanin in an animal model of multiple sclerosis, i.e. Experimental Autoimmune Encephalomyelitis (EAE). This study examined brain tissues during the second week post EAE induction, and found an increased in Galanin-positive fibers. Furthermore, our studies found that Galanin was not expressed in brain astrocytes and oligodendrocytes. Since Galanin-positive nerves can normally be detected only in a few dorsal root ganglion neurons and they are dramatically up regulated after peripheral nerve injury in both rat and monkey (Zhang et al., 2006), the upregulation of brain Galanin-positive nerves during EAE may be secondary to the nerve injury taking place during this disease. Experiments are in progress to establish time course of brain Galanin regulation and whether brain Galanin has a protective or a deleterious role. Zhang X, Xu ZO, Shi TJ, Landry M, Holmberg K, Ju G, Tong YG, Bao L, Cheng XP, Wiesenfeld-Hallin Z, Lozano A, Dostrovsky J, Hökfelt T. (1998) Regulation of expression of galanin and galanin receptors in dorsal root ganglia and spinal cord after axotomy and inflammation. Ann N Y Acad Sci. 863:402-13. Wraith DC, Pope R,

Butzkueven H, Holder H, Vanderplank P, Lowrey P, Day MJ, Gundlach AL, Kilpatrick TJ, Scolding N, Wynick D.(2009) A role for galanin in human and experimental inflammatory demyelination. Proc Natl Acad Sci U S A. 106:15466-71. doi: 10.1073/pnas.0903360106. Epub 2009 Aug 26.

Disclosures: P. LoPresti: None.

Poster

223. Demyelinating Disorders: Human and Animal Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 223.21/F38

Topic: C.09. Demyelinating Disorders

Support: Applied Psychology Center Research Development Grant

Title: Effects of brief aquatic exercise on cardiovascular fitness and cerebral oxygenation in Multiple Sclerosis

Authors: J. PETERSEN[1], D. CALVO[2], H. GERHART[1], M. SPITZNAGEL[2], *A. L. RIDGEL[1]; [1]Exercise Physiol., [2]Psychology, Kent State Univ., Kent, OH

Abstract: Multiple Sclerosis (MS) is the leading cause of non-traumatic neurologic disability in adults. Demyelination of neuronal axons results in inflammation, focal plaque formation, decreased oxygen utilization, and reduced absolute cerebral blood flow. As a result, people with MS are less physically active and deconditioned which further compromises activities of daily living. Although physical rehabilitation and exercise are an important treatment component in MS, fatigue and overheating limit the abilities of this population. The impact of high intensity exercise on fitness and cerebral oxygenation has not been well studied in MS. We examined the effects of seven consecutive days of aquatic aerobics on cardiovascular fitness, assessed with the two minute step test, and cerebral oxygenation, measured with near-infrared spectroscopy (NIRS). Although fitness improvements were minimal, there was a 45-65% increase in cerebral oxyhemoglobin after the seven day intervention. In addition, there was a 35-55% decrease in cerebral deoxyhemogloblin. These findings suggest that a high intensity aquatic aerobics program can increase cerebral blood flow, and potentially improve quality of life in Multiple Sclerosis.

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Poster

223. Demyelinating Disorders: Human and Animal Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 223.22/F39

Topic: C.09. Demyelinating Disorders

Title: The birth seasonality in multiple sclerosis: Implications from a similar seasonality found among gay men and an opposite seasonality found among both lesbian women and powerful baseball hitters

Authors: *G. MARZULLO;
Per Aspera Res. Fndn., New York, NY

Abstract: Multiple sclerosis, which occurs 2 or 3 times more often in women than in men and more often at higher (darker) than at lower latitudes, has also shown a birth seasonality with an excess around May and an equally significant deficit six months later around November. We recently found this same birth seasonality among gay men and a diametrically opposite seasonality among both lesbian women and more powerful classes of professional baseball hitters (Marzullo, 2014). Based on evidence that two major maternal messengers of short day-lengths (which are also major antioxidants), melatonin (MEL) and reduced glutathione (GSH), can both suppress fetal testosterone (T) formation and/or function in mammalian species, we proposed a sunlight hypothesis prompted by the coincidence of 17th or 18th-week peak stages of T-dependent fetal male-female differentiation with the summer solstice (SS) in early-Nov births and the winter solstice (SW) in early-May births. This held that the seasonality of sexually-dimorphic development in the newborn reflected the sunlight-dependent seasonality of T activity in fetus. A more in-depth version of this same "solstitial" hypothesis may also explain the MS birth seasonality. This follows from evidence that the processes of neuronal myelination (NM) are intertwined in time as well as in cell death-dependent mechanisms with those responsible for sexually dimorphic neurogensis (SDN). NM was thus found to peak in the human fetus around the same 18th post-conceptional week as the peaks in T formation and sexual differentiation. NM and SDN were also both found to be critically dependent on apoptotic cell death and therefore on the action of Bax proteins in triggering the apoptotic reaction cascade. Bax-/- mice mutants thus failed to properly myelinate as well as to sexually differentiate. Most interestingly, GSH and MEL were found to play critical roles in, respectively, the first and the last event in the apoptotic cascade. The cascade was thus found to be triggered by a precipitous extrusion of intracellular GSH with a resulting relative rise in oxidized GSH (GSSG). Higher extracellular levels of GSH were found to oppose this sudden loss of cellular redox potential and cause apoptotic resistance. Lower extracellular GSH levels did the opposite. MEL was found to inhibit the last reaction in the chain, one that activates fatal mitochondrial caspases. GSH and Mel thus appeared to act synergistically in ways that minimized apoptosis near the WS and maximized it near the SS. Such "sunlight messenger" functions of MEL and GSH in multicellular species appeared derived from their primary functions as sunlight-protective antioxidants in earlier species.

Disclosures: **G. Marzullo:** None.

Poster

223. Demyelinating Disorders: Human and Animal Studies

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Program#/Poster#: 223.23/F40

Topic: C.09. Demyelinating Disorders

Support: ARSEP (association pour la recherche sur la sclérose en plaques)

Title: Immunointervention in experimental autoimmune encephalomyelitis by targeting the protease site of endothelial NMDA receptor

Authors: ***R. M. MACREZ**[1], C. ORTEGA[2], A. FOURNIER[1], S. VAN DER POL[3], A. MERHA[1], E. MAUBERT[1], F. LESEPT[1], A. CHEVILLEY[1], B. HAELEWYN[4], F. DE CASTRO[2], E. DE VRIES[3], D. VIVIEN[1], D. CLEMENTE[2], F. DOCAGNE[1];
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Abstract: Background: Recent advances suggest a role for NMDAR in immunological mechanisms of brain diseases. Here, we tested an original therapeutic strategy of immunointervention in animal models of multiple sclerosis (MS) based on a newly developed therapeutic anti-NMDAR antibody. This antibody targets a regulatory site of the GluN1 subunit sensitive to the protease tPA (tissue plasminogen activator). We aimed at identifying the cell targets with a particular focus on immune cells and components of the blood brain barrier (BBB). Methods: Antibody was injected in C57/Bl6 mice subjected to EAE, a model of MS. Neurological assessment was performed via clinical score scale, and using automated activity measurement. Inflammation in animals was studied by 3D-T2* molecular MRI. Immune cells were analysed by flow cytometry (FACS) and immunohistology. Modulation of NMDAR function by our antibody was characterized by Ca2+ videomicroscopy in HEK cells expressing NMDAR. Its impact was evaluated *in vitro* on splenocytes and leukocyte transcytosis assay. Findings: We show that our antibody blocks tPA-induced potentiation of NMDA-R function without affecting basal NMDA-R activity. In EAE animals, it reduced neurological impairments (delayed onset and reduced clinical score) and inflammation (reduced signal void in MRI, reduced leukocyte infiltration, BBB preservation and switches in splenic cell populations). *In vitro*, our antibody reduces leukocyte transmigration, and we investigated its effects on lymphocyte activation, proliferation and survival. This study argues for a critical function in MS of NMDAR expressed outside the nervous system, and highlights the therapeutic potential of anti-NMDA-R based immunointervention.

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Poster

223. Demyelinating Disorders: Human and Animal Studies

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Title: Galectin-3 is a possible target molecule for anti-brain microvascular endothelial cell antibodies in patients with secondary progressive multiple sclerosis

Authors: *H. NISHIHARA, F. SHIMIZU, Y. SANO, Y. TAKESHITA, M. ABE, T. MAEDA, T. KANDA;
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Abstract: Objective: We previously reported that autoantibodies against human brain microvascular endothelial cells (hBMECs) from secondary progressive multiple sclerosis (SPMS) patients could compromise the blood-brain barrier (BBB). In this study, we investigate a target for anti-brain microvascular endothelial cell antibodies in patients with SPMS. Methods: Proteins from hBMECs were separated by 2-dimentional electrophoresis. Two dimentional immunoblots were used to compare serum reactivities from pooled sera of 7 SPMS patients and those of controls (9 relapsing-remitting MS (RRMS), 14 neuromyelitis optica (NMO), 9 amyotrophic lateral sclerosis (ALS), 10 myositis patients, and 20 healthy controls). Proteins were identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Afterwards individual serum reactivities against target antigens were confirmed by western blot analysis. Identified protein functions in hBMECs were examined using small interfering RNA (siRNA)

method. Result: We identified galectin-3 as a target antigen in SPMS patients group. Positivity of the anti-galectin-3 antibodies was significantly higher in patients with SPMS (90 %, 9 of 10) than in patients with RRMS (30 %, 3 of 10). The anti-galectin-3 antibodies were not found in all the patients with NMO, ALS, myositis, or in healthy controls. Protein and mRNA levels of NFκB and ICAM-1 in hBMECs were elevated after the downregulation of galectin-3. Sera from the patient with SPMS also increased the protein amounts of NFκB and ICAM-1 in hBMECs. Conclusion: In SPMS patients, anti-galectin-3 antibody might compromise BBB by upregulating NFκB and downstream target of NFκB like ICAM-1.

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Poster

223. Demyelinating Disorders: Human and Animal Studies

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Title: Diverse nicotinic mechanisms in a model of multiple sclerosis

Authors: *R. J. LUKAS[1], Q. LIU[1], L. LUCERO[1], A. R. SIMARD[2], P. WHITEAKER[1], B. J. MORLEY[3], F.-D. SHI[1];
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Abstract: Several lines of evidence implicate acetylcholine (ACh) in modulation of immune system as well as nervous system function. This testifies to progressive specialization of ACh from its primordial roles in chemical signaling early in the evolution of life forms. Focusing on nicotinic mechanisms, our earlier work defined nicotinic ACh receptor (nAChR) subunit gene expression patterns in mouse and human immune system cells and demonstrated nicotinic suppression of T cell development and differentiation. This suggested that nicotine could have

immunomodulatory and anti-inflammatory effects. Further studies using experimental autoimmune encephalomyelitis (EAE) in mice as a model for multiple sclerosis (MS), coupled with exploitation of a range of nAChR subunit knock out mice, suggests that different nAChR subtypes play disease exacerbating or protective roles in different stages in disease progression and recovery, building upon initial observations that nicotine protects against EAE. We have extended our characterization of nAChR subunit mRNA levels in different immune cell types in the periphery or infiltrating into the brain, including T cells, B cells, dendritic cells, macrophages, and microglia, finding remarkably widespread expression that changes with disease stage and cell microenvironment. Results continue to support the hypotheses: (1) that peripheral immune cell nAChR containing α9 subunits (α9*-nAChR) play disease initiating/exacerbating roles as revealed by their attenuation in α9 subunit knockout mice or via nicotine's antagonism of α9*-nAChR, (2) that there are neuroprotective roles of α7-nAChR, probably expressed by brain cell types, and made particularly evident in α7/α9 double knock out mice, and (3) that β2-nAChR are involved in recovery from EAE, which is absent in β2 subunit knock out mice. Adoptive transfer studies support these hypotheses, and additional nAChR subunits and subtypes also could play roles. Taken together, our results suggest that modulation of disease-exacerbating or disease-ameliorating inflammatory and/or immune processes is possible through therapeutic approaches targeting nicotinic signaling. There are exciting possibilities that improved treatment of hyperimmune and inflammatory disorders such as MS, lupus and arthritis, and even stroke, brain cancer or neurodegenerative diseases, could result from nAChR modulation.

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Poster

223. Demyelinating Disorders: Human and Animal Studies

Location: Hall A

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Myelin Repair Foundation Grant 31677

Title: Oligodendrocyte death in the DTA mouse model results in late-onset immune-mediated CNS demyelination

Authors: ***M. TRAKA**[1], J. R. PODOJIL[2], D. P. MCCARTHY[2], S. D. MILLER[2], B. POPKO[1]; [1]Univ. of Chicago, Chicago, IL; [2]Microbiology-Immunology and Interdepartmental Immunobiology Ctr., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Multiple sclerosis (MS) is an inflammatory neurodegenerative disease characterized by CNS demyelination induced by myelin-specific T lymphocytes. Although the primary etiology of MS remains unknown, the main hypothesis suggests that a primary dysregulation of the immune system exists that leads to an autoreactive response against myelin-sheath components, which secondarily leads to a breakdown of the blood-brain barrier. This is followed by infiltration of the CNS by T cells, leading to the focal inflammation and demyelination that characterize MS lesions. An alternative hypothesis is based on pathological evidence showing that oligodendrocyte loss and myelin defects occur in the brains of MS patients even in the absence of apparent signs of inflammation. Thus, the loss of oligodendrocytes and subsequent demyelination might trigger the autoreactivity against myelin antigens and, secondarily, lead to inflammation and demyelination in the CNS. To investigate the second hypothesis, we used the DTA (PLP/CreERT;ROSA26-eGFP-DTA) mouse model (Traka et al., Brain 2010 Oct;133(10):3017-29), in which activation with tamoxifen causes pervasive oligodendrocyte loss resulting in widespread CNS demyelination, that peaks at 5 weeks and resolves by 10 weeks. Strikingly, a fatal, secondary demyelinating disease follows the early disease around 40 weeks post-activation. The late-onset disease in DTA mice is characterized by the presence of focal, MS-like, actively demyelinating lesions that progress to extensive myelin and axonal loss at later disease stages and is associated with increased numbers of activated CD4+ T cells in the CNS and myelin oligodendrocyte glycoprotein (MOG)-specific T cells in peripheral lymphoid organs. We confirmed the pathogenic potential of the MOG-specific DTA-derived T cells by adoptively transferring them to naïve Rag1-deficient mice that produce no mature T cells and B cells; this approach resulted in neurological defects in the DTA cell-recipient mice that correlated with CNS white matter inflammation. Furthermore, we significantly ameliorated the late-onset disease symptoms in the DTA mice by immune tolerization against MOG. Overall, these data suggest that primary oligodendrocyte death is sufficient to trigger an adaptive autoimmune response against myelin, raising the possibility that a similar process can occur in the pathogenesis of MS, consistent with the 'inside-out' hypothesis.

Disclosures: **M. Traka:** None. **J.R. Podojil:** None. **D.P. McCarthy:** None. **S.D. Miller:** None. **B. Popko:** None.

Poster

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Title: Visuomotor delay adaptation reduces intention tremor in multiple sclerosis: a case series

Authors: *M. HEENAN[1], R. SCHEIDT[1,2,3], S. BEARDSLEY[1,4,5];
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Abstract: We have previously shown that intention tremor in MS may be due to an inability to adapt to increased visual processing time. This inability creates a prediction error that the neuromotor controller attempts to minimize, which results in tremor. We characterized and simulated neuromotor feedback control in compensatory and pursuit tracking tasks. Preliminary results suggest that subjects with intention tremor may be trapped within locally-optimal region of control space and are unable to minimize error further because doing so would require accepting an initial increase in movement error. Simulations suggest that adaptation to gradually increasing feedback delays may induce subjects to learn an internal model of the visual response delay that matches their actual response delay. We now examine whether a visual delay adaptation task - as our simulations suggest - can be used to reduce tremor by gradually shifting subjects' expected delays. Subjects used a 1-D robot to track a target during a series of random step displacements. Cursor position had no added feedback delay (Sham Adaptation Session) or was delayed from hand position (Adaptation Session) using 3 delays that were specific to subjects' deficits. Tremor was examined before, immediately after, and 24-48 hours after the Sham Adaptation and Adaptation Sessions using spiral tracing, handwriting, the Nine Hole Peg Test (9HPT), and a step-tracking task. For three subjects with MS and moderate to severe intention tremor, no significant improvements across subjects were seen before and after the Sham Adaptation Session. Sumulations suggest that during the Adaptation Session, three additional feedback delays would be sufficient to induce adaptation of predicted visual response delay. Subjects adapted to each new feedback delay (ranging from 50-300 ms of additional delay) for approximately 15 minutes, followed by an "adaptation" to a no delay condition. After this time, submovement interval (a proxy for predicted visual delay) increased by 55.6±15.7% (mean±SE) but fell short of subjects' actual response delays by 25.2±7.2%. Tremor power decreased by 65.7±12.3% Movement endpoint error decreased by 22.7±13.4%. 9HPT scores improved by 7.5±4.5% in the dominant hand and by 23.2±3.2% in the non-dominant hand. Although there were not significant improvements in handwriting speed, handwriting quality improved in 2/3 subjects. In all subjects, at least one of these improvements persisted into the following day. These results suggest that visual delay adaptation can be used therapeutically to increase predicted visual delay and decrease intention tremor in MS.

Disclosures: **M. Heenan:** None. **R. Scheidt:** None. **S. Beardsley:** None.

Poster

223. Demyelinating Disorders: Human and Animal Studies

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Topic: C.09. Demyelinating Disorders

Support: CIHR MOP 97847

Title: Dominant influence of mood on neurophysiological responses to exercise in people with multiple sclerosis

Authors: ***L. M. KOSKI**[1], A. YUSUF[2];
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Abstract: BACKGROUND: Multiple sclerosis (MS) is associated with increased fatigability during exercise, which can have a significant impact on functional outcome. Neurophysiological studies of changes in corticomotor excitability during exercise may yield insights into potentially modifiable causes of this fatigability. This study aimed to identify predictors of abnormal motor evoked potentials (MEPs) responses to exercise among patients with relapsing-remitting MS. METHODS: Muscle strength, MEP amplitudes, and recovery of motor threshold were assessed in 36 MS patients and 14 healthy age- and sex-matched controls (HC) before and after a series of 30 maximum voluntary contractions (3-s on, 3-s off). Age, sex, self-reported fatigue, MS Functional Composite test, Hospital Anxiety and Depression Scale, and measures of cortical excitability (motor threshold, cortical silent period duration, short-interval intracortical inhibition) were also obtained at baseline. RESULTS: The difference in electromyographic activity during exercise was comparable in both groups (HC: -10.6 =/- 18.7; MS: -11.4 +/-38.6), as was the time to recovery of motor threshold post-exercise. After recovery of motor threshold, MEPs were facilitated in the HC group but not in the MS group. Among all potential contributing variables, only depressive symptoms predicted post-exercise facilitation, with higher depressive symptoms predicting lower post-exercise facilitation in the MS group ($r = 0.39$, $p = 0.04$). CONCLUSIONS: Similar results have been reported in the depression literature, highlighting the need to control for mood when investigating neurophysiological contributors to fatigue in MS.

Disclosures: **L.M. Koski:** None. **A. Yusuf:** None.

Poster

223. Demyelinating Disorders: Human and Animal Studies

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Program#/Poster#: 223.29/G2

Topic: C.09. Demyelinating Disorders

Title: Brain endothelium differs between mouse and humans with adrenoleukodystrophy

Authors: J. M. T. SNYDER[1], *P. L. MUSOLINO[3], Y. GONG[1], J. LOK[2], E. LO[2], F. S. EICHLER[1];
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Abstract: Cerebral X-linked adrenoleukodystrophy (ALD) is a progressive inflammatory demyelinating disease caused by mutations in the ABCD1 gene. A prominent perivascular infiltrate at the leading edge suggests that blood brain barrier disruption plays an important role in lesion progression. Recent in-vitro cell work indicates that ABCD1 deficiency causes upregulation of adhesion molecules and decreased tight junction protein expression, resulting in increased adhesion and permeability of human brain endothelium. A major barrier to progress in the field is that the current ALD mouse model, an ABCD1 KO mouse, never develops the cerebral form of the disease. Therefore, we set out to determine what molecular differences exist between human and mouse brain endothelium and between human brain and other organ specific endothelium. Using primary human brain microvascular (HBMEC), human umbilical vein (HUVEC) and human dermal microvascualar (HDMEC) endothelial cells, ABCD1 was silenced via siRNA. Molecular characterization was performed using RT-PCR arrays, western blot, CL-CTMS. Adhesion and transmigration assays were used to assess monocyte-endothelial cells interactions. We found that silencing ABCD1 in HUVEC and HDMEC does not cause significant changes in adhesion molecules, tight junction protein expression and adhesion of monocytes, suggesting that the molecular and functional changes in human endothelium due to ABCD1 deficiency are brain specific. Moreover, unlike human brain endothelium, ABCD1 deficiency in mouse brain endothelium results in an increase in tight junction protein expression and no change in adhesion molecule expression as compared to controls, even following endothelial activation with inflammatory cytokines. Together these data demonstrate that endothelial dysfunction characterized by increased adhesion and permeability to monocytes caused by the deficiency of ABCD1 is selective to human brain. Our findings also provide potential molecular targets for future intervention as well as creating an improved mouse model and functional assays for the cerebral form of ALD.

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Poster

224. Demyelinating Disorders: Animal Studies

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Topic: C.09. Demyelinating Disorders

Support: the Conrad N. Hilton Foundation

Title: Enhanced GABAA receptor-mediated tonic conductance in hippocampal CA1 pyramidal neurons in experimental autoimmune encephalomyelitis

Authors: *W. WEI[1], L. KAMMEL[1], R. R. VOSKUHL[1], T. J. O'DELL[2];
[1]Dept Neurol, [2]Dept Physiol, UCLA Sch. Med., Los Angeles, CA

Abstract: A number of recent studies have found that phasic inhibitory synaptic transmission mediated by synaptic $GABA_A$ receptors ($GABA_ARs$) is altered in experimental autoimmune encephalomyelitis (EAE), a mouse model of Multiple Sclerosis (MS). Importantly, persistent activation of extrasynaptic $GABA_ARs$ by ambient levels of GABA gives rise to a distinct form GABAergic inhibition known as tonic inhibition and it is unclear whether this form of inhibition is also altered in EAE. Here we investigated $GABA_AR$-mediated inhibitory transmission in hippocampal CA1 pyramidal neurons using whole-cell patch-clamp recordings to determine whether tonic inhibition is altered in EAE. We find that the $GABA_AR$-mediated tonic conductance is significantly enhanced in EAE (0.43 ± 0.07 pA/pF, n = 13 in control vs. 0.76 ± 0.06 pA/pF, n = 28 in EAE, $p < 0.01$). To determine whether reduced GABA transporter function and elevated levels of extracellular GABA might contribute to enhanced tonic inhibition in EAE, we examined tonic currents in the presence of the GABA transporter inhibitor nipecotic acid (10 μM). Although nipecotic acid strongly enhanced tonic inhibition, tonic inhibitory currents were still significantly larger in pyramidal cells from EAE mice (5.60 ± 0.49 pA/pF, n = 10 in control vs. 7.93 ± 0.62 pA/pF, n = 10, in EAE, $p < 0.01$). This suggests that reduced GABA transporter function is unlikely to account for enhanced tonic inhibition in EAE. In contrast, L-655,708 (5 μM), an inverse-agonist of the α5 subunit-containing $GABA_ARs$ responsible for tonic inhibition in CA1 pyramidal cells, reduced tonic inhibition to a greater extend in pyramidal cells from EAE mice (tonic inhibition was reduced by 6.8 ± 1.4% in control cells, n = 9 compared to 34.6 ± 8.1% in cells from EAE mice, n = 11, $p < 0.01$). However, the delta subunit-containing $GABA_AR$ agonist DS2 (2 μM) had a similar effect on tonic inhibition in both control and EAE cells ($p > 0.05$, n = 14 and 11, respectively). This suggests that enhanced tonic inhibition in EAE may be due to increased expression and/or function of α5 subunit-containing $GABA_ARs$. We also find that phasic inhibitory synaptic transmission is enhanced in EAE (spontaneous IPSC frequency was 18.13 ± 1.11 Hz, n = 12 in control vs. 24.64 ± 1.06 Hz, n = 28 in EAE, $p < 0.01$). Together our results indicate that multiple forms of inhibitory synaptic transmission are altered in EAE and identify additional targets for pharmacological interventions aimed at restoring normal synaptic and circuit function in MS.

Disclosures: W. Wei: None. **L. Kammel:** None. **R.R. Voskuhl:** None. **T.J. O'dell:** None.

Poster

224. Demyelinating Disorders: Animal Studies

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Topic: C.09. Demyelinating Disorders

Support: UCLA LNE Training Grant (5T32HD007228)

Conrad N. Hilton Foundation

Title: Increased GABAergic inhibition contributes to impaired hippocampal synaptic plasticity in experimental autoimmune encephalomyelitis

Authors: *L. G. KAMMEL[1], W. WEI[2], S. W. THORNTON[1], T. J. O'DELL[2], R. R. VOSKUHL[1];
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Abstract: Hippocampal synaptic dysfunction contributes to cognitive impairment experienced by multiple sclerosis (MS) patients. A number of findings, both in post-mortem MS brain tissue and from mouse models of MS (experimental autoimmune encephalomyelitis or EAE) indicate that GABAergic neurotransmission may be particularly vulnerable to disruption in MS. However, the functional consequence of impaired GABAergic neurotransmission in MS remains poorly understood. Thus, using electrophysiological techniques we examined inhibitory synaptic transmission in the CA1 region of hippocampal slices prepared from control and EAE mice. Although the number of parvalbumin positive cells in the hippocampal CA1 region is reduced in slices from EAE mice (44.5 ± 1.8 in control, n=5, compared to 37.1 ± 2.2, n=5 in EAE, $p < 0.05$), we find that the frequency of spontaneous IPSCs in CA1 pyramidal cells is significantly enhanced in EAE (n = 28) compared to control mice (n = 12, $p < 0.01$ compared to control). Moreover, tonic inhibition mediated by extrasynaptc GABA receptors was strongly enhanced in CA1 pyramidal cells from EAE mice (currents were 0.43 ± 0.07 pA/pF in controls, n = 13 and 0.76 ± 0.06 pA/pF in cells from EAE mice n = 28, $p < 0.01$). Based on these findings we hypothesize that the inflammatory, demyelinating milieu in MS generates changes in GABAergic inhibition that could strongly oppose the induction of activity-dependent forms of synaptic plasticity at excitatory synapses, such as long-term potentiation (LTP). Consistent with this, the induction of LTP by 150 pulses of 5 Hz presynaptic fiber stimulation was significantly reduced in hippocampal slices from EAE mice (fEPSPs were potentiated to 137.2 + 4.4% of baseline in control, n = 5, compared to 120.8 + 3.0% in slices from EAE mice, n = 6, $p < 0.05$). We conclude that decreased synaptic plasticity seen in hippocampal synapses during EAE may be due to increased GABAergic inhibition. Together these data suggest that targeting GABAergic neurotransmission may be a strategy for treating cognitive impairments in MS associated with impaired hippocampal synaptic plasticity.

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Poster

224. Demyelinating Disorders: Animal Studies

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Topic: C.09. Demyelinating Disorders

Support: Korea Healthcare Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (A111345)

Title: Pathological involvement of astrocyte-derived lipocalin-2 in the rodent model of demyelinating optic neuritis

Authors: *J.-H. KIM[1], B. CHUN[2], K. SUK[1];
[1]Brain Sci. and Engin. Institute, and Depar, Daegu, Korea, Republic of; [2]Dept. of Ophthalmology, Kyungpook Natl. Univ. Sch. of Med., Daegu, Korea, Republic of

Abstract: The current study was carried out to determine the role of lipocalin-2 (LCN2) in the pathogenesis of demyelinating optic neuritis using an experimental autoimmune optic neuritis (EAON) model. EAON was induced by subcutaneous immunization with an emulsified mixture of myelin oligodendrocyte glycoprotein (MOG_{35-55}) peptide in mice. LCN2 expression was examined in the optic nerve after MOG peptide injection. The expression of LCN2 was notably increased in the optic nerve after EAON induction and colocalized with reactive astrocytes. In comparison of degree of demyelination, inflammatory infiltration, glial activation, and expression profile of inflammatory mediators in the optic nerve between LCN2 knockout (KO) animals and wild-type littermates by histological analysis and real-time PCR following EAON induction, a significant reduction of demyelination, inflammatory infiltration, and gliosis was demonstrated in the optic nerve of LCN2 KO mice. In addition, LCN2 KO mice also showed markedly reduced gene expression associated with the M1-polarized glia phenotype and toll-like receptor signaling in the optic nerve. Their visual ability measured by photopic Flash VEP Recording showed that VEPs of EAON-induced WT mouse were diminished or not recordable but the impaired conductivities were attenuated in EAON-induced LCN2 KO mouse. Next, plasma levels of LCN2 in patients with optic neuritis were measured by ELISA. The LCN2 levels in plasma were significantly higher in optic neuritis patients (71.6 ± 10.6 ng/ml) compared to healthy controls (37.4 ± 9.1 ng/ml) ($p=0.0284$). In this study, we demonstrated a significant induction of LCN2 expression in astrocytes of the optic nerve following EAON induction. Our results imply that astrocyte-derived LCN2 may play a pivotal role in the development of demyelinating optic neuritis, and LCN2 can be a therapeutic target to alleviate immune and inflammatory damage in optic nerve.

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Poster

224. Demyelinating Disorders: Animal Studies

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Topic: C.09. Demyelinating Disorders

Support: NS30800

Myelin Repair Foundation

Title: Opposing roles of astrocytes in developmental myelination and early myelin repair

Authors: *R. TOGNATTA[1], M. KARL[1], C. KANTOR[2], R. R. DIGIACOMO[2], G. SHANO[2], P. LEAHY[2], S. L. FYFFE-MARICICH[3], R. H. MILLER[1];
[1]George Washington Univ., Washington, DC; [2]Case Western Reserve Univ., Cleveland, OH; [3]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Understanding the mechanisms regulating oligodendrocyte development and myelination in the vertebrate CNS is becoming increasingly important, not only to facilitate myelin repair but also to further define the roles of oligodendrocytes and myelin in the maintenance of axonal integrity and neuronal connectivity. *In vitro* studies have implicated astrocytes in influencing the development of oligodendrocytes and myelination, however their roles *in vivo* remain poorly defined. In this study we utilize GFAP-iCP9 transgenic animals that allow us for the first time to selectively eliminate non-proliferative GFAP+ astrocytes. In these animals the GFAP promoter drives coordinated expression of DsRed and iCP9 in greater than 90% of GFAP+ astrocytes. Localized delivery of CID results in selective loss of DsRed+/GFAP+ cells. Astrocyte ablation during postnatal spinal cord development results in a transient loss of astrocytes and a concomitant delay in myelination, demonstrating a critical role for astrocytes in promoting the timing of developmental myelination. By contrast, in the adult CNS localized ablation of astrocytes 2 days after the induction of a demyelinating lesion resulted in myelin repair in both the spinal cord and the corpus callosum, although through fundamentally different cellular pathways. In spinal cord, remyelinating cells exhibited a characteristic Schwann cell phenotype, while in the corpus callosum preservation of CC1+ OLs was seen. The proportion of BrdU+ OPCs was actually somewhat decreased in astrocyte depleted lesions compared to controls suggesting the higher number of OLs reflects either enhanced survival or differentiation rather than increased OPC proliferation. The potential preservation of oligodendrocytes in astrocyte depleted lesions is consistent with the diminished immune response in such lesions. This protection against demyeliantion was associated with increased numbers of oligodendrocytes that persisted for at least 1-month post lesion, providing axonal support. Microarray analysis reveals astrocytic NF-kB signaling pathway as a major contributor to the pathological events compromising myelin repair in the brain, suggesting cellular responses to astrocyte loss are temporally and regionally specific.

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Poster

224. Demyelinating Disorders: Animal Studies

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Support: VIEP-BUAP

Title: Analysis of chemokines and receptors expression profile in the myelin mutant taiep rat

Authors: G. GARCIA-ROBLES[1], G. SOTO-RODRIGUEZ[5], J.-A. GONZALEZ-BARRIOS[7], D. MARTINEZ-FONG[6], V.-M. BLANCO-ALVAREZ[1], J.-R. EGUIBAR[2], A. UGARTE[3], F. MARTINEZ-PEREZ[8], E. BRAMBILA[1], L. MILLAN-PEREZ PEÑA[4], N.-G. PAZOS-SALAZAR[1], M. TORRES-SOTO[1], C. TOMAS-SANCHEZ[1], *B. LEON CHAVEZ[9]; [1]Facultad en Ciencias Quimicas, [2]Fisiología, [3]Inst. de fisiologia, [4]Ctr. de quimica, Benemerita Univ. Autonoma de Puebla, Puebla, Mexico; [5]Fisiologia, Biofisica y Neurociencias, Ctr. de Investigaciones y de Estudios Avanzados, Cd. México, Mexico; [6]Fisiologia, Biofisica y Neurociencias, Ctr. de Investigaciones y de Estudios Avanzados, Cd. Mexico, Mexico; [7]Medicina Genomica, Hosp. regional 1 de Octubre ISSSTE, Cd. Mexico, Mexico; [8]Lab. de Genomica de Celomados, Univ. Industrial de Santander, Bucaramanga, Colombia; [9]Benemerita Univ. Autonoma Puebla, Puebla, Mexico

Abstract: Taiep rat has a failure in myelination and remyelination processes leading to a condition of hypomyelination throughout its life. Chemokines, which are known to play a role in inflammation, are also involved in the remyelination process. We aimed to demonstrate that remyelination-stimulating factors are altered in the brainstem of 1- and 6-months old taiep rats. We used a rat RT2 Profiler PCR array to assess mRNA expression of 84 genes coding for cytokines, chemokines and theirs receptors. We also evaluated protein levels of CCL2, CCR1, CCR2, CCL5, CCR5, CCR8, CXCL1, CXCR2, CXCR4, FGF2, and VEGFA by ELISA. Sprague-Dawley rats were used as a control. Our results using PCR Array procedure showed that pro-inflammatory inflammatory cytokines were not upregulated in the taiep rat. In contrast, some mRNA levels of beta- and alpha chemokines were upregulated in 1-month old rats, but CXCR4 was downregulated at their 6 months of age. ELISA results showed that CXCL1, CCL2, CCR2, CCR5, CCR8 and CXCR4 protein levels were decreased in brainstem at age of 6 months. These results suggest the presence of a chronic neuroinflammation process with deficiency of remyelination-stimulating factors (CXCL1, CXCR2 and CXCR4), which might account for the demyelination in the taiep rat.

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Poster

224. Demyelinating Disorders: Animal Studies

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Support: Consejo Nacional de Investigaciones Científicas Técnicas - PIP 0567

Universidad de Buenos Aires - Proyecto 20020120100132BA

Title: Notch ligand-selective activation in CNS demyelination-remyelination

Authors: P. MATHIEU, L. GÓMEZ PINTO, F. ALMEIRA GUBIANI, *A. M. ADAMO; Facultad De Farmacia Y Bioquímica. Univ. of Buenos Aires. IQUIFIB-CONICET, CABA, Argentina

Abstract: In the CNS, myelination is a physiological process driven by oligodendroglial cells, while demyelination is a pathological process characterized by myelin loss around axons. Demyelination is followed by remyelination, which solves functional deficits. This work focuses on Notch1 ligands and their role in the demyelination-remyelination process in a cuprizone (CPZ)-induced demyelination model. Twenty-one-day-old Wistar rats were fed a diet containing CPZ during 2 weeks. Demyelinated and control animals were sacrificed 7d before CPZ withdrawal (-7d), the day of CPZ withdrawal (0d), 7, 14 and 21d (+7d, +14d and +21d) after CPZ withdrawal and experiments were conducted for each survival time. The levels of F3/contactin and Jagged1 were determined by Western blot in the subventricular zone (SVZ) and corpus callosum (CC) of control and CPZ animals. To evaluate the participation of SVZ in the remyelination process in the CC, primary neurosphere cultures from SVZ dissected from control and CPZ animals were carried out. The cellular populations in these cultures were characterized with specific markers, i.e. Nestin for neural precursor cells, GFAP for astrocytes and NG2 for oligodendroglial progenitor cells (OPCs). Notch signaling activation and ligands F3/contactin and Jagged1 expression were determined. Results show an increase in F3/contactin expression at -7d, +7d and +21d in CC of CPZ animals as compared to controls, whereas in the SVZ the increase in F3/contactin was observed at +14d. In addition, a greater proportion of Nestin+ cells was detected in neurosphere cultures from SVZ of demyelinated and spontaneously remyelinating animals (0d, +7d) as compared to controls, which indicates an increase in OPC proliferation in response to injury. Notch signaling activation (NICD levels) was observed in

NG2+ OPCs, together with an increase in the percentage of Jagged1-expressing cells, which suggests that the pool of OPCs remains preserved in these cultures. In turn, the increase observed in the percentage of F3/contactin+ cells and the concomitant decrease in NG2+ OPCs in neurosphere cultures carried out at 0d and +7d might prove F3/contactin participation in inducing OPC maturation. Worth pointing out, F3/contactin was found to colocalize with OPC marker NG2 but went undetected in GFAP+ cells. Finally, co-immunoprecipitation experiments prove F3/contactin binding to Notch, thus confirming its role as a non-canonical ligand.

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Poster

224. Demyelinating Disorders: Animal Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 224.07/G9

Topic: C.09. Demyelinating Disorders

Title: Vitamin C facilitates pluripotent stem cell maintenance of MSCs and promotes its neurotrophic functions

Authors: Y. LIU, B. ULLOA, M. ALAHIRI, *S. A. SADIQ;
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Abstract: Background: Ascorbate (Vitamin C) is best known for its role in scurvy, in which the hydroxylation of collagen catalyzed by dioxygenases is incomplete due to ascorbate deficiency. DNA methylation is a heritable epigenetic modification involved in gene silencing. In early embryo and the germ line, global DNA demethylation were mediated by Tet (ten eleven translocation) enzymes, which convert 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC). Vitamin C (Vit C), a potential cofactor for Tet enzymes, could promote the generation of induced pluripotent stem cells (iPSCs) and facilitates pluripotent stem cell maintenance. Objective: In this study, we added Vit C into the culture media of mesenchymal stem cells (MSCs) to investigate its role on the morphology, proliferation, differentiation and function of MSCs. Methods: MSCs from human multiple sclerosis patients were cultured and treated with Vit C at 50μg/ml. Genomic DNA extracted from MSCs were quantified and used for dot-blot . FACS was carried out to detect the cell surface or intracellular markers of MSCs after Vit C treatment. RNA was extracted and quantitative PCR was performed using 7900HT fast real-time PCR system and relative quantification was determined using RQ manager software. Adipocytes and osteocytes were differentiated by induction media with or without Vit C added. Results: Vit C significantly enhanced Tet -mediated generation of 5hmC. Vit C did not significantly increase the proliferation of MSCs and did not alter the pluripotent markers of MSCs. Vit C did not induce MSC differentiation into adipocytes and osteocytes. Treatment with Vit C significantly

increases the levels of MSC expression of HGF and IGF. Conclusion: Our study provides the indirect evidence of Vitamin C on production of HGF and IGF which are important in the neurotrophic function of MSCs without inducing the unwanted mesodermal differentiation. Vit C should be investigated further for its role on MSCs used for transplantation to treat multiple sclerosis.

Disclosures: Y. Liu: None. **B. Ulloa:** None. **M. Alahiri:** None. **S.A. Sadiq:** None.

Poster

224. Demyelinating Disorders: Animal Studies

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Topic: C.09. Demyelinating Disorders

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Title: Pericyte deficiency leads to white matter tract damage, demyelination and axonal degeneration

Authors: *A. M. NIKOLAKOPOULOU, A. MONTAGNE, G. SI, A. P. SAGARE, Z. ZHAO, B. V. ZLOKOVIC;
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Abstract: White matter damage is a characteristic of a plethora of neurodegenerative diseases, such as Alzheimer's disease (AD) and Multiple Sclerosis (MS). Pericytes, the vascular mural cells located within the basement membrane of blood microvessels, regulate blood brain barrier (BBB) permeability and cerebral blood flow while they participate in clearance of toxic byproducts. Pericyte loss has been implicated in BBB breakdown, neuronal degeneration, cognitive impairment, inflammation, synaptic loss and effects on cerebral blood flow (CBF). In this study, we used $Pdgfr\beta^{F7/F7}$ mice to examine the effects of pericyte-deficiency on white matter tracts and more specifically in the corpus callosum. Our data show that pericyte-deficiency leads to early BBB leakage and CBF deficits, which precede demyelination and axonal degeneration; one-month-old animals show no defects in myelin and axonal integrity, although accumulation of blood byproducts is visible (IgG and fibrin). However, young adult $Pdgfr\beta^{F7/F7}$ mice (2-4m old) exhibit white matter volume reduction and demyelination as determined by diffusion tensor magnetic resonance imaging (DTI-MRI) and by trace weighted imaging (TWI) compared to neurologically-intact controls. In particular, radial diffusivity, axial diffusivity and fractional anisotropy are increased, and fiber tract maps are significantly impaired

in *Pdgfrβ*$^{F7/F7}$ mice compared to controls. Furthermore, *Pdgfrβ*$^{F7/F7}$ mice show impaired fiber tract integrity that affects brain connectivity, as demonstrated by reduction in projection length. In addition, immunohistological, flow cytometry, and electron microscopy data show a decrease in myelin thickness and myelin-protein expression both in young adult and old animals. Taken together, our results show that pericyte-deficiency causes BBB leakage and CBF abnormalities at very early stages, followed by myelin destruction and axonal degeneration, thus emphasizing the importance of the neurovascular unit.

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Poster

224. Demyelinating Disorders: Animal Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: C.09. Demyelinating Disorders

Title: Studying lipocalin 2 as a novel player in the pathophysiology of the disease

Authors: ***F. MARQUES**, S. NEVES, C. FERREIRA, S. MESQUITA, C. SERRE-MIRANDA, L. BELARD, M. CORREIA-NEVES, J. SOUSA, J. CERQUEIRA, J. PALHA;
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Abstract: Multiple sclerosis (MS) is an immune-mediated demyelinating disease of the central nervous system (CNS), characterized by the presence of demyelination plaques, inflammation and gliosis that consequently lead to axonal damage. The sequence of events that leads to demyelination remains unclear and the pathophysiological mechanisms are diverse. Recently we found that lipocalin 2 (LCN2), an acute phase protein that is a part of the defense system against bacteria by binding to iron-loaded siderophores, was increased in cerebrospinal fluid (CSF) and serum of MS patients, when compared to control subjects. Similarly, using the experimental autoimmune encephalomyelitis (EAE) mouse model, LCN2 was detected in brain parenchyma astrocytes, in regions typically affected in MS patients. This expression by astrocytes, together with an increased LCN2 level in the CSF, occurs during the active phases of the disease, which could point towards a role for LCN2 secreted by astrocytes in the mediation of inflammatory responses in the EAE model. To further understand the role of LCN2 in MS pathology we are using the EAE mouse model and inducing EAE both in LCN2-null mice and in WT littermate controls. Non-induced EAE animals are being used as controls. LCN2-null mice induced with EAE do not show major alterations in terms of the clinical score when compared with WT littermate controls also induced with EAE. Regarding the activation of astrocytes, we quantified the percentage of area GFAP+ and did a 3D morphology reconstruction of astrocytes, in the white matter of the cerebellum of LCN2-null mice and WT littermates induced with EAE. The

EAE animals present an increase in the GFAP+ area, but we only detected on tendency to decreased activation in LCN2-null mice. We also evaluated the role of LCN2 in demyelination, in the cerebellum, using the histochemical coloration luxol fast blue, and the LCN2-null mice with EAE show a tendency to present less demyelination than their induced WT littermates.

Disclosures: **F. Marques:** None. **S. Neves:** None. **C. Ferreira:** None. **S. Mesquita:** None. **C. Serre-Miranda:** None. **L. Belard:** None. **M. Correia-Neves:** None. **J. Sousa:** None. **J. Cerqueira:** None. **J. Palha:** None.

Poster

224. Demyelinating Disorders: Animal Studies

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Topic: C.09. Demyelinating Disorders

Support: FP/2007-2013 ERC grant 310932

Title: ROS-initiated calcium-influx drives axonal degeneration in an animal model of multiple sclerosis

Authors: ***M. WITTE**[1], A.-M. SCHUMACHER[2], C. MAHLER[2], J. BEWERSDORF[2], P. R. WILLIAMS[3], O. GRIESBECK[4], T. MISGELD[3], M. KERSCHENSTEINER[2];
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Abstract: Multiple sclerosis (MS) is a neuroinflammatory disorder driven by an autoimmune response against myelin components in the central nervous system (CNS). Besides demyelination, infiltration of immune cells into the CNS also leads to axonal degeneration, which in turn causes irreversible neurological disability. Previously, we identified a shared mechanism of axonal degeneration in MS and its animal model, experimental autoimmune encephalomyelitis (EAE), which we termed focal axonal degeneration (FAD). Essentially, FAD is a two-step process of focal axonal swelling followed by axonal fragmentation. Interestingly, a substantial proportion of swollen axons recovered and returned back to normal morphology. Here we set out to identify the underlying molecular mechanism of neuroinflammation-induced FAD.First, we investigated intra-axonal calcium levels in EAE mice *in vivo*, by using two-photon microscopy in the spinal cord of transgenic mice expressing a FRET-based ratiometric calcium sensor in neurons. This revealed that many axons in neuroinflammatory lesion have elevated levels of intracellular calcium, whereas calcium levels were tightly controlled in healthy animals. Importantly, high-calcium levels were also found in axons with normal morphology, suggesting that a rise in intra-axonal calcium precedes and perhaps initiates FAD. To address this

question, we followed changes in axonal calcium levels and morphology over time in a large number of axons in EAE animals. Our results indicate that intra-axonal calcium levels predict both swelling and fragmentation of axons in a neuroinflammatory environment. Next, we set out to identify potential causes for this rise in intra-axonal calcium. Of the previously implicated damage mechanisms in MS/EAE, only oxidative/nitrosative stress was able to directly induce an increase in axonal calcium levels and a FAD-like process in the spinal cord of healthy mice, while glutamate excitoxicity and acidosis did not markedly alter axonal calcium levels *in vivo*. Taken together, our experiments point to a crucial role for reactive oxygen/nitrogen species (ROS/RNS) in increasing intra-axonal calcium levels and, driving axonal degeneration in MS and EAE. We are currently exploring pathways involved in the ROS/RNS-induced rise in intra-axonal calcium.

Disclosures: M. Witte: None. **A. Schumacher:** None. **C. Mahler:** None. **J. Bewersdorf:** None. **P.R. Williams:** None. **O. Griesbeck:** None. **T. Misgeld:** None. **M. Kerschensteiner:** None.

Poster

224. Demyelinating Disorders: Animal Studies

Location: Hall A

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Topic: C.09. Demyelinating Disorders

Title: Exosomal protein changes of cerebrospinal fluid from neuromyelitis optica and multiple sclerosis patients

Authors: *S. HWANG[1], J. LEE[1], K. Q. MCKINNEY[1], A. J. PAVLOPOULOS[1], J. M. CONWAY[2], H.-J. KIM[3];
[2]Neurol., [1]Carolinas Healthcare Syst., Charlotte, NC; [3]Neurol., Res. Inst. and Hosp. of Natl. Cancer Ctr., Goyang-si, Korea, Republic of

Abstract: Microvesicles including exosomes are believed to play a significant role in intercellular signaling by delivering molecules via membrane vesicle trafficking. These processes then influence immune system cells, such as dendritic cells and B cells, and mediate adaptive immune responses to pathogens and tumors. Cerebrospinal fluid (CSF) is the most valuable biological specimen for the determination of neurodegenerative diseases since it contains corresponding molecular mediators being kept separated from systemic circulation. Comparative proteomics profiling of CSF exosomes isolated from neuromyelitis optica (NMO) and multiple sclerosis (MS) patients detected potential distinctive disease markers capable of discriminating NMO from MS. Differential centrifugation provided good yields of exosomes from CSF pooled by cytokine expression pattern. Immunological pre-classification of CSF by cytokine level gave advantages in target molecule identification by providing enhanced fold

change ratio and lower p-values when the subsets with high-cytokines were compared to each other. In this study, proteomics profiling of the exosomes isolated from the CSF of NMO patients identified disease specific molecular markers by comparison to the CSF exosome profiles of control and MS subjects. A SEQUEST search employing Swiss-Prot protein database (Human, reviewd) identified 470 proteins from the technical duplicate LC-MS/MS analysis of CSF exosomal proteins from control, NMO and MS subjects. The refined proteomic datasets identified putative disease specific markers, glial fibrillary acidic protein (GFAP) for NMO and fibronectin for MS. Among the target proteins, GFAP was observed from intact exosomes by flow cytometry and transmission electron microscopy (TEM) analysis in combination with immunogold staining and its expression level was measured by label-free quantification, which were in good agreement with western blot analysis results. The ELISA assay measurement of target proteins from individual CSF demonstrated their diagnostic applicability by clustering disease groups successfully. The current comprehensive study also suggests that this approach may be utilized to detect other neuronal diseases involved in inflammation, degeneration, demyelination, differentiation of oligodendrocytes, and neurofilament remodeling, etc.

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Poster

224. Demyelinating Disorders: Animal Studies

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Topic: C.09. Demyelinating Disorders

Support: NIH Grant NS34939

Title: CHOP knockdown protects oligodendrocytes in mouse models of immune-mediated demyelination

Authors: ***Y. DZHASHIASHVILI**[1], H. BOMMIASAMY[2], R. B. KUNJAMMA[1], B. POPKO[1]; [1]Neurol., Univ. of Chicago, Chicago, IL; [2]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), characterized by focal demyelination. A central feature in the development of MS lesions is the death of oligodendrocytes, the cells responsible for generating and maintaining the myelin sheath. We have previously shown that genetic impairment of the integrated stress response (ISR), a cytoprotective mechanism that maintains cellular homeostasis, significantly exacerbates oligodendrocyte death and demyelination during an inflammatory attack. In addition, we have shown that the genetic or pharmacological enhancement of the ISR provides increased

protection against inflammatory demyelination. CHOP (C/EBP homologous protein) is a key ISR pro-apoptotic transcription factor that becomes activated under conditions of sustained endoplasmic reticulum (ER) stress, which has been implicated in MS lesion formation. We demonstrate here that genetic CHOP deletion protects oligodendrocytes against inflammatory demyelination, and alleviates disease in experimental autoimmune encephalomyelitis (EAE), a mouse model of MS. Our results implicate CHOP as a potential target for therapeutic intervention in MS, aimed to prevent oligodendrocyte loss and demyelination in the inflammatory CNS environment.

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Poster

224. Demyelinating Disorders: Animal Studies

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Topic: C.09. Demyelinating Disorders

Title: Application of a novel cell ablation for dissecting the role of immune cells in the pathogenesis of experimental allergic encephalomyelitis

Authors: *X. QIN, SR[1], F. LIU[2], Y. OHTAKE[3], D. FUNG[4], S. DAI[2], X. PENG[2], B. GAO[4], S. LI[3];
[1]Neurosci., Temple Univ. Sch. of Med., Philadelphia, PA; [2]Neurosci., Temple Univ. Sch. of Med., Philadephia, PA; [3]Dept. of Anat. and Cell Biol., Shriners Hosp. Pediatric Res. Ctr., Philadephia, PA; [4]Natl. Inst. on Alcohol Abuse and Alcoholism, Lab. of Liver Dis., Bethesda, MD

Abstract: The roles of various immune cells in the pathogenesis of experimental allergic encephalomyelitis (EAE), a rodent model widely used for multiple sclerosis study have been previously investigated with different methodologies. However, the relative roles of different immune cells have not been studied with the same model system. Here, we utilized intermedilysin (ILY)-mediated cell ablation model to compare the pathogenic roles of immune cells in EAE. ILY, secreted by *Streptococcus intermedius* (SI), binds exclusively to human membrane protein CD59 (hCD59) but not to CD59 of any other species. Once bound, ILY rapidly and potently lyses the targeted cells. Using this unique feature, we previously developed a novel cell ablation method. To streamline this approach, we recently generated the floxedSTOP-hCD59 or inducible hCD59 knock-in mice (ihCD59) where hCD59 expression only occurs following Cre-mediated recombination. To express the hCD59 in each type of the T cells, monocytes, and B cells, we crossed ihCD59 with Lck-Cre, Lyz-Cre or CD19-Cre mice respectively to generate three different strains of the mice: ihCD59/Lck-Cre, ihCD59/CD19-Cre

or ihCD59/Lyz-Cre. As expected, the specific Cre expression in T cells, monocytes, or B-cells mediated the hCD59's expression in each strain of these mice. Systemic ILY administration to each strain specifically ablated the corresponding cells. ILY administration was initiated 3 days after immunization with myelin oligodendrocyte glycoprotein peptide and continued for 14 days (daily i.p.) to ablate T cells, monocytes and B cells, respectively. By monitoring the clinical EAE scores in these mice, we demonstrated that ablation of T cells completely prevented EAE development and that ablation of monocytes largely attenuated the EAE scores, in contrast to moderate reduction of EAE scores in B-cell ablated mice. Therefore, for the first time, we have successfully dissected the roles of different immune cells with the same approach and demonstrated their distinct functions in EAE's pathogenesis.

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Poster

224. Demyelinating Disorders: Animal Studies

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Legacy of Angels

Title: Exosomal secretion of a lipid neurotoxin from neural progenitors

Authors: ***G. SCESA**, A. L. MOYANO, L. D'AURIA, K. PITUCH, M. I. GIVOGRI, E. R. BONGARZONE;
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Abstract: Exosomes are small vesicles secreted from most mammalian cells via the multivesicular endosomal pathway. Multiple functions have been associated with this secretory pathway. It is unknown whether exosomes contribute to detoxification and also to the mobilization of neurotoxins. Krabbe's disease is an autosomal recessive genetic disorder affecting central and peripheral nervous systems, due to mutations in beta-galactosyl-ceramidase gene (GALC). As a result of GALC deficiency, the normal degradation pathway of several galactosyl-sphingolipids is impaired. One of these lipids, galactosyl-sphingosine or psychosine, exerts cytotoxic effects on neurons and myelin forming cells. Considering that psychosine is associated with lipid rafts in plasma membranes and that raft components are secreted via exosomes, we hypothesized that the neurotoxin psychosine may be secreted through this mechanism. Using a neural stem cell (NSC) *in vitro* paradigm, we show that NSC deficient in GALC (GALC-/- NSC) accumulate psychosine significantly more than wild-type cells upon

differentiation into the main three neural lineages. Upond differentiation GALC+/+ and GALC-/- cultures released detectable levels of exosomes to the medium, but psychosine was only detected in exosomes derived from deficient cells. Furthermore, levels of psychosine correlate with days of *in vitro* differentiation, suggesting an intracellular accumulation prior to exosomal release. Medium containing exosomes from differentiated GALC-/- NSC was sufficient to impair the differentiation of wild-type primary cultures of oligodendrocytes, suggesting a mechanism of trans-toxicity. These results suggest that mammalian cells have mechanisms to secrete toxic metabolites associated with lipid raft microdomains like psychosine through the exosomal pathway. This process may contribute to long-range toxicity in disease conditions and may also be part of a more general detoxifying mechanism.

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Poster

224. Demyelinating Disorders: Animal Studies

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Legacy of Angels Foundation (ERB)

Title: Psychosine causes cellular toxicity by downregulating the PI3K/Akt pathway in motoneurons

Authors: *T. SURAL-FEHR, L. CANTUTI-CASTELVETRI, H. ZHU, M. MARSHALL, E. R. BONGARZONE;
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Abstract: Deficiency of the lysosomal galactosyl-ceramidase (GALC) enzyme results in the accumulation of the toxic lipid psychosine, and is the underlying cause for the hereditary Krabbe's disease. Psychosine causes fast axonal transport defects through abnormal activation of GSK3b and results in a dying-back pathology in neurons. However, the exact mechanism leading to this activation and the associated cellular toxicity is unknown. We previously showed that psychosine preferentially accumulates in lipid rafts within cell membranes disrupting their structure. Lipid rafts are essential platforms for cellular signaling pathways, therefore we hypothesized that psychosine accumulation would lead to a deregulation of intracellular signaling pathways affecting key mediators of integrity in neuronal axons. Using the neuronal-

like NSC34 cell culture system, we have systematically analyzed the downstream effects of psychosine, particularly on cellular signaling. We have found that exogenous psychosine administration causes a significant downregulation of the PI3K/Akt and MAPK pathways downstream of the IGF receptor. Inhibition of phospho-Akt takes place mainly at the plasma membrane and can be fully recovered through either stimulation of the IGF receptor by IGF-1, or using a small molecule activator (SC-79) of Akt, implying that the short-term effects of psychosine toxicity may be reversible. We conclude that the mechanism of psychosine toxicity in motoneurons goes through modulation of the cell's ability to sense growth factor availability in its environment and appropriately adjust its intracellular response. This effect possibly goes through perturbing the structure within membrane lipid rafts, thereby altering associated survival signals that depend on them, such as Akt activation coupled to IGF receptor signaling. Therefore, the use of Akt activators such as IGF-1 or SC-79 may represent potential therapies to alleviate the aspects of this disease regulated by this pathway.

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Poster

224. Demyelinating Disorders: Animal Studies

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Title: Functional characterization of IPSC-derived brain cells as a model for X-linked adrenoleukodystrophy

Authors: *I. SINGH[1], A. SINGH[2], M. KHAN[2], M. BAARINE[2];
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Abstract: X-linked Adrenoleukodystrophy (X-ALD) is an inherited neurodegenerative disorder where mutations in ABCD1 gene result in clinically diverse phenotypes, the fatal disorder of cerebral ALD (cALD) or a milder disorder of adrenomyeloneuropathy (AMN). The different models used to study the pathobiology of X-ALD disease lack the appropriate presentation for different phenotypes of cALD vs AMN. This study demonstrates that induced pluripotent stem

cell derived brain cells astrocytes (Ast), neurons and oligodendrocytes (OLs) express morphological and functional activities of the respective brain cell types. The excessive accumulation of saturated VLCFA a “hallmark” of X-ALD was observed in both AMN OLs and cALD OLs but higher levels were in cALD OLs than AMN OLs. The levels of ELOVL1 parallel the VLCFA load in these cell types. Secondly, cALD Ast expressed higher levels of proinflammatory cytokines than AMN Ast and control Ast with or without stimulation with lipopolysaccharide. These results document that IPSC-derived Ast and OLs from cALD and AMN fibroblasts mimic the respective biochemical disease phenotypes and thus provide an ideal platform to investigate the mechanism of VLCFA load in cALD OLs and VLCFA-induced inflammatory disease mechanisms of cALD Ast and thus for testing of new therapeutics for AMN and cALD disease of X-ALD.

Disclosures: I. Singh: None. **A. Singh:** None. **M. Khan:** None. **M. Baarine:** None.

Poster

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Topic: C.09. Demyelinating Disorders

Support: NIH R01NS060017

National Multiple Sclerosis Society research grant RG3975

Title: Deletion of signal transducer and activator of transcription 3 (STAT3) in myeloid cells confers resistance to experimental autoimmune encephalomyelitis

Authors: *H. LU, A. STEELMAN, S. KIM, B. ZHOU, J. LI;
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Abstract: Multiple sclerosis (MS) is a debilitating disease characterized by demyelination and neurodegeneration in the CNS. Both activated microglia and infiltrating myeloid cells are thought to contribute to disease initiation and progression of experimental autoimmune encephalomyelitis (EAE), a widely used animal model of MS that involves immunization of animals with a myelin antigen. STAT3 regulates multiple cellular functions including cell differentiation, survival, and inflammation and is a critical signaling molecule for the interleukin-6 family of cytokines. To investigate the role of STAT3 in myeloid cells and neuroinflammation, we generated conditional knockout mice with STAT3 selectively disrupted in myeloid cells and investigated their susceptibility to myelin oligodendrocyte glycoprotein (MOG)-induced EAE. Whereas $STAT3^{f/f}$ control mice developed typical signs of EAE with characteristic tail and hind limb paralysis, myeloid-specific STAT3 deficient mice were resistant to MOG-induced EAE. In contrast to $STAT3^{f/f}$ mice that elicited MOG-specific T_H1 and T_H17 responses at disease onset,

myeloid STAT3 deficient mice did not develop MOG-specific T cell responses. However, at later time points, the STAT3 mutant mice developed MOG-specific specific T_H1 and T_H17 responses in the peripheral lymphoid organs comparable to that of $STAT3^{f/f}$ mice, but failed to develop classic EAE and had minimum leukocyte infiltration into the CNS even after the peak disease phase. Our results suggest a duo role of myeloid STAT3 signaling in T cell polarization and myeloid cell function in modulating CNS autoimmunity. *Supported in part by NIH R01NS060017 and the National Multiple Sclerosis Society research grant RG3975*

Disclosures: H. Lu: None. **A. Steelman:** None. **S. Kim:** None. **B. Zhou:** None. **J. Li:** None.

Poster

224. Demyelinating Disorders: Animal Studies

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UBA 2014-2017, 20020130100673BA

Title: Indomethacin effect on BMMC migration: Prostaglandin synthesis inhibition or PPARɣ activation?

Authors: V. USACH, C. I. CASALIS, G. M. PIÑERO, L. PARRA, K. WEBER, P. A. SOTO, M. FERNÁNDEZ-TOMÉ, *P. C. SETTON-AVRUJ;
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Abstract: Demyelination is one of the hallmarks of the Wallerian degeneration (WD) process and cell therapy is among the strategies under study to induce remyelination. Results from our group obtained in a reversible model of WD induced by the crush of the rat sciatic nerve demonstrated the spontaneous migration of endogenous or transplanted bone marrow mononuclear cells (BMMC) exclusively to the injured nerve. Once in the ipsilateral nerve, some BMMC colocalized with Schwann cell markers and nerve fiber markers, which accelerated the regeneration process. On the basis of these results, the aim of the present work was to evaluate whether prostaglandins (PGs), one of the molecules generated during the inflammatory process associated with injury, is one of the signals involved in the migration and recruitment of BMMC to the demyelinated nerve. To this end, adult Wistar rats were submitted to sciatic nerve crush and one group of animals was immediately transplanted BMMC through the sacra artery. The presence of BMMC in the injured nerve was evaluated through confocal microscopy 24 h, 3 and 5 days post injury; the expression of ciclooxygenase 2 (Cox-2) was evaluated at 24 and 72 h and the synthesis of PGs was evaluated between 0 and 24 h post crush, through Western blot and PG radioconversion, respectively. Besides, the effect of a non-steroidal anti-inflammatory drug as

indomethacin on the migration of BMMC and PG biosynthesis was analyzed by treating animals with a subcutaneous injection of indomethacin 50 mg/kg/day the day of the lesion and the previous day, and 5 mg/kg/day the subsequent days. The results obtained show that, as soon as 24 h post injury, BMMC arrived at the edges of the ipsilateral nerve, and after 3 days they became part of it. Our results demonstrate the biosynthesis of PGE2, PGD2 and PGJ2 in sciatic nerve homogenates, and that their levels did not change significantly as a consequence of the lesion. Although indomethacin inhibited the migration of transplanted BMMC to the injured sciatic nerve, the biosynthesis of PGE2 and PGD2 was not affected. Surprisingly, indomethacin promoted a significant increase in PGJ2 both in the contralateral and the ipsilateral nerves as well as in the control nerve. This increase was dose-dependent, as rats treated with 12.5 or 25 mg/kg showed a smaller increase in PGJ2 synthesis. In the light of these results, indomethacin action on BMMC migration may be thought to occur through an independent PG-mediated mechanism such as the PPARɣ pathway. Further experiments are necessary to more precisely elucidate indomethacin effect on BMMC migration and on the degeneration-regeneration process.

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Poster

224. Demyelinating Disorders: Animal Studies

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Topic: C.09. Demyelinating Disorders

Support: Alberta Innovates - Health Solutions CRIO Team Program

Title: Aging exaggerates myelin disruption in an experimental model of demyelination

Authors: ***N. MICHAELS**, K. RAWJI, M. B. KEOUGH, J. R. PLEMEL, V. W. YONG; Univ. of Calgary, Calgary, AB, Canada

Abstract: Multiple sclerosis (MS) is an inflammatory and degenerative condition that destroys the myelin and axons of the central nervous system (CNS). It clinically begins most commonly with a relapsing-remitting (RRMS) disease course in young adults with the majority of these cases becoming progressively disabling. Age has been identified as an independent predictor for the time to the onset of the progressive stage of the disease; therefore, age-associated differences in the response to acute demyelination may underlie the progressive compromise of axons (neurodegeneration) and myelin (demyelination) with aging. We sought to test the hypothesis that the extent of early myelin disruption to a demyelinating injury would be greater in aging compared to young mice; if so, this would provide a model to examine the proximal mechanisms

of aging-enhanced neural injury. We have used the toxin lysophosphatidylcholine (LPC, lysolecithin) to induce demyelination, and have focused on myelin disruption over the first 72h in order to model aspects of an early MS lesion. LPC (0.5 µl of 1% solution) was deposited into the ventral column of the mouse spinal cord as described previously (Keough et al., JOVE 2015). Young (6 weeks old) and aging (8 month) mice were compared. At 3h post-LPC, disruption of myelin detected by eriochrome cyanine staining was apparent but not obviously different in both age groups. However, at 24h post-LPC, we found an exaggerated amount of myelin disruption in aging compared to young animals, and this exacerbation of lesion was still evident at 3 days. Current results indicate that the older animals after demyelination have greater microgliosis, as evaluated using Iba1 immunoreactivity. These results not only emphasize a greater demyelinating injury in aging compared to young mice in response to LPC, but they also provide a model to identify mechanisms that contribute to exaggerated myelin disruption in an effort to develop neuroprotective therapeutic strategies to delay progression in MS patients.

Disclosures: **N. Michaels:** None. **K. Rawji:** None. **M.B. Keough:** None. **J.R. Plemel:** None. **V.W. Yong:** None.

Poster

224. Demyelinating Disorders: Animal Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 224.20/G22

Topic: C.09. Demyelinating Disorders

Title: Increased levels of colony stimulating factor-1 and its receptors in mouse spinal cords with experimental autoimmune encephalomyelitis

Authors: *X. BO[1], S. GUSHCHINA[2], W. J. B. ATKINS[2], G. PRYCE[2], G. GIOVANNONI[2], D. BAKER[2];
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Abstract: Multiple sclerosis (MS) is generally considered as a chronic autoimmune disease, resulting from the attack of autoaggressive T cells on the myelin sheath or oligodendrocytes in the brain and spinal cord. Recently the contribution of microglia to various stages of the development of MS is being recognized. It is known that activated microglia are involved in antigen presentation, phagocytosis, and release of various pro-inflammatory molecules. Colony stimulating factor-1 (CSF-1) and interleukin-34 (IL-34) are two crucial molecules responsible for proliferation and activation of microglia. The main aim of the project was to study whether the expression levels of CSF-1, IL-34, and their receptors (CSF1R) were increased in the mouse spinal cords with experimental autoimmune encephalomyelitis (EAE). Using ELISA we found that the level of CSF-1 was significantly higher in the spinal cords of acute and chronic EAE

mice than the control mice, while the levels of CSF-1 in the plasma of the three groups were not significantly different, which may indicate increased local synthesis of CSF-1 in the spinal cords of EAE mice. The level of IL-34 in the spinal cords of the control group was not significantly different from the acute EAE group, but significantly higher than the chronic group, implicating that IL-34 may play a lesser important role in microglia activation in the EAE mice. IL-34 levels in the plasma of the three groups were also not significantly different. Preliminary data from immunohistochemistry showed that there were more CSF-1R positive microglia in the spinal cords of the acute and chronic EAE mice than the control group. The results from the current study implicate that locally synthesized CSF-1 in the central nervous system may play an important role in the activation of microglia in EAE, contributing to neuroinflammation and neurodegeneration.

Disclosures: X. Bo: None. **S. Gushchina:** None. **W.J.B. Atkins:** None. **G. Pryce:** None. **G. Giovannoni:** None. **D. Baker:** None.

Poster

224. Demyelinating Disorders: Animal Studies

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Topic: C.09. Demyelinating Disorders

Support: NMSS RG4439-A-2

CCTS Pre-Doctoral Education for Clinical and Translational Scientist Fellowship 2014 and the University of Illinois at Chicago

Title: Notch-1 signaling in a mouse model of metachromatic leukodystrophy

Authors: *F. MAROTTOLI;
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Abstract: Metachromatic leukodystrophy (MLD) is an inherited lysosomal storage disease caused by a deficiency in the catabolic enzyme arylsulfatase-A (ARSA). This deficiency leads to the accumulation of sulfatides in glial and neuronal cells, resulting in demyelination in the central and peripheral nervous systems. The pattern of sulfatide accumulation seen in this disease has been recapitulated in a knock-out (KO) mouse model, in which a delay in the onset of myelination has been observed. The molecular mechanism behind this delay is unknown. While the process of myelination is governed by a number of pathways working in concert, previous work from various groups including ours indicates that activity of Notch signaling pathway represses the differentiation of oligodendrocytes. Importantly, previous studies in *Drosophila* have also found that this highly conserved signaling pathway is positively stimulated by glycosphingolipids under certain conditions. Therefore, we hypothesized that sulfatides

aberrantly activate the Notch1 pathway, contributing to block oligodendrocyte differentiation and to the delayed onset of myelination in the MLD mouse. We have been testing this hypothesis by measuring the levels of the various signaling components of the Notch pathway, including its downstream targets (Hes genes). This was done via RT-qPCR and western blot in the cortex and corpus callosum of MLD KO and wild-type mice during the peak of myelination. We found a significant increase in the notch intracellular domain (NICD) in our western blot analysis at P21 in MLD KO mice accompanied by a significant increase in Hes 5 mRNA expression, which correlates with the delay in myelin gene expression. Currently we have incorporated a sensitive luciferase-based reporter system to specifically measure the effect of sulfatides on the activity of Hes1 and Hes5 promoters in primary glial cultures. Abberant Notch activation during the period of oligodendrocyte maturation in the MLD mouse appears to be a possible mechanism contributing to the observed delay in myelination.

Disclosures: F. Marottoli: None.

Poster

224. Demyelinating Disorders: Animal Studies

Location: Hall A

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Topic: C.09. Demyelinating Disorders

Support: NIH Grant RO1NS073743

NIH Grant R21NS082725

The Intramural Research Program of NINDS

Title: Elimination of mitochondrial anchoring protects dysmyelinating shiverer: implications for progressive MS

Authors: *D. C. JOSHI[1], C.-L. ZHANG[1], T.-M. LIN[1], A. GUSAIN[2], M. G. HARRIS[3], Z.-H. SHENG[4], Z. FABRY[3], R. J. DEMPSEY[2], S. CHIU[1];
[1]Neurosci., [2]Ddepartment of Neurolog. Surgery, [3]Dept. of Pathology and Lab. Med., Univ. of Wisconsin, Madison, Madison, WI; [4]4Synaptic Functions Section, The Porter Neurosci. Res. Ctr., Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD

Abstract: The demyelinating disease multiple sclerosis (MS) has an early inflammatory phase followed by a late phase with subdued inflammation. Unlike the first phase, the late phase resists all known treatments and has poorly understood mechanisms for neurodegeneration. Recent studies have suggested an emerging role for mitochondria in the pathogenesis of MS. In particular, demyelination/dysmyelination triggers an increased axonal mitochondrial density and numbers at lesion sites which is said to a beneficial adaptive response to match an increase in

metabolic demand in demyelinated axons. Further, it has been suggested that mitochondrial anchoring contributes to the increased axonal mitochondrial content in demyelination, evidenced by increased expression of a neuron specific protein Syntaphilin (SNPH). However it is not well studied if the increased mitochondrial contents and SNPH expression are beneficial or detrimental. To examine whether a SNPH-mediated increase in axonal mitochondrial content is beneficial in MS, we genetically eliminated SNPH from the *Shiverer* mice, a dysmyelinating mutant that approximates the late phase of MS in lacking inflammation, show increased mitochondrial density / SNPH expression and cerebellar degeneration. We generated a *Shiverer*-SNPH knock out (Shi-SNPH-KO) mice by crossbreeding *Shiverer* into SNPH-KO mice anticipating worsen of disease course and life span of *Shiverer*. Surprisingly, SNPH-KO significantly prolonged the survival of *Shiverer* and reduced the cerebellar grey and white matter degeneration in these mice. Biochemical and fluorometric analysis revealed an improved mitochondrial health and decreased oxidative stress in Shi-SNPH-KO mice. In contrast, SNPH deletion did not benefit clinical symptoms in experimental autoimmune encephalomyelitis (EAE), a model for early-phase MS. Our results provide the critical link of mitochondrial mobility to MS mice model. Further, because SNPH deletion exerts neuroprotection in the non-inflammatory *Shiverer* model, we suggests that mitochondrial anchoring is a novel site for therapeutic intervention in late phase MS.

Disclosures: **D.C. Joshi:** None. **C. Zhang:** None. **T. Lin:** None. **A. Gusain:** None. **M.G. Harris:** None. **Z. Sheng:** None. **Z. Fabry:** None. **R.J. Dempsey:** None. **S. Chiu:** None.

Poster

224. Demyelinating Disorders: Animal Studies

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: C.09. Demyelinating Disorders

Support: the Guthy Jackson Charitable Foundation

Title: Effects of anti-AQP4 and co-existing autoantibodies at the Blood-Brain Barrier in Neuromyelitis optica

Authors: ***Y. TAKESHITA**[1], B. OBERMEIER[2], F. SHIMIZU[2], A. COTLEUR[2], S. SPAMPINATO[3], T. KRYZER[4], V. LENNON[4], Y. SANO[1], T. KANDA[1], R. M. RANSOHOFF[2]; [1]Neurosci., Yamaguchi Univ. Grad. Sch. of Med., Ube, Japan; [2]Biogen, Cambrige, MA; [3]Univ. of Catania, Catania, Italy; [4]Mayo Clin., Rochester, MN

Abstract: [Background] Neuromyelitis optica (NMO), an autoimmune inflammatory astrocytopathy, is caused by antibodies to the astrocyte water channel aquaporin 4 (AQP4). AQP4 is expressed widely on astrocytes. The IgG plasma fraction of NMO patients (NMO-IgG)

contains AQP4 antibodies. 30% of NMO patients also have well-known co-existing autoantibodies (like anti-phospholipid antibody). It is suggested that NMO-IgG might contain some unknown co-existing autoantibodies to disrupt the blood-brain barrier (BBB) (Shimizu F et al., 2011). It remains uncertain how AQP4 and these co-existing autoantibodies affect on the BBB. [Aim] We examined effects of anti-AQP4 and these co-existing autoantibodies on endothelial cells and astrocytes. [Method] We prepared the total NMO-IgG (T-NMO-IgG) and individual NMO-IgG (I-NMO-IgG). T-NMO-IgG was pooled from therapeutic plasma exchange from total 50 NMO patients and I-NMO-IgG came from each NMO patient (N=5). All NMO-IgG were purified by IgG prep. Conditionally immortalized human brain microvascular endothelial cell line (EC) or human astrocyte cell line which express AQP4 (A4) or don't express AQP4 (A) were cultured on the slide chamber. After exposing NMO-IgGs, we evaluated ICAM-1 expression of EC by quantity PCR and immunocytochemistry, IL-6 expression of EC, A4 and A by ELISA. We also demonstrated immunocytochemistry with T-NMO-IgG or I-NMO-IgG as primary antibodies. [Results] T-NMO-IgG and some I-NMO-IgG increased ICAM-1 of EC and IL-6 of EC and A4 but not A and recognized unknown molecules on EC. The other I-NMO-IgGs didn't induce ICAM-1 expression and detect anything on EC although they elevated IL-6 in A4. [Conclusion] These results indicate that 1) co-existing autoantibodies clearly exerts vascular effects, causing upregulation of ICAM1 and IL-6; and 2) Anti AQP4-IgG itself induces IL-6 in astrocytes via AQP4.

Disclosures: Y. Takeshita: None. **B. Obermeier:** None. **F. Shimizu:** None. **A. Cotleur:** None. **S. Spampinato:** None. **T. Kryzer:** None. **V. Lennon:** None. **Y. Sano:** None. **T. Kanda:** None. **R.M. Ransohoff:** None.

Poster

224. Demyelinating Disorders: Animal Studies

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Program#/Poster#: 224.24/G26

Topic: C.09. Demyelinating Disorders

Support: NIH R01NS060017

the National Multiple Sclerosis Society research grant RG3975

Title: Role of Caspase-8 in multiple sclerosis

Authors: *S. J. KIM[1], F. LU[1], A. STEELMAN[1], J. LI[1], B. ZHOU[2];
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Abstract: Recent studies suggested non-canonical functions of caspase-8 in immune cells. To investigate the role of caspase-8 in regulating immune reactions, we generated conditional

knockout mice with Casp8 selectively disrupted in myeloid cells including activated microglia through lysozyme M-driven Cre recombination, and subjected these mice to experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS). We report here that caspase-8 is activated in microglia/macrophages in active MS lesions and that myeloid specific Casp8 deletion (Casp8LysM) exacerbates neuroinflammation and clinical symptoms of EAE with enhanced Th1 specific T cell responses in the CNS. Mice with Casp8 selectively ablated in microglial cells (Casp8CX3CR1-CerERT) failed to show enhanced EAE progression when compared to their littermate controls, suggesting that caspase-8 activation in peripheral myeloid cells restricts EAE progression. Consistent with our *in vivo* findings, Caspase-8 deficiency in bone-marrow-derived macrophages and dendritic cells produced significantly more IL-1β after innate challenges, which was independent of any cell death. The heightened IL-1β production in Casp8-deficient macrophages/dendritic cells further promoted Th1 cell activation and production of interferon gamma (IFN-γ). The enhanced IL-1β production by caspase-8 deficient macrophages and dendritic cells was completely abolished in Rip3/Casp8 LysM double knockout cells or by necrostatin-1 that prevents RIP1 and RIP3 dimerization, suggesting a function link between RIP3 and IL-1β processing that is negatively regulated by caspase-8. Moreover, exaggerated neuroinflammatory responses and clinical EAE symptoms were ameliorated by Casp8 LysM/Rip3-/-double deletion. Taken together, our studies provide the first evidence that caspase-8 negatively regulates IL-1β production and neuroinflammation during autoimmune demyelination, and suggest potential targets for developing therapeutics in the acute phase of relapsing remitting MS.

Disclosures: **S.J. Kim:** None. **F. Lu:** None. **A. Steelman:** None. **J. Li:** None. **B. Zhou:** None.

Poster

224. Demyelinating Disorders: Animal Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 224.25/G27

Topic: C.09. Demyelinating Disorders

Support: VA Merit

Title: Simultaneous measurement of hnRNP A1 fibril formation by dynamic light scattering (DLS)

Authors: ***M. MOTIWALA**[1], S. LEE[2], H. SALAPA[3], Y. SHIN[2], J. DOUGLAS[3], H. CHO[3], M. LEVIN[2];
[1]Neurol., VA Med. Ctr., Memphis, TN; [2]Neurol., [3]Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Heterogeneous nuclear ribonucleoprotein (hnRNP) A1 is a functionally variable RNA-binding protein (RBP). It affects transcription and mRNA splicing using both RNA interactions and nuclear pore transport. HnRNP A1 is required by neurons for normal functioning, and moreover, inadequate levels of the RBP, whether caused by transcription errors or as a result of autoimmune reactions, result in the dysregulation of normal RNA processing. Indeed, this type of dysregulation is well established. It plays an important role in several neurodegenerative conditions, but its link to hnRNP A1 represents a fairly novel insight and may involve other associated proteins. Notably, when stress granules form as a result of mutations in genes coding for stress granule-associated RNA binding proteins such as hnRNP A1, fused in sarcoma (FUS), transactive response DNA-binding protein (TDP43), etc., the aggregation of proteins is exacerbated. This dysregulated aggregation caused by genetic mutations in RBPs can initiate degenerative disease. Furthermore, depleted hnRNP A1 is associated with a symptomatological increase in numerous neurodegenerative diseases, including Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS) and HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP). In the context of our own research, point mutations in the genetic sequence for hnRNP A1 are shown to inhibit normal binding by transportin-1 at the M9 region, and this condition eventually contributes to the pathogenesis of MS. In this study, we utilized the dynamic light scattering (DLS) method to examine the protein-protein interaction between transportin-1 and hnRNP A1. DLS measures intensity fluctuations of scattering light in order to calculate the molecular radius of protein complexes in solution. Successful application of this method confirmed normal function of the RNA-binding proteins, demonstrating proof of concept for DLS as a higher throughput approach to evaluate protein binding. Because defective and aggregated cellular proteins are a feature of a number of neurodegenerative diseases, DLS represents a novel technique to explore their significance without the costly and time consuming constraints of the standard protein-protein interaction assays (GST pull- down assay, co-immunoprecipitation, proximity ligation assay (PLA), etc.). By utilizing the DLS approach, numerous samples of abnormal hnRNP A1 could be quickly screened for binding with transportin-1 or other proteins, further clarifying the functional significance of these defects in the context of MS.

Disclosures: **M. Motiwala:** None. **S. Lee:** None. **H. Salapa:** None. **Y. Shin:** None. **J. Douglas:** None. **H. Cho:** None. **M. Levin:** None.

Poster

224. Demyelinating Disorders: Animal Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 224.26/G28

Topic: B.11. Glial Mechanisms

Support: Mayo Clinic CMSDD Grant

NIH 5T32AI007047-33

Koltan Pharmaceuticals

Title: Cross-talk between astrocytes and mast cells is a neuro-immune mechanism for disease exacerbation in neuromyelitis optica

Authors: *M. CAULFIELD, Y. GUO, H. KITA, C. HOWE, C. LUCCHINETTI;
Mayo Clin., Rochester, MN

Abstract: Neuromyelitis optica (NMO) is an inflammatory CNS autoimmune disorder characterized by recurrent attacks of the optic nerve, spinal cord, and brain. The identification of a putative pathogenic autoantibody, NMO IgG, has enabled reliable diagnostic distinction between NMO and other similar disorders. Pathogenesis is likely initiated by binding of NMO IgG to the surface of target cells expressing aquaporin-4 (AQP4), the principle CNS water channel. Astrocytes are a primary target in NMO due to the high expression of AQP4 on perivascular and peripial endfeet. In addition to lytic events, we have found immunopathological evidence of a spectrum of early, sublethal, non-lytic, widespread astrocytic alterations in NMO human tissues including early granulocytic infiltration, even in the absence of terminal complement deposition and demyelination, suggesting that early astrocyte responses to NMO IgG may drive recruitment. In an *in vitro* culture system, stimulation of astrocytes with NMO IgG drives a rapid and robust pro-granulocytic cytokine and chemokine response. Strikingly, mast cells, members of the innate immune system, co-localize with both astrocyte foot processes and AQP4 expression throughout the CNS. Mast cells are early responders to insults in both infectious and autoimmune settings where they act to recruit other innate immune cells to sites of inflammation. Recent studies have shown that mast cells and astrocytes interact directly via CD40:CD40L interactions or via soluble mediators that induce the release of histamine, leukotrienes, and numerous cytokines and chemokines. Therefore, we asked if cross-talk between astrocytes and mast cells contributes to the exacerbation of inflammation in NMO. Microarray analysis revealed that astrocyte cultures upregulate a variety of mast cell-associated transcripts within 24 hours of stimulation by NMO IgG. Preliminary examination of mast cells in human tissue using H&E and tryptase immunohistochemistry in 4 NMO cases (29 regions) and 7 healthy controls revealed: i) increased frequency of mast cells in NMO meninges and periplaque white and gray matter (PPWM/PPGM); ii) mast cells in early non-destructive, vacuolated NMO lesions with AQP4 loss; iii) mast cell degranulation in meninges, PPWM, PPGM and NMO lesions; and iv) mast cells co-localized with granulocytic infiltrates. Together these data suggest that mast cells are actively involved in early NMO lesions where they may play a pivotal role in granulocyte recruitment, and suggest that cross talk with astrocytes may provide the impetus for this novel mechanism of early NMO pathogenesis.

Disclosures: M. Caulfield: None. **Y. Guo:** None. **H. Kita:** None. **C. Howe:** None. **C. Lucchinetti:** None.

Poster

224. Demyelinating Disorders: Animal Studies

Location: Hall A

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Topic: B.11. Glial Mechanisms

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Adelson Medical Research Foundation

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Novartis Institute for Biomedical Research

NHMRC (Australia)

Title: A1 reactive astrocytes are strongly neurotoxic inducing apoptosis of neurons and oligodendrocytes and are induced by M1 microglia via IL1α, TNFα and C1q signaling

Authors: *S. A. LIDDELOW[1,2], A. E. MÜNCH[1], L. E. CLARKE[1], B. A. BARRES[1]; [1]Neurobio., Stanford Univ., Stanford, CA; [2]Pharmacol. and Therapeut., The Univ. of Melbourne, Melbourne, Australia

Abstract: Astrocytes undergo profound changes in morphology and gene expression in response to brain injury and disease. But whether reactive astrocytes are harmful or helpful has been unclear. We recently found that the genes induced in reactive astrocytes depends on the nature of the inducing injury. After ischemia, reactive astrocytes upregulate neurotrophic factors suggesting they may be beneficial, whereas after systemic injection of lipopolysaccharide (LPS) they strongly upregulate multiple complement cascade components needed to drive synapse destruction suggesting they may be detrimental. These findings suggest that, like macrophages which exist on a spectrum from bad (M1) to good (M2) states, reactive astrocytes also exist in bad (A1) and good (A2) states. Here we show that LPS-induced M1 microglia are sufficient to induce A1 reactive astrocytes. M1 microglia do this by releasing IL1α, TNFα and C1q, which together are sufficient to induce A1 (bad) reactivity in purified astrocytes within 24h and are all required for M1 microglia to induce the A1 state. Using IL1α, TNFα and C1q together, allowed us to create the first defined serum-free cultures of pure A1 reactive astrocytes enabling us to investigate their function. By directly comparing the function of normal astrocytes with A1 astrocytes *in vitro*, we found that A1 astrocytes are unable to promote neuronal survival, axon outgrowth, synapse formation or synapse function, and have lost the ability to phagocytose synaptosomes and myelin debris. In addition to loss of their normal functions, A1 reactive astrocytes gained a powerfully neurotoxic function, releasing a toxic protein that specifically induces apoptosis of neurons and oligodendrocytes. Drugs that prevent the formation of A1 reactive astrocytes or inhibit this toxic protein may have great potential to treat neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Multiple Sclerosis.

Disclosures: **S.A. Liddelow:** None. **A.E. Münch:** None. **L.E. Clarke:** None. **B.A. Barres:** None.

Poster

224. Demyelinating Disorders: Animal Studies

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 224.28/G30

Topic: C.09. Demyelinating Disorders

Support: NIH Grant 5R01NS080976-02

Title: Ultrastructural analysis of hippocampal synapses following demyelination

Authors: ***S. JAWAID**[1], G. J. KIDD[1], R. DUTTA[1], L. ROHOLT[1], S. DECKARD[1], E. BENSON[1,2], A. CHOMYK[1], B. D. TRAPP[1];
[1]Dept. of Neurosci., Lerner Res. Inst., Cleveland, OH; [2]Renovo Neural, Inc., Cleveland, OH

Abstract: Memory dysfunction and hippocampal demyelination is common in Multiple Sclerosis (MS) patients. Hippocampal demyelination has been associated with significant decrease in expression of pre- and post-synaptic proteins specific for glutamatergic synapses and neuronal proteins that modulate synaptic plasticity and memory without obvious loss of neurons. To investigate how demyelination impacts synaptic function, we utilized a rodent animal model of hippocampal demyelination/remyelination that uses the oligodendrocyte toxin cuprizone (C) and rapamycin (R), an inhibitor of oligodendrocyte differentiation. C/R treatment for 12 weeks led to ~95% reduction of myelin staining; followed with 6 weeks of normal diet led to myelin levels ~63% of control. Remyelination of the hippocampus in this model completely reversed the memory dysfunction observed following demyelination. To understand the ultrastructural changes associated with hippocampal demyelination and remyelination, we used a serial blockface electron microscopy (SBF-SEM) and investigated synaptic alterations in the distal stratum radiatum of CA1 pyramidal neurons. Dendrites in this region produce excitatory synaptic spines which are highly dynamic and are involved in learning and memory. Imaging and reconstruction of primary dendrites from chronic demyelinated hippocampus showed increase (almost double) in median spine volume, spine lengths (35%) and larger post synaptic densities (PSD area/Spine area; 25%) compared to Rap-only control. These changes were a response to chronic demyelination as the dendritic spine morphology showed reversal towards control levels following remyelination. As astrocytes are integral part of synaptic functioning, we determined extent of astrocyte contacts in CA1 synapses. In demyelinated hippocampus, the frequency of astrocyte processes at synaptic cleft doubled and recovered towards Rap-only control after 6w remyelination. Larger spines participated more in the formation of these tripartite synapse. Collectively, our results suggest that demyelination affects synaptic plasticity in rodent

hippocampus through alteration of the dendritic spine morphology and increased astrocyte-synapse interactions.

Disclosures: **S. Jawaid:** None. **G.J. Kidd:** None. **R. Dutta:** None. **L. Roholt:** None. **S. Deckard:** None. **E. Benson:** None. **A. Chomyk:** None. **B.D. Trapp:** None.

Poster

224. Demyelinating Disorders: Animal Studies

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Program#/Poster#: 224.29/G31

Topic: C.09. Demyelinating Disorders

Support: T32DC012280-01

Title: Myelin disruption leads to targeted behavioral deficits

Authors: *E. A. GOULD, N. BUSQUET, D. RESTREPO, W. MACKLIN;
Univ. of Colorado Sch. of Med., Aurora, CO

Abstract: Myelin is required for proper nerve conduction and has an important role in normal axonal function. Alteration in myelin structure and quantity is observed in various neurological diseases. Polymorphism in myelin genes is associated with conditions including depression, schizophrenia, and bipolar disorder. However, little is known about the neuronal and behavioral consequences of myelin disruption alone and the role of myelin genes in pathology. To address the contribution of myelin function to behavioral and cognitive deficits, we used a mouse line lacking the myelin proteolipid protein (PLP). These mice generate myelin but exhibit progressive myelin dysfunction and eventual axonal degeneration. We tested 3 and 8 month-old PLP knockout PLP(-/Y) male mice in a battery of behavioral tests. No motor deficits were observed in 3 and 8 month old PLP(-/Y) mice on the Rotarod, a classical test of motor function. Altered emotionality was observed in 3 and 8 month PLP(-/Y) mice. 3 month PLP(-/Y) mice spent more time in open arms of the zero maze (175 ±17 vs 92±21 seconds, $p<0.05$), a test of anxiety, while no change was observed at 8 months (126±27 vs 117 ±26 s, $p=0.81$). 8 month PLP(-/Y) mice spent less time in the center of an open field (159±23 seconds vs. 239±25 seconds, $p <0.05$), while exploration of the walls was increased (105.3±9 vs 74.1±10 seconds, $p<0.05$). PLP(-/Y) mice demonstrated a decrease in the motivation to bury marbles in the marble burying task (mean number of marbles buried: 3 month = 2.9±0.3 vs. 6.1±0.6; 8 month = 0.3±0.2 vs. 4.2±0.9, $p<0.001$). Performance on the Y maze, a test of spatial memory and hippocampal function, was normal in 3 and 8 month old PLP(-/Y) mice. In the Puzzle Box, a test of problem-solving and executive function, 3 and 8 month PLP(-/Y) mice displayed longer latency to reach the goal box when presented with a new challenge (repeated measure ANOVA, $p=0.02$), indicating deficits in higher cognition. Intriguingly, a significant increase in immobility and a lack of coordinated

swimming was observed when 8 month PLP(-/Y) mice were placed in water (distance moved = 140 ± 5 vs 339 ±11 in 30 second swimming trial, $p<0.01$). Our data indicate that myelin dysfunction results in targeted behavioral deficits and cognitive dysfunction even long before significant axonal degeneration can be observed. This suggests that there could be a myelin-specific dimension to certain neurological disorders, which may warrant specific therapeutic interventions. Ongoing investigation aims at better characterizing those deficits and linking them to structural alteration of myelin in specific parts of the brain, in order to understand the underlying mechanisms.

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Poster

224. Demyelinating Disorders: Animal Studies

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Program#/Poster#: 224.30/G32

Topic: C.09. Demyelinating Disorders

Support: Dartmouth SYNERGY

Title: Correlation of spinal cord and CSF biomarkers to clinical and radiological measures of CNS injury in a mouse model of multiple sclerosis

Authors: *F. A. GILLI[1], X. CHEN[2], A. R. PACHNER[1], B. GIMI[2];
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Abstract: OBJECTIVE: To develop biomarkers relevant to inflammatory, demyelinating CNS injury in the Theiler's encephalomyelitis virus-induced demyelinating disease (TMEV-IDD) model of MS by correlating gene expression in the spinal cord and CSF with MRI and clinical measures of spinal cord injury. BACKGROUND: A major gap in our understanding of MS is a lack of information about the pathogenesis of demyelination and axonal injury that are the pathological hallmarks of the disease. This has led to the lack of availability of useful clinical biomarkers of inflammation and disease. The TMEV-IDD model of MS reproduces both the progressive spastic disability and the pathology of MS, and thus serves as an ideal model to improve our understanding of mechanisms of CNS injury in MS. DESIGN/METHODS: We used gene expression measures of spinal cord tissue and CSF to correlate potential biomarkers to spinal cord injury as measured by MRI (T2 and diffusion tensor imaging) and Rotarod. SJL/J mice with TMEV-IDD with varying levels of clinical disease and anti-TMEV antibodies in the serum were serially imaged using a 9.4T horizontal bore small animal MRI scanner. Mice were then necropsied; blood, CSF, and spinal cord tissue were obtained. Gene expression of a range of host and viral proteins were analyzed by real time RT-PCR for mRNA of tissue, and multiplex

bioassay for proteins in the CSF. Correlation was then performed between potential biomarkers and MRI and Rotarod. RESULTS: A number of potential biomarkers, including molecules upregulated in CNS antibody production, were identified using this approach. Many of these potential biomarkers are classified as innate immune molecules. CONCLUSIONS: Correlation of CNS tissue and CSF biomarkers to clinical and MRI measures of disease in the spinal cord in TMEV-IDD represents a sound approach to development of clinically useful biomarkers in MS.

Disclosures: **F.A. Gilli:** 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.; Biogen Idec. **X. Chen:** None. **A.R. Pachner:** 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.; Biogen Idec, EMD Serono, Genzyme, Sanofi-Aventis. **B. Gimi:** None.

Poster

225. Traumatic Brain Injury: Human Studies II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 225.01/G33

Topic: F.03. Motivation and Emotion

Support: CIHR to DE

Title: Long-term psycho-affective outcomes in male athlete with a history of concussion

Authors: ***W. SAUVE**, R. D. MOORE, D. ELLEMBERG;
Kinesiology, Neurodevlab, Montreal, QC, Canada

Abstract: Mild or concussive brain injuries are known to cause psycho-affective changes (Jorge, 2004). However, these disturbances are believed to resolve within 7 to 10 days of injury (McCrory, 2013), and few studies have evaluated the persistent psycho-affective outcomes of concussion in collegiate athletes. As such, this study evaluated the long-term psycho-affective outcomes of concussions in collegiate athletes and the relation between the number of injuries and their psycho-affective functioning. Seventy-seven collegiate male athletes (43 concussed, age = 21.23 ± 1.43; 34 controls, age = 21.82 ±1.83) completed the Beck's Depression Inventory-II (BDI-II) and the Profile of Mood States (POMS). All participants were asymptomatic at the time of testing and those with a concussion history were 1+ year post-injury (26.58 months ± 14.67). Analyses revealed that athletes with a concussion history has significantly higher scores on the BDI-II (5.72 ± 3.92) relative to controls (3.91 ± 3.40; $p<0.05$, $d = 0.49$). In addition, athletes with a history of concussion had a significantly higher score on the total mood disturbance scale of the POMS (17.81 ± 24.11) relative to controls (5.82 ± 16.94; $p<0.05$, d =

0.58). Further, the anger subscale is also significantly higher for the concussed athletes(8.30 ± 6.37) relative to controls (4.05 ± 3.77; $p<0.05$, $d = 0.81$). Bivariate correlations failed to reveal a relation between number of injuries and scores on either the BDI-II or POMS ($p's \geq 0.20$). Thus, a single concussion may be sufficient to cause small, yet persistent, alterations in psycho-affective functioning.

Disclosures: W. Sauve: None. **R.D. Moore:** None. **D. Ellemberg:** None.

Poster

225. Traumatic Brain Injury: Human Studies II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 225.02/G34

Topic: F.01. Human Cognition and Behavior

Support: R01 EB008432

R01 AG040060

U54 EB020403

R01 NS080655

UCLA BIRC

NS027544

NS05489

Title: Disruptions to white matter microstructure of the default mode network in pediatric traumatic brain injury

Authors: *V. G. BABOYAN[1,2,3], A. MEZHER[1], E. L. DENNIS[1], M. DAIANU[1], Y. JIN[1], T. BABIKIAN[2], C. GIZA[3], R. F. ASARNOW[2], P. M. THOMPSON[1];
[1]USC, Marina Del Rey, CA; [2]Dept. of Psychiatry and Biobehavioral Sci., [3]Dept. of Neurosurg., UCLA, Los Angeles, CA

Abstract: Traumatic Brain Injury (TBI) has been shown to disrupt Intrinsic Connectivity Networks (ICNs) in studies examining the Default Mode Network (DMN). This disruption may interfere in switching between task-dependent and task-independent networks, leading to various neurocognitive deficits. Reduced connectivity in the DMN may even cause or be driven by structural changes in white matter (WM) tracts connecting DMN hubs. Here we tested thickness measures in the cingulate gyrus - the underlying *cortical* structure of the DMN - and microstructural changes in the cingulum bundle - the underlying *subcortical* structure of the DMN. Neuropsychological tests, T1-weighted MRI scans, and high angular resolution diffusion

weighted imaging (HARDI) were obtained from 24 chronic (13-19 months post-injury) TBI participants (mean age: 15.65±3) and 26 healthy controls (mean age: 16.79±2.8). Principal Components Analysis (PCA) was used to generate a single performance index based on tests from several cognitive domains that are sensitive to brain injury. Multi-atlas based fiber clustering was performed on whole-brain tractography to extract 4 fibers (bilateral cingulum and their parahippocampal components), for which we extracted measures of WM integrity - FA (fractional anisotropy) and MD (mean diffusivity). We computed cortical thickness for 4 subdivisions of the cingulate gyrus (rostral and caudal anterior, posterior, and isthmus) along with the precuneus and parahippocampal gyri using FreeSurfer v5.3. To test for group differences in the microstructural integrity of the cingulum and separately, thickness measures across gyri, we ran a random effects regression, covarying for age, sex, and scanning site. We also tested relationships between DMN structure and cognitive function. Significant group differences were found in mean MD measures across all 4 fibers examined (FDR critical P= $p<0.05$, FDR corrected) - most pronounced in the left cingulum ($p<0.01$, corrected), with TBI participants having greater mean MD. Cortical thickness measures were not significantly different between groups and no relationships were found between gray/WM measures and the performance indices. Consistent with past findings, mean diffusivity of DMN WM was a sensitive biomarker of structural abnormalities in TBI. Understanding the structural and functional dynamics of the brain's ICN's in TBI may inform clinical decision making if therapies could target specific brain networks. Future work examining tract-wise WM microstructure of the cingulum and commissural fibers as well as vertex-wise gray matter volume will help evaluate their contributions to DMN function.

Disclosures: V.G. Baboyan: None. **A. Mezher:** None. **E.L. Dennis:** None. **M. Daianu:** None. **Y. Jin:** None. **T. Babikian:** None. **C. Giza:** None. **R.F. Asarnow:** None. **P.M. Thompson:** None.

Poster

225. Traumatic Brain Injury: Human Studies II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 225.03/G35

Topic: C.10. Trauma

Support: Canada Excellence Research Fund

Title: Processing of complex, real-world information as awareness fades under sedation

Authors: *L. NACI, E. HOULDIN, R. CUSACK, M. ARANGO, C. HARLE, A. M. OWEN; Brain and Mind Inst., Western Univ., London, ON, Canada

Abstract: Anesthesia provides a controlled manipulation for abolishing consciousness in a reliable manner in healthy individuals. When combined with functional neuroimaging, it provides a unique approach for studying the brain mechanism of consciousness and how they go awry when consciousness is lost. The majority of anesthesia studies have used a stimulus- and task-free paradigm, known as the resting state, to investigate changes in neural activity as the brain transitions from the wakeful to the anesthesia-induced unconscious state. However, these studies cannot address the question of how processing of external information that evolves over time is affected by sedation. For the first time, we investigate the cognitive response to complex naturalistic stimuli in states of incremental sedation, from awake, to mild, and to deep sedation, a state characterized by lack of behavioral responsivity. We used a novel paradigm that presented a rich, auditory narrative to participants (N=17) in the functional Magnetic Resonance Imaging (fMRI) scanner. The level of propofol sedation was assessed with the Ramsay clinical scale (Ramsay 1974). Assessments of Ramsay 1, 3, or 5 determined awake, mild, or deep sedation, respectively. Perception and higher-order cognition were investigated by modeling the fMRI response with Statistical Parametric Mapping. Independent Component Analysis further characterized brain networks. In the awake state, we found robust responses in brain networks involved in sound processing, language, visual imagery, and executive function ($p<0.05$, FEW cor). In mild sedation, the sound processing and language networks were preserved, but the visual imagery and executive function networks were abolished. In deep sedation, the sound network was preserved, whereas the language network showed weak residual activity. No activity was observed in the visual and executive function networks. These results suggest a dose-dependent effect of sedation on some, but not all aspects of the brain response to complex naturalistic information. Moreover, they suggest hierarchical impairment of cognition in the unconscious state, with incremental impairment from sound to language processing, and a complete loss of visual imagery and executive function. By contrast to resting state studies, which suggest present albeit diminished activity across different brain networks, these data paint a more nuanced picture of loss and preservation of brain function in the unconscious state.

Disclosures: **L. Naci:** None. **E. Houldin:** None. **R. Cusack:** None. **M. Arango:** None. **C. Harle:** None. **A.M. Owen:** None.

Poster

225. Traumatic Brain Injury: Human Studies II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 225.04/G36

Topic: C.10. Trauma

Support: James S. McDonnell Foundation Scholar Award

Title: Restoration of thalamo-cortical connectivity after severe brain injury: recovery consciousness or passage of time?

Authors: *M. M. MONTI;
UCLA, Los Angeles, CA

Abstract: What mechanisms underlie the loss and recovery of consciousness after severe brain injury? Conceived as a network-level impairment, thalamo-frontal connectivity is generally believed to be a central aspect for the maintenance (and restoration) of consciousness after severe brain injury. Indeed, in a landmark case report, it has been shown that thalamo-frontal connectivity appeared to be restored in concomitance with the return of awareness (Laureys et al., 2000). Yet, to date, the well accepted notion of the parallel restoration of thalamo-cortical functional connectivity and awareness after severe brain injury rests mainly on that single case report. We thus followed prospectively 11 patients surviving severe traumatic brain injury and acquired functional resting state data within the first week of insult and at six-months post-trauma. Data were acquired on a 3T Siemens TimTrio system, and included structural T1-weighted (MPRAGE) as well as T2*-weighted data. The blood oxygenation level dependent (BOLD) signal was measured, at both the acute and chronic timepoints, with a "resting-state" design. Data were preprocessed according to established procedures, including anatomical registration, slice time correction, motion correction, nuisance signal regression, temporal filtering ans smoothing (8mm FWHM). A seed based analysis, employing bilateral thalamus as the seed mask, was performed independently for each session (for each subject) and then entered in a paired-samples group analysis. At the group level, the variance was modeled employing a mixed-model approach, and statistical maps were thresholded using a cluster correction of $Z > 2.3$ and $p < 0.05$ (corrected). The results show that, as compared to the acute timepoint, greater thalamo-frontal connectivity was present across all patients -- replicating the original case report. However, contrary to the original report, the increase in thalamo-cortical connectivity was observed regardless of the change in consciousness state of the patient. Indeed, the degree of connectivity restoration in patients who recovered consciousness was comparable to that observed in patients who were already (minimally) conscious during the acute scan and remained so through the chronic scan. These findings suggest that while thalamo-cortical connectivity does return with time -- as shown in the original finding -- it might not necessarily be connected to the return of consciousness. Reference Laureys, S., Faymonville, M. E., Luxen, A., Lamy, M., Franck, G., & Maquet, P. (2000). Restoration of thalamocortical connectivity after recovery from persistent vegetative state. The Lancet, 355(9217), 1790-1791.

Disclosures: M.M. Monti: None.

Poster

225. Traumatic Brain Injury: Human Studies II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 225.05/G37

Topic: C.10. Trauma

Support: James McDonnell Foundation

Mind Science Foundation

European Space Agency

University and University Hospital of Liège

Title: Intrinsic fMRI functional architecture differentiates single patients after severe brain injury

Authors: *A. DEMERTZI[1,2], G. ANTONOPOULOS[2], L. HEINE[2], H. U. VOSS[4], J. S. CRONE[6,7,8], C. DE LOS ANGELES[9], M. BAHRI[3], C. DI PERRI[2], F. GOMEZ[10], A. VANHAUDENHUYSE[11], V. CHARLAND-VERVILLE[2], M. KRONBICHLER[7,7], E. TRINKA[8], C. PHILLIPS[3], L. TSHIBANDA[12], A. SODDU[13], N. D. SCHIFF[14,5], S. WHITFIELD-GABRIELI[9], S. LAUREYS[2];
[1]Inst. Du Cerveau Et De La Moelle Épinière - ICM, PARIS, France; [2]Coma Sci. Group, Cyclotron Res. Ctr. & Neurol. Dept., [3]Cyclotron Res. Ctr., Univ. of Liège, Liège, Belgium; [4]Dept. of Radiology and Citigroup Biomed. Imaging Ctr., [5]Dept. of Neurol. and Neurosci., Weill Cornell Med. Col., New York, NY; [6]Dept. of Psychology, Ctr. for Neurocognitive Res., Salzburg, Austria; [7]Neurosci. Inst. and Ctr. for Neurocognitive Research, Christian-Doppler-Klinik, [8]Dept. of Neurology, Christian-Doppler-Klinik, Paracelsus Private Med. Univ., Salzburg, Austria; [9]Martinos Imaging Ctr. at McGovern Inst. for Brain Res., MIT, Cambridge, MA; [10]Computer Sci. Dept., Univ. Central de Colombia, Bogota, Colombia; [11]Dept. of Algology – Palliative Care, [12]Dept. of Radiology, CHU Univ. Hosp. of Liège, Liège, Belgium; [13]Brain & Mind Institute, Dept. of Physics and Astronomy, Western Univ., London, ON, Canada; [14]Dept. of Neurosci., Weill Cornell Grad. Sch. of Med. Sci., New York, NY

Abstract: Background: Resting state acquisitions are by definition appropriate to assess non-communicating subjects. We here evaluated the clinical relevance of systems-level resting state fMRI in patients with disorders of consciousness with the aim to promote single-patient diagnostics. Methods: Seventy three patients in minimally conscious state (MCS), vegetative state/unresponsive wakefulness syndrome (VS/UWS) and coma were scanned in 3 different centers. The main analysis was performed on the dataset coming from one centre (Liège, 51 patients; 26 MCS, 19 VS/UWS, 6 coma; 16 traumatic, 32 non-traumatic, 3 mixed; 35 patients assessed >1 month post-insult) for whom the clinical diagnosis with the Coma Recovery Scale-Revised (CRS-R) was congruent with positron emission tomography scanning. Using a multiple-seed correlation approach, group-level functional connectivity was investigated for the default mode, frontoparietal, salience, auditory, sensorimotor and visual networks. Between-group inferential statistics and machine learning were used to identify each network's capacity to discriminate between patients in MCS and VS/UWS. Data from 22 patients independently scanned in two other centres (Salzburg: 10 MCS, 5 VS/UWS; New York: 5 MCS, 1 VS/UWS, 1 emerged from MCS) were used to validate the classification with the indetified features. Results:

CRS-R total scores correlated with key regions of each network reflecting their involvement in consciousness-related processes. Although all networks had a high discriminative capacity (>80%) for separating patients in MCS and VS/UWS, the auditory network was ranked the most highly. Specifically, bilateral auditory and visual cortices of the auditory network were more functionally connected in patients in MCS compared to VS/UWS. Connectivity values in these three regions discriminated congruently 20 out of 22 independently assessed patients. Conclusions: These findings highlight the significance of preserved multisensory integration and top-down processing in minimal consciousness which are seemingly supported by auditory-visual crossmodal connectivity, and promote the clinical utility of the resting paradigm for single-patient diagnostics.

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Poster

225. Traumatic Brain Injury: Human Studies II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 225.06/G38

Topic: C.10. Trauma

Title: Default mode network anticorrelations are associated with better clinical outcome in patients with disorders of consciousness

Authors: *S. GABRIELI;
MIT, Cambridge, MA

Abstract: Introduction: Resting state functional connectivity (rs-fcMRI) reveals intrinsic, spontaneous networks that elucidate the functional organization of the human brain. These resting state networks offer insight into residual brain function in patients with disorders of consciousness (DOC), such as minimally conscious state (MCS) and vegetative state/unresponsive wakefulness syndrome (VS/UWS). During rest, the BOLD signal fluctuations in regions of the default mode network (DMN) strongly correlate with each other, whereas they are anticorrelated (negatively correlated) to regions such as the dorsolateral prefrontal cortex (DLPFC) in the “task positive network”(TPN). The DMN is thought to be engaged in “internal awareness” whereas the TPN is engaged in “external awareness” and the magnitude of the DMN/TPN anticorrelations may reflect an ability to toggle between internal and external modes of processing. Here we examined the DMN rs-fcMRI in 71 participants: 27 Control, 22 MCS and 22 VS/UWS participants. We hypothesized that the DMN positive correlations would remain in

patients with DOC while the anticorrelations would be selectively extinguished. Methods: 10-minute resting-state fMRI scans were collected and analyses were performed using a seed-driven approach in Conn, (http://www.nitrc.org/projects/conn). Group statistics were performed on the Fisher transformed correlation maps. Clinical outcome of the patients was measured with Glasgow Outcome Scale-Extended (GOSE). DMN rs-fcMRI was correlated with GOSE. Results: Controls had robust DMN correlations/anticorrelations. Both patient groups had significant positive DMN correlations however DMN anticorrelations were statistically eliminated. However, variation of DMN-DLPFC rs-fcMRI predicted clinical outcome such that more negative correlations were associated with better outcome. Although on average, the DMN-DLPFC antircorrelations were not significantly different from zero for both patient groups, the individual differences significantly related to clinical outcome both within and across groups. Conclusions: Patient heterogeneity of intrinsic functional networks may provide useful neuromarkers for clinical outcome.

Disclosures: S. Gabrieli: None.

Poster

225. Traumatic Brain Injury: Human Studies II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 225.07/G39

Topic: C.10. Trauma

Support: James S. McDonnell Foundation

NIH #UL1 TR000043

The Stavros Niarchos Foundation

Title: Neocortical dynamics of ongoing EEG reflects mesocircuit integrity in acute anoxic brain injury after cardiac arrest

Authors: *P. B. FORGACS[1,2], H.-P. FREY[3], E. MEYERS[3], A. G. VELAZQUEZ[3], S. THOMPSON[3], S. PARK[3], S. AGARWAL[3], M. C. FALO[3], M. SCHMIDT[3], N. D. SCHIFF[1,2], J. CLAASSEN[3];
[1]BMRI - Dept. of Neurol., Weill Cornell Med. Col., New York, NY; [2]The Rockefeller Univ., New York, NY; [3]Dept. of Neurology, Div. of Critical Care, Columbia Univ. Med. Ctr., New York, NY

Abstract: Functional changes of the anterior forebrain mesocircuit (AFM) (Schiff, 2010) measured in EEG and PET have been correlated with spontaneous recovery of consciousness (Forgacs et al. 2014, Fridman et al. 2014) and with observed effects of therapeutic interventions in patients with chronic brain injuries (Schiff et al. 2007, Williams et al. 2013). The AFM model

predicts graded transitions in neocortical dynamics as measured by the EEG based on the degree of functional or structural deafferentation of the corticothalamic system (Drover et al. 2011, Schiff, Nauvel and Victor, 2014). The goal of this study was to assess EEG markers of AFM integrity and correlate them with short-term recovery of consciousness in patients in the Intensive Care Unit (ICU) as determined by daily standardized behavioral exam (Coma Recovery Scale - Revised [CRS-R]) and clinical outcome at hospital discharge (Cerebral Performance Categories Scale [CPC]). EEGs of 54 patients in ICUs after cardiac arrest were analyzed using multi-taper method. Frequency spectra were categorized using a coarse-grained 4 point scale based on the AFM model (Schiff et al. 2014). All EEG analyses were performed blinded to all clinical variables. 36 (66.6%) patients could be classified into our pre-defined categories on at least one day of EEG analysis and had a known CPC. The non-classified patients included: 3 with unknown CPC, 4 with status epilepticus, 8 with other spectral features, 3 with technical problems limiting analysis. We did not find significant differences in age, time to return of spontaneous circulation, CRS-R and CPC between the two sub-cohorts. In the 36 classified patients, the level of AFM integrity correlated with behavioral exam (CRS-R). Importantly, Fisher's exact test revealed significant correlation ($p=0.0011$) between AFM markers and clinical outcome. These results demonstrate that neocortical dynamic features are linked to the degree of corticothalamic deafferentation as predicted by the AFM model and may provide new insights into cellular and network level mechanisms involved in short-term recovery of consciousness in the acute stages of severe anoxic brain injury. These results suggest the possible use of these measures to track recovery and the effects of neuroprotective or other specific restorative therapeutic interventions employed in very early stages of brain injury.

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Poster

225. Traumatic Brain Injury: Human Studies II

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 225.08/G40

Topic: C.10. Trauma

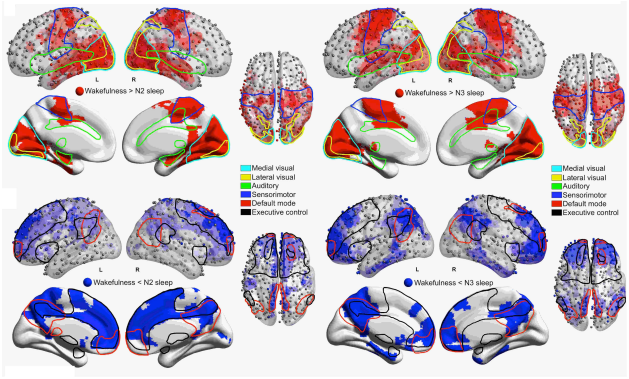
Support: Bundesministerium für Bildung und Forschung (grant 01 EV 0703)

LOEWE Neuronale Koordination Forschungsschwerpunkt Frankfurt (NeFF)

Title: Dynamics, connectivity and anatomical-functional coupling during loss of consciousness in deep sleep

Authors: *E. TAGLIAZUCCHI[1], N. CROSSLEY[3], E. BULLMORE[4], H. LAUFS[2]; [2]Neurol. Department,, [1]Univ. of Kiel, Kiel, Germany; [3]Inst. for Biol. and Med. Engin., Pontificia Univ. Catolica de Chile, Santiago de Chile, Chile; [4]Dept. of Psychiatry, Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Daily, we experience deep sleep as a state of reversible loss of consciousness. Why is brain activity during sleep not associated with conscious awareness, and which mechanisms allow the re-emergence of wakefulness? In response, we derive three fundamental principles of deep sleep from electrophysiology-combined functional neuroimaging data: 1) In contrast to wakefulness, activity in frontoparietal regions exhibits random, uncorrelated dynamics; 2) Large-scale functional connectivity changes in this set of regions - compared to wakefulness - represent a localised departure from the underlying constraints imposed by anatomical connectivity; 3) A simple model unfolding over the network of anatomical connections can accommodate both observations above, provided that a change in the threshold for activity propagation occurs. Our results highlight network-specific, diminished complexity of spatiotemporal dynamics as a correlate of loss of consciousness. They also suggest that deep sleep actively disrupts default patterns of functional connectivity in associative cortices, which are essential for the conscious access of information, and that anatomical connectivity acts as an anchor for the restoration of frontoparietal functionality upon awakening. This last observation may be the key for the understanding of conditions characterized by an irreversible loss of consciousness like coma or the vegetative state.



System-specific changes of anatomical-functional coupling during deep sleep.
Red: Regions where increased coupling was observed.
Blue: regions where decreased coupling was observed.

Disclosures: **E. Tagliazucchi:** None. **N. Crossley:** None. **E. Bullmore:** None. **H. Laufs:** None.

Poster

225. Traumatic Brain Injury: Human Studies II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

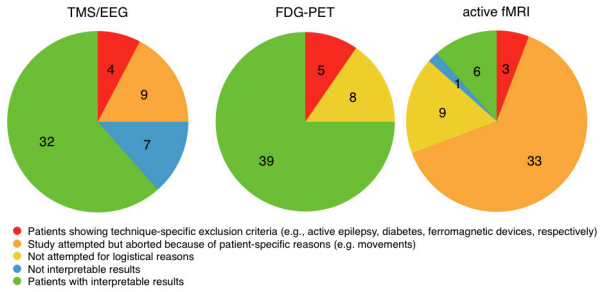
Program#/Poster#: 225.09/G41

Topic: C.10. Trauma

Title: Multimodal TMS/EEG, FDG-PET and active fMRI assessments in patients with disorders of consciousness and locked-in syndrome

Authors: *O. BODART[1,2], S. WANNEZ[1,2], A. THIBAUT[1,2], J. ANNEN[1,2], A. G. CASALI[3], S. CASAROTTO[4], O. GOSSERIES[2,5], M. ROSANOVA[4], M. MASSIMINI[4], S. LAUREYS[1,2]; [1]Neurol., CHU Sart Tilman, Liege, Belgium; [2]Cyclotron Res. Ctr., University of Liege, Belgium; [3]Fac. of Med. Clinics Hosp., Univ. of Sao Paulo, Sao Paulo, Brazil; [4]Dept. of Biomed. and Clin. Sci. 'Luigi Sacco', Univ. of Milano, Milano, Italy; [5]Dept. of Psychiatry, Univ. of Wisconsin, Madison, WI

Abstract: Introduction: Both transcranial magnetic stimulation coupled to electroencephalography (TMS/EEG), positron emission tomography (PET) of cerebral metabolism, and active fMRI paradigms may identify signs of residual cognition in severely brain damaged patients. However the clinical feasibility and diagnostic accuracy of the combination of all three techniques in patients with disorders of consciousness (DOC) have never been assessed. Methods: 52 patients with severe brain damage leading to a period of coma were studied by standardised behavioural assessments (coma recovery scale revised - CRS-R), TMS/EEG (perturbational complexity index - PCI - calculated as in Casali et al 2013), FDG-PET (regional changes in metabolism assessed as in Stender et al 2014) and active functional MRI - fMRI - performed as in Monti et al 2010. 15 unresponsive wakefulness syndrome/vegetative state, 26 minimally conscious state - MCS, 7 exit-MCS and 4 locked-in syndrome - LIS - participated. Results: Eight patients were behaviourally unambiguously unresponsive but had indirect signs of consciousness detected by TMS/EEG (5), PET (7), both TMS/EEG and PET (4) or active fMRI (1). All studied patients who emerged from MCS and all patients with LIS showed high TMS/EEG PCI values and relatively preserved cerebral metabolism. Clinical feasibility is shown in figure 1. The sensitivity to MCS of TMS/EEG was 91.7%, of FDG-PET 100%, and of active fMRI 33.3%, while the congruence with the CRS-R was 74%, 79%, and 40%, respectively. Conclusion: TMS/EEG permits the detection of covert consciousness in patients with chronic DOC with a similar accuracy but slightly lower feasibility than FDG-PET. Both TMS/EEG and FDG-PET seem more feasible and accurate as compared to active fMRI examinations in this challenging patient population.



Disclosures: **O. Bodart:** A. Employment/Salary (full or part-time):; Research fellow at FRS-FNRS. **S. Wannez:** None. **A. Thibaut:** None. **J. Annen:** None. **A.G. Casali:** None. **S. Casarotto:** None. **O. Gosseries:** A. Employment/Salary (full or part-time):; Post-doctoral fellow

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Poster

225. Traumatic Brain Injury: Human Studies II

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Program#/Poster#: 225.10/G42

Topic: C.10. Trauma

Support: NIH R01 MH095984

NIH R01 MH064498

Belgian American Education Foundation

Wallonie-Bruxelles International

Title: Differentiating consciousness from unconsciousness in non-rapid eye movement sleep using transcranial magnetic stimulation and high-density EEG

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Abstract: Introduction: Combined transcranial magnetic stimulation and electroencephalography (TMS-EEG) allows for directly and non-invasively perturbing the brain and measuring the subsequent cortical response. Previous TMS-EEG studies have shown clear-cut differences between conscious and unconscious states. However, these studies did not investigate if TMS-EEG was sensitive to variations in the level of consciousness within the same physiological state, for example, non-rapid eye movement (NREM) sleep. Previously, it has been shown that subjects awakened from NREM sleep throughout the night report having conscious dream-like experiences in about 50% of the time. We here hypothesize that TMS-EEG responses differ depending on the presence or absence of a conscious experience in NREM sleep. Methods: Five healthy participants underwent TMS-EEG during NREM sleep (5 nights per subject). Brain activity was measured using a 60-channel TMS-compatible EEG amplifier and single-pulse TMS was applied to the superior parietal cortex. After each TMS session, subjects were awakened to ask for a dream report. Preprocessing analyses included bad-trials rejection and filtering the data (1.5 to 45 Hz). TMS-evoked potential were then averaged over the last 15 seconds before the awakenings and the phase locking factor was computed to compare between conscious and non-conscious conditions. Results: Our results reveal increased bistability when subjects do not report any conscious experience during sleep, as compared to when they do. Specifically, at

around 150-200 ms after TMS, the negative peak amplitude of the TMS-evoked response is larger, which probably reflects a downstate. Phase locking factor is of longer duration when subjects report conscious experiences as compared to when they do not. Conclusion: Our findings suggest that variations in the level of consciousness within the same physiological state - NREM sleep - are predicted by changes in the bistability of cortical networks revealed by TMS-EEG responses. This study shows that TMS-EEG is able to differentiate between conscious and unconscious states within the same physiological state.

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Poster

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Topic: C.10. Trauma

Support: NIH Grant UL1TR000457

Title: Characterization of synaptic dopaminergic deficits in post-traumatic brain injury (TBI) minimally conscious states

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Abstract: Pharmacological stimulation can markedly improve some minimally conscious states (MCS) following severe TBI, restoring goal-directed behaviors. Previously we have shown that down-regulation of anterior forebrain function grades with level of consciousness (Fridman et al. 2014, PNAS). These findings may reflect both loss of long-range glutamatergic neurons and dopaminergic projection neurons in MCS. No prior studies have provided direct measurements of presynaptic and postsynaptic dopamine deficits of the mesolimbic and mesothalamic pathways in MCS. Here, we assessed the availability of dopamine D2-like receptors (D2LR) at rest and following dopamine reuptake blockade with dextroamphetamine (to test presynaptic level) in 10 normal volunteers (NV) and 4 MCS subjects, utilizing [11C]raclopride-PET. We manually defined substructures of the anterior forebrain mesocircuit in high-resolution MRI: the striatum was divided into 3 functional subparts: ventral striatum (VST), associative striatum (AST) and sensorimotor striatum (SMST); globus pallidus (GP) was a single structure; and, the central thalamus (c-TH). We analyzed the signal using the kinetic SRTM2 with cerebellum as reference tissue to extract binding potential nondisplaceable (BPnd) from ROIs. The mean BPnd at rest and mean delta % change (Δ BPnd) between pre- and post-AMPH were calculated and

analyzed using ANOVA (BPnd and Δ BPnd as dependent variables and group as independent variables in each ROI). Results at baseline showed that BPnd obtained from MCS and NV did not significantly differ in VST, AST, SMST and GP while significant differences were observed in c-TH and GP [c-TH: NV= .58 ± 0.1, MCS= 0.38 ± 0.1; F= 15.5, p = <0.001; GP: NV= 1.41 ± 0.1, MCS= 1.93 ± 0.3; F= 6.2, p = <0.02]. Following administration of dextroamphetamine the pattern of response of the presynaptic dopaminergic neurons showed a clear stimulus-response deficit in MCS patients compared to NV: mean Δ BPnd of MCS were significantly reduced in VST, SMST and c-TH [VST: NV= -22.8 ± 3.1, MCS= -7.6 ± 7.0; F= 5.2, p = <0.05; SMST: NV= -23.3 ± 2.5, MCS= -11.3 ± 1.1; F= 9.4, p= <0.01; c-TH: NV= -13.6 ± 2.6, MCS= 2 ± 6.7; F= 7.3, p = <0.05]. We suggest: 1) that the relative "normal-high" BPnd of MCS at baseline represents a partial presynaptic dopamine depletion; 2) that lower BPnd in c-TH of MCS patients reflects direct postsynaptic damage; 3) that the stimulus-response deficit observed in VST, SMST and c-TH reflects a presynaptic neuronal deficit in the synthesis and release of dopamine. This deficit may reflect partial loss of background presynaptic dopamine neuron activity in post-traumatic MCS that contributes to anterior forebrain down-regulation.

Disclosures: E.A. Fridman: None. **J.R. Osborne:** None. **D.P. Mozley:** None. **N.D. Schiff:** None.

Poster

225. Traumatic Brain Injury: Human Studies II

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Program#/Poster#: 225.12/G44

Topic: C.10. Trauma

Support: This work was supported in part by Riddell Sports

Title: Extracellular RNA sequencing to identify RNA biomarkers of head impact in college athletes

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Abstract: Objective Each year in the United States, over two million traumatic brain injuries are diagnosed. This number is likely much greater if it were to include undiagnosed TBIs, as well as more mild forms of TBI. What is needed is an objective way to assess brain injury through the use of a test that is rapid and is based in an easily obtainable biological specimen. To work towards that goal, we studied college-aged football players using a combination of in-helmet monitoring and biospecimen based RNA biomarker profiling. Methods Urine samples were collected from Arizona State University Football players weekly within 24 hours following the completion of a game. Control urine samples were collected from a group of non-contact athletes of similar age as well as from every enrolled player prior to their first full contact practice. Head impacts were measured using the Riddell SRS at every contact practice and game. This system quantitatively measures every impact in real time. Extracellular RNA was isolated from urine with the NORGEN Total RNA Purification Maxi Kit. 2ng of total RNA was reverse transcribed with the Clontech SMARTer Universal Low Input RNA kit, and sequencing libraries were prepared with the Clontech Low Input Library Prep kit. This kit analyzes the whole RNA transcriptome. Libraries were sequenced on the Illumina HiSeq2500 with paired-end 83bp runs. Gene counts were computed and normalized with DESeq2. Classification of samples into case and control groups was performed using a Random Forest approach. Linear regression was used to identify genes that correlate with hit frequency and magnitude. Results From an average urine specimen, we generated 39 million reads and identified 41 thousand transcripts. Using linear regression, 29 genes were identified that significantly correlated with total number of hits sustained. 27 genes were identified that significantly correlated the magnitude of hits sustained. Using a Random Forest algorithm we classified cases and controls for highest magnitude hits and highest frequency hits. 25 genes with the highest information gain were selected for classification. Using these genes we correctly classified 100% of the highest frequency hit samples from baseline samples, and 81% of the highest magnitude hits from baseline samples. Conclusions This is an early attempt to assess brain injury by quantitative transcriptome analysis. Accurate, unbiased diagnosis of TBI is critical to the safety of athletes and others experiencing brain injury. These data suggest that extracellular RNA expression changes following head impacts can be detected in peripheral biofluids and may serve as biomarkers of TBI.

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Poster

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Support: Central Norway Regional Health Authority 46056907

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Central Norway Regional Health Authority 46039500

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Title: Very low birth weight and preterm birth: Impacts on subcortical volumes and white matter tracts by middle childhood

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Abstract: Preterm birth is a worldwide problem, affecting 15 million newborns each year and burdening many survivors with lifelong physical, cognitive, and psychological challenges. Individuals born preterm with very low birth weight (VLBW: birth weight ≤1500 grams) are at an increased risk of perinatal brain injury and neurodevelopmental and cognitive problems. Cerebral white matter injury and neuronal and axonal abnormalities are considered the dominant neuropathologies in preterm-born infants and are believed to underlie many of these cognitive deficits. We investigated group differences in subcortical brain structure volumes and white matter tract properties between VLBW children and controls, as well as possible relationships between brain structure and IQ scores, birth weight, and gestational age. METHODS: 103 term-born children participating in the Norwegian Mother and Child Cohort Study and 37 VLBW children born between 2001 and 2007 underwent 1.5 T MRI and age-appropriate cognitive testing with Wechsler tests (mean age=8 years). We used FreeSurfer software version 5.3.0 to extract volumes of subcortical structures and diffusion tensor imaging (DTI) for assessment of white matter. We used the general linear model for between-group analyses of subcortical volumes and partial correlations for morphometric data and IQ scores, controlling morphometry analyses for age at scan, sex, and estimated total intracranial volume. RESULTS: Compared to controls, the VLBW group had reduced volumes of thalamus, right globus pallidus, right hippocampus, cortical white matter and brain stem, while the ventricular system was enlarged compared with controls. Uncorrected IQ scores were significantly lower ($p<0.001$) in the VLBW group (mean=98.6; SD 9.7) than in controls (mean=108.1; SD 13.6). Among all participants, IQ score correlated with volumes in cortical white matter, both thalami, right hippocampus, and right ventral DC; only in right hippocampus among controls; no correlations in VLBW group. A smaller number of subcortical volumes correlated ($p<0.05$) to birth weight and gestational age. DTI analysis showed group differences in mean diffusivity and axial diffusivity in major longitudinal association fibers; we did not find differences in fractional anisotropy.

DISCUSSION: Volumes of several subcortical structures and properties of white matter tracts in both hemispheres are associated with very low birth weight and preterm birth. These persistent structural differences may be due to perinatal brain injury or secondary postnatal events and likely influence cognitive, behavioral, and mental health as children enter adolescence and later life.

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Poster

225. Traumatic Brain Injury: Human Studies II

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Program#/Poster#: 225.14/H2

Topic: C.10. Trauma

Support: Office of Naval Research (SAA 402925)

Title: Oculometric assessment of traumatic brain injury

Authors: *D. B. LISTON[1,2], L. R. WONG[1,2], L. S. STONE[1];
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Abstract: Diffuse tissue damage from impact or blast TBI degrades information processing throughout the brain. As precise oculomotor behavior depends on an extensive network of cortical and subcortical visuomotor brain areas, eye movements have long been known to be useful in the clinical assessment of brain pathology (e.g., Diefendorf & Dodge, 1908). To this end, we developed a 15-minute test that yields ten metrics of the oculomotor tracking response to visual motion, quantifying movement initiation, steady-state tracking, direction tuning, and speed tuning, and have collected a baseline database of “normal” human responses from 41 subjects (Liston & Stone, 2014). Methods. Subjects (N=30) with a clinically-diagnosed impact TBI in their past provided a numerical self-assessment of the current state of their impairment on a scale from 1 to 10 (with 1 defined as "back to the way I was prior to my injury" and 10 defined as "completely disabled"). They then ran our oculometric assessment test consisting of 180 trials of Rashbass (1961) step-ramp motion. The speed, direction, onset-timing, and initial location of target motion were randomized to promote uniform distribution of attention across space, time, and direction and to discourage predictive movements. We then processed their eye-movement responses to compute the ten test metrics. Finally, we measured the average pattern of oculomotor impairments associated in our TBI population (i.e., the TBI vector) and used this to quantify the severity of each individual’s impairment as a single scalar value (i.e., the “impairment index” or projection onto the TBI vector). Results. Our TBI subjects’ self-reported

severity values ranged from 1 to 7 (median: 3). Comparing them to our published control population, we observed significant impairments in: latency, acceleration, gain, catch-up saccade amplitude, proportion of smooth movement, and speed responsiveness (all p0.05). The ability of our test to discriminate TBI from control subjects increased as a function of self-reported severity (Pearson's R, $p<0.05$), ranging from an ROC area of ~0.6 for subjects subjectively reporting no residual impairment to an ROC area >0.90 for subjects reporting residual impairment of 5-7. Conclusion. Our 15-minute clinical test delivers ten metrics that quantitatively characterize several aspects of dynamic visual function and can detect mild impairments of neural function associated with TBI. Our method also quantifies the severity of an individual's impairment. We conclude that oculomotor tests could be used to screen for signs of TBI, quantify the extent of functional impairment, and monitor the time course of recovery.

Disclosures: D.B. Liston: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); DL holds a provisional patent, which is the subject matter of this publication, not licensed or otherwise commercialized.. **L.R. Wong:** None. **L.S. Stone:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); LS holds a provisional patent, which is the subject matter of this publication, not licensed or otherwise commercialized..

Poster

225. Traumatic Brain Injury: Human Studies II

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Topic: C.10. Trauma

Support: BioImaging Research Center, University of Georgia

John and Mary Franklin Foundation

Department of Kinesiology, University of Georgia

Title: Lack of volumetric differences in brain regions of former athletes with multiple concussions

Authors: D. TERRY[1], M. FERRARA[3], *L. S. MILLER[2];
[1]Psychology, [2]Univ. of Georgia, Athens, GA; [3]Univ. of New Hampshire, Durham, NH

Abstract: Recent reports suggest that people with a history of head trauma, even of the form of a concussion, may experience brain atrophy in regions sensitive to head injury, as well as memory deficits, later in life. Other studies have failed to find these alterations. We aimed to investigate whether these volumetric differences are evident many years after multiple concussions occurred

and how these volumes are related to cognition. Two groups of community-dwelling males currently ages 40-65 who played high school football, but not college or professional sports, were recruited. The experimental group included 21 participants reporting a history of two or more concussions in the context of high school football participation on an empirically validated semi-structured interview (median=3; range=2-15). The control group included 20 similar participants who denied any concussive events. Participants underwent memory testing (California Verbal Learning Test-II, the Logical Memory subtest of the WMS-IV), completed a self-report index of current concussive symptomatology, and underwent structural MRI brain scanning. High-resolution T1-weighted images were analyzed using the Freesurfer software package, which produced automated volumetric results. A priori regions of interest (ROIs) were based on previous studies and included total intracranial volume (ICV), total gray matter, total white matter, bilateral anterior cingulate cortex, and bilateral hippocampi. ROIs were corrected for head size using a normalization method that takes ICV into account. The two groups were well matched on age, education, estimated premorbid IQ, and current concussive symptomatology. In the concussed group, 29.0% of the concussions were associated with loss of consciousness and 29.0% were associated with professional medical attention. Repeated measures ANOVAs failed to reveal volumetric group differences across the ROIs, main effects of hemisphere, or interactions despite a sample size larger than in previous studies with positive findings. Further, there were no differences on any memory indices across groups. Bivariate correlations between normalized hippocampal volume, memory indices, and number of concussions were non-significant in this sample. These data suggest that multiple sports-related concussions in the context of high school football may not be associated with measurable brain atrophy or worse memory later in life. This finding may suggest that the severity of injury and chronicity of sport exposure may be especially important when measuring long-term neuroanatomical and neuropsychological differences.

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Poster

225. Traumatic Brain Injury: Human Studies II

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Topic: C.10. Trauma

Support: NIHR RP 011048

Guarantor's of Brain Clinical Fellowship

Title: Disruption to the dopaminergic system following traumatic brain injury

Authors: *P. O. JENKINS[1], S. DE SIMONI[1], J. J. FLEMINGER[1], A. E. JOLLY[1], J. H. COLE[1], D. TOWEY[2], D. J. SHARP[1];
[1]Computational, Cognitive and Clin. Neuroimaging Lab., Imperial Col., London, United Kingdom; [2]Radiological Sci. Unit, Imperial Col. Healthcare NHS Trust, London, United Kingdom

Abstract: *Background.* Cognitive impairments are a common cause of disability following traumatic brain injury (TBI). One potential cause is disruption of the neuromodulatory systems (e.g. dopamine), which are known to modulate cognitive functioning. Previous studies have identified dopaminergic dysfunction following TBI. It is unclear whether this dysfunction is related to damage to the dopaminergic nuclei in the substantia nigra or their ascending projections in the nigrostriatal tract. *Objectives.* To investigate: 1) whether TBI reduces striatal dopamine transporter (DAT) levels; 2) whether TBI causes structural changes to the nigrostriatal ascending pathways and/or atrophy of the substantia nigra or striatum; 3) whether there is a relationship between atrophy of the substantia nigra, structural changes to the nigrostriatal tract and DAT levels in the striatum. *Methods.* 19 subjects who had suffered a TBI at least 6 months previously and had persistent cognitive problems were compared with 10 healthy controls. All subjects underwent an ioflupane (^{123}I) single-photon emission computed tomography (SPECT) scan and magnetic resonance imaging (MRI) including diffusion tensor imaging. DAT levels were measured from the SPECT scan in the striatum, and voxel-based morphometry methods were used to calculate substantia nigra and striatal volumes. A nigrostriatal tract mask created from 100 participants from the Human Connectome Project was used to calculate mean fractional anisotropy measures. *Results.* Patients showed reduced striatal DAT levels and also demonstrated reduced fractional anisotropy in the nigrostriatal tract and reduced substantia nigra and striatal volumes. There was no significant correlation between DAT levels and either fractional anisotropy in the nigrostriatal tract or substantia nigra volumes. *Conclusions.* A proportion of patients who have suffered a TBI and have persisting cognitive problems have reduced DAT levels implying a disruption to their dopaminergic systems. We found no evidence for a clear relationship between damage to the ascending nigrostriatal tracts or substantia nigra and DAT levels. As the dopaminergic system is known to modulate certain cognitive functions, those patients with evidence of damage to their dopaminergic system may benefit from dopaminergic therapies.

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Poster

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Topic: C.10. Trauma

Support: NIHR Clinical Lectureship

Title: White matter disruption in the cingulum relates to apathy following traumatic brain injury

Authors: *N. GORGORAPTIS, A. JOLLY, P. O. JENKINS, C. FEENEY, G. SCOTT, J. H. COLE, S. DE SIMONI, D. J. SHARP;
Imperial Col. London, London, United Kingdom

Abstract: Apathy refers to a set of behaviours including reduced motivation, disinterest and lack of initiative. It is common following traumatic brain injury (TBI) and it has a detrimental impact on functional recovery, autonomy and response to rehabilitation. Diffuse axonal injury, leading to disruption of white matter pathways, is a common effect of TBI. Such white matter damage can be quantified using diffusion tensor imaging (DTI), and it relates to disruption of functional connectivity and to cognitive impairment following TBI. However white matter disruption in TBI has not been examined in relation to apathy. We used tract-based spatial statistics (TBSS) to examine microstructural white matter changes relating to apathy following TBI. Apathy was quantified using the Lille Apathy Rating Scale (LARS) and the apathy subscore of the Frontal Systems Behaviour Scale (FrSBe-A). 41 TBI patients [5 female, median age: 44 years (range: 20-65)] and 14 healthy participants [3 female, median age: 44 years (range: 21-61)] were studied. Apathy scores were significantly higher in patients than in controls. Higher apathy scores, as quantified by the caregiver-based version of the LARS, relate to lower fractional anisotropy (FA) values in the cingulate bundle in TBI patients, based on our TBSS analysis. No relationship was found between self-reported measures of (self-reported version of LARS and FrSBe-A) and changes in white matter microstructure. Using a voxelwise approach, we demonstrated that changes to the white matter structure of the cingulum relate to caregiver-reported apathy following TBI. This result is in keeping with DTI studies in other neurological conditions, such as Alzheimer's disease, as well as focal lesion mapping results from penetrating TBI, linking apathy with cingulum bundle disruption. Assessment of cingulum bundle integrity may have a role as a surrogate marker of apathy with potential use for patient stratification in future interventional studies.

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Poster

225. Traumatic Brain Injury: Human Studies II

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Topic: C.10. Trauma

Support: James S. McDonnell Foundation “Scholar Award”

Title: Subcortical atrophy and EEG in disorders of consciousness

Authors: *E. S. LUTKENHOFF[1], C. SCHNAKERS[2], P. VESPA[2], M. MONTI[3];
[1]Psychology-Clinical, [2]Neurosurg., [3]Psychology, UCLA, Los Angeles, CA

Abstract: In this work, we employ conventional T1-weighted magnetic resonance imaging (MRI) and electroencephalography (EEG) in a sample of patients with disorders of consciousness (DOC) in an effort to bridge the gap between clinical assessments of consciousness and underlying brain damage. The sample included 19 patients who survived severe brain injury and developed a disorder of consciousness without developing epilepsy or showing signs of seizure activity. To assess local brain atrophy on the basis of T1-weighted MR images, we employed a technique referred to as "shape (or vertex) analysis," available in FMRIB Software Library (FSL). The analysis included 3 preprocessing steps: (1) data were brain-extracted, using optiBET, (2) subcortical structures of interest were segmented, on an individual basis, and reconstructed into 3-dimensional vertex meshes and each segmentation was visually inspected, (3) to account for the effect of head size variability across individuals, we calculated each subject’s total normalized brain volume using SIENAX and included this measure as a covariate in all analyses. Segmentation and mesh construction were performed on all of the following brain regions, separately for each hemisphere: thalamus, caudate nucleus, putamen, globus pallidus, hippocampus, and brainstem. The EEG data was separated into spectra according to frequency bandwidths: delta (< 4 Hz), theta (4-8 Hz), and alpha (8-15 Hz). Then the mean and variance of the spectra over 24 hours were used in the analyses. Since the frequency bandwidths and demographics were collinear, a PCA factor analysis was used to separate the factors of interest across demographics, spectra averages, and spectra variances. The demographic information (post-injury day, days between MRI and EEG sessions, age, gender, and sedation level) was separated into 3 factors. The average spectra factors were comprised of alpha as one component and delta+theta as the second component. The spectra variance factors were also comprised of two components: delta variance and theta variance while alpha variance washed out. All the factors were included in a randomise (FSL) model, a nonparametric permutation test, to measure the correlation of the factors with subcortical atrophy. Initial findings indicate strong correlations between the average alpha factor and the theta variance factor and atrophy in various subcortical structures previously implicated in impairments of consciousness after severe brain injury.

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Poster

225. Traumatic Brain Injury: Human Studies II

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Topic: C.10. Trauma

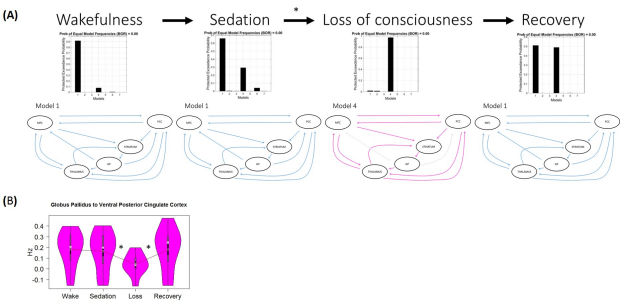
Support: James S. McDonnell Foundation

Title: The role of the thalamus during propofol-induced loss of consciousness: New evidence from resting-state fMRI using dynamic causal modeling

Authors: *J. S. CRONE[1], E. LUTKENHOFF[2], B. BIO[2], S. LAUREYS[3], M. MONTI[2]; [1]UCLA, Los Angeles, ; [2]UCLA, Los Angeles, CA; [3]Univ. of Liege, Liege, Belgium

Abstract: Understanding the neural mechanisms of consciousness has been a focus of neuroscience in the last decades. Despite the fact that the mystery is far from being solved, the neural complexity of conscious experience has been related to network connectivity of distinct brain regions comprising areas within the medial frontal and posterior cortex as well as basal ganglia and thalamus. Especially thalamo-cortical connectivity has been considered to play a critical role in impaired consciousness. In this study, we have investigated the effects of propofol induced loss of consciousness on the effective network interaction between medial frontal cortex, posterior cingulate, globus pallidus, striatum and thalamus using spectral dynamic causal modeling (DCM) for resting state fMRI. 18 volunteers underwent 4 fMRI scans with different levels of consciousness (wakefulness; mild sedation; loss of consciousness; recovery). DCM was performed with the following steps: (1) extraction of fMRI time series from each subject using individual anatomically defined masks and individual coordinates obtained from independent component analysis; (2) specification and estimation of the model space with 7 plausible models; (3) implementation of Bayesian model selection routine (BMS) to identify the best model for each condition; (4) implementation of BMS between conditions to find significant differences. The results show significant differences in the “best” model between sedation and loss of consciousness (Fig. 1A). Remarkably, the efferent and afferent connectivity of the thalamus is not effected by loss of consciousness as has been proposed. Instead, the effective connectivity strength from the globus pallidus towards the ventral posterior cingulate is significantly reduced during loss of consciousness compared to sedation and recovery (Fig. 1B). This study demonstrates that - rather than connectivity of the thalamus - the driving influence of the globus pallidus on the ventral posterior cingulate cortex plays a crucial role in propofol induced loss of consciousness.

Figure 2. (A) Results of the Bayesian model selection routine showing the best model for each condition; (B) Significant differences between level of consciousness in connectivity strength (Hz) from the globus pallidus to ventral posterior cingulate cortex; * indicate significant differences between conditions



Disclosures: **J.S. Crone:** None. **E. Lutkenhoff:** None. **B. Bio:** None. **S. Laureys:** None. **M. Monti:** None.

Poster

225. Traumatic Brain Injury: Human Studies II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 225.20/H8

Topic: C.10. Trauma

Support: STIC-AmSud Complex

Title: Signatures of awareness in the resting-state brain activity dynamics. An EEG/fMRI study of similarities and differences between wakefulness, anesthesia and disorders of consciousness

Authors: ***J. D. SITT**[1,2], G. DECO[3], P. BARTTFELD[1], A. TAUSTE[3], F. RAIMONDO[4], L. NACCACHE[2], S. DEHAENE[1];
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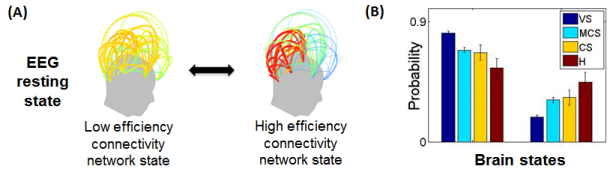
Abstract: At rest, the brain is traversed by spontaneous functional connectivity patterns. Two hypotheses have been proposed for their origins: they may reflect a continuous stream of ongoing cognitive processes as well as random fluctuations shaped by a fixed anatomical connectivity matrix. In this presentation I will show that both sources contribute to the shaping of resting-state networks, yet the level of consciousness modulates the respective contributions. Using a quantification of the distinct dynamical patterns of functional connectivity, extracted from the resting state functional MRI and EEG, I will show that wakefulness is characterized by a sequential exploration of a rich repertoire of functional configurations. These dynamical states are often dissimilar to the underlying anatomical connectivity structure, and comprising positive and negative correlations among brain regions. Conversely, under anesthesia the more frequent functional connectivity patterns inherit the structure of anatomical connectivity, exhibit fewer small-world properties, and lack negative correlations. This is also the case for the disorders of consciousness (DOC) - a set of pathological conditions like the vegetative state (VS) or the minimally conscious state (MCS) – where patients dynamically fluctuate between low and high efficiency information integration states (Fig 1A) but the rate of occurrence of the high efficiency state parametrically increases with the clinical level of consciousness (Fig 1B). These results reconcile theories of consciousness with observations of long-range correlation in the unconscious brain and show that a rich functional dynamics might constitute a signature of consciousness, with potential clinical implications for the detection of awareness in anesthesia and brain-lesioned patients. Figure caption. (A) Connectivity states maps extracted from EEG resting state recordings of DOC patients and normal controls. (B) Modulation of the dynamical

states rate of occurrence by the clinical state of the subjects (VS, MCS, CS -confusional- and H – healthy-)



Disclosures: J.D. Sitt: None. **G. Deco:** None. **P. Barttfeld:** None. **A. Tauste:** None. **F. Raimondo:** None. **L. Naccache:** None. **S. Dehaene:** None.

Poster

225. Traumatic Brain Injury: Human Studies II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 225.21/H9

Topic: C.10. Trauma

Support: The Hospital For Sick Children Endowment Fund

Ontario Institute for Cancer Research

Late Effects of Childhood Cancer Initiative

Title: Characterizing the effect of chemotherapy on the developing brain and associated cognitive functions

Authors: *E. VAN DER PLAS[1], S. ITO[2], R. SCHACHAR[3], B. J. NIEMAN[4];
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Abstract: Over 90% of children with acute lymphoblastic leukemia (ALL) will be cured of their illness. Unfortunately, the chemotherapy treatment necessary to eradicate leukemia is also associated with long-term side effects. Between 40 - 60% of ALL survivors experience impairments in executive functions, including attention and working memory. These impairments are thought to be related to chemotherapy-induced alternations in brain development; however, there is limited literature exploring the relationship between brain morphology and cognitive impairments in ALL survivors. We sought to determine whether chemotherapy exposure alters brain structure and associated cognitive functions. ALL survivors (n=21) and age- and sex-matched controls (n=18) between 8 - 18 years old underwent 3T structural MRI (data collection ongoing). T1-weighted anatomical scans were processed using the CIVET pipeline. We performed volumetric measurements of gray matter and white matter,

and performed measurements of cortical thickness, surface area and volume. Attention and working memory abilities were assessed with the Stop Signal Task (SST) and the N-Back Task. The SST measures the ability to cancel an ongoing response (response inhibition), which is a sensitive parameter of attention. The N-Back presents participants with a sequence of stimuli that need to be remembered, capturing working memory. Linear regression models with age and group as explanatory variables were performed to explore the impact of chemotherapy on brain morphology and cognitive function. Frontal white matter volume was significantly reduced in ALL survivors relative to controls (FDR-corrected p-value, $q < 0.1$). Cortical surface volume deficits in ALL survivors were evident in the temporal lobes and the occipital lobes ($p < 0.01$). Relative to controls, ALL survivors showed impairments in response inhibition ($t = -2.82$, $p < 0.01$) and working memory ($t= 3.43$, $p < 0.01$). Deficits in response inhibition were significantly correlated with reduced white matter volume in the frontal lobes and the temporal lobes (average $r = 0.34$, $p < 0.05$). Working memory performance correlated significantly with cortical surface volume in the inferior orbitofrontal cortices and superior regions of the temporal poles (average $r = 0.42$, $p < 0.05$). Chemotherapy is neurotoxic to the developing brain and resulting changes in brain morphology may explain some of the cognitive impairments commonly observed in ALL survivors. Identification of these links will help reveal the pathophysiology of chemotherapy-induced brain damage, which will ultimately lead to remedies and prevention.

Disclosures: E. Van der Plas: None. **S. Ito:** None. **R. Schachar:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Equity in BNAS (psychological software company), Owns patent on Stop Signal Task. F. Consulting Fees (e.g., advisory boards); Highland Therapeutics, Purdue Pharma, Lilly Corp. **B.J. Nieman:** None.

Poster

225. Traumatic Brain Injury: Human Studies II

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 225.22/H10

Topic: C.10. Trauma

Support: W81XWH-14-2-0151

Title: The return-to-play recovery time for female concussion sufferers at a military academy

Authors: *C. J. D'LAURO[1], B. R. JOHNSON[2], E. WILLSON[2], J. JACKSON[2], D. ALLRED[2], G. MCGINTY[2], D. CAMPBELL[2];
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Abstract: Concussions have become a topic of popular interest as well as an important public health issue over the past decade. While women's interest in sport and integration into the

military have both steadily increased, the amount of research dedicated to female concussion and recovery time still lags behind that of men. In particular, the heavy focus on contact sports and the military, both heavily male endeavors, continues to influence the proportion of research addressing concussions in female athletes. Some studies have shown greater susceptibility to concussion in women or more severe immediate post-impact symptoms, but data on the full time course of recovery is lacking. The United State Air Force Academy (USAFA) provides a unique data collection environment for studying concussions: healthcare is on-site and free, physical education and training is mandatory, cadets must be medically excused to miss a class, and they must be medically cleared to return to class or sports following a concussion. In total, these regulations produce a concussion-susceptible and highly-compliant population that is much more frequently assessed than typical athletes recovering from sports-related concussions. The USAFA return-to-play protocol is based on the Zurich 2012 Consensus Statement on Concussions and on US Department of Defense protocols. Before cadets can be fully returned to sport, they must be symptom-free, pass a medical exam, and exhibit a normal computerized neurocognitive assessment (i.e. ImPACT). The sports medicine personnel at the USAFA Concussion Clinic began detailed tracking of concussion causes and recovery times beginning in Fall 2012 and continuing to the present. During the time period analyzed, clinic staff recorded 245 concussions with complete recovery data. Women comprise 23% of the cadet population and produced 64 (26.1%) of these concussions. All concussions were regressed on full return-to-play time with sex entered as a between-subjects factor. Women took significantly more time (m=42.3 days ±31.3) to complete return-to-play than men (m=28.4 days±24.7), $F(1,243)=12.88$, $p<.001$. Other studies have shown sex-based differences in concussion symptoms, but this study is the first to comprehensively track and compare male and female concussions and demonstrate a lengthier RTP for women.

Disclosures: **C.J. D'Lauro:** None. **B.R. Johnson:** None. **E. Willson:** None. **J. Jackson:** None. **D. Allred:** None. **G. McGinty:** None. **D. Campbell:** None.

Poster

226. Spinal Cord Injury: Restorative Strategies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 226.01/H11

Topic: C.10. Trauma

Support: Veterans Administration

NIH S042291

NIH EB014986

Adelson Medical Research Foundation

Title: Adult myelin stimulates neurite outgrowth from neural progenitor cells

Authors: *G. H. POPLAWSKI[1], R. LIE[1], P. LU[2], C. GEOFFROY[1], R. KAWAGUCHI[3], G. COPPOLA[3], B. ZHENG[1], M. TUSZYNSKI[1,2];
[1]Neurosci., Univ. California SD, La Jolla, CA; [2]VAMC, San Diego, CA; [3]Neurol., Univ. of California Los Angeles, Los Angeles, CA

Abstract: Adult central nervous system white matter inhibits axon outgrowth from adult neurons. However, grafts of multipotent neural progenitor cells exhibit extensive axonal elongation through adult white matter, suggesting either that early stage neurons are not inhibited by adult white matter, or are actually stimulated by white matter. To address these possibilities, we cultured multipotent neural progenitor cells from E14 rat spinal cords on crude adult myelin extracts and compared findings to adult DRG cultures. Whereas adult DRG neurons exhibited the predicted 60% reduction in neurite outgrowth on myelin compared to laminin substrates ($p < 0.001$), neuronal progenitor cell (NPC) cultures exhibited a significant 86% increase in neurite length compared to cells cultured in the absence of myelin ($p < 0.001$). While adult neurite growth inhibition is mediated in part by interactions with Nogo receptors, we found no change in neurite outgrowth when neural progenitor cells were plated on Nogo-deficient myelin, suggesting that the inhibitory and stimulatory pathways of myelin may be mediated by distinct molecules. In contrast, another class of neurite outgrowth inhibiting molecules, chondroitin sulfate proteoglycans, significantly inhibited neurite outgrowth from NPCs ($p < 0.01$). Neurite outgrowth stimulation from NPCs on myelin declined in cells isolated from embryonic day 16, suggesting that growth pathways are differentially regulated as maturation proceeds. We are currently investigating differences in the transcriptome of neural progenitors cultured on myelin-containing vs. myelin-free substrates to identify specific molecular species enhancing neurite-outgrowth of NPCs on myelin. Collectively, these findings indicate that at least a portion of the ability of multipotent neural progenitor cell grafts to extend very long axons on adult myelin may result from facilitation of growth on this substrate. Insight into mechanisms underlying this effect may generate novel strategies for promoting adult axonal regeneration through myelin.

Disclosures: **G.H. Poplawski:** None. **R. Lie:** None. **P. Lu:** None. **C. geoffroy:** None. **R. Kawaguchi:** None. **G. Coppola:** None. **B. Zheng:** None. **M. Tuszynski:** None.

Poster

226. Spinal Cord Injury: Restorative Strategies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 226.02/H12

Topic: C.10. Trauma

Support: Veterans Administration, NIH (NS09881 and EB014986)

Craig H. Neilsen Foundation

California Institute for Regenerative Medicine

Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

CNPQ-Brazil

Title: Caudal projection patterns of neural stem cells grafted to sites of spinal cord injury

Authors: *W. GOMES-LEAL, SR[1,2], S. ANIL[1,3], Y. WANG[1], D. WU[1], M. TUSZYNSKI[1], P. LU[1];
[1]Univ. of California (UCSD), San Diego, CA; [2].Institute of Biol. Sciences. Federal Univ. of Para, Belem, Brazil; [3]Neuroprosthetics and Brain Mind Inst., Lausanne, Switzerland

Abstract: Previously we reported that neural stem cells (NSCs) transplanted to sites of spinal cord injury (SCI) differentiate into multiple neural cell types, including neurons, and extend large numbers of axons over very long distances (Lu Cell 2012; Neuron 2014). Here we sought to determine the origin of axons within grafts that emerge and extend caudally into the host spinal cord: do caudally projecting axons arise from all levels of the graft and give rise to monosynaptic relays across lesion sites, or do they primarily arise from cells located only in the caudal sections of grafts, and therefore more likely give rise to polysynaptic relays across lesion sites? Rat embryonic day14 spinal cord derived-multipotent neural progenitor cells (NPCs) expressing GFP were implanted into C5 lateral hemisection lesions. 3 weeks later, the retrograde tracer fluorogold (FG) was injected 3mm caudal to the lesion/graft site, and animals were perfused 3 days later. Histological examination indicated that the greater proportion (~55%) of retrogradely labeled NPC neurons were located within 600μm of the caudal graft/host interface; the remaining 45% of retrogradely labeled NPCs were located at all rostral-caudal levels of the grafts. In a second experiment, rat embryonic day 14 NPCs were grafted into C5 lateral hemisection lesion sites, but only 1% of all grafted cells were labeled for GFP prior to implantation. This allowed examination of projection patterns of individual grafted neurons to caudal aspects of the host spinal cord. 3-dimensional analysis of these results is underway. Preliminary analysis indicates that NPC-derived axons extending caudal to the lesion site are most likely to originate from neurons located in caudal aspects of the graft; however, some neurons present at more rostral levels of the graft also project caudal to the lesion site. Thus, relays across the lesion most likely represent a mix of monosynaptic and polysynaptic projections; additional studies are in progress.

Disclosures: W. Gomes-Leal: None. **S. Anil:** None. **Y. Wang:** None. **D. Wu:** None. **M. Tuszynski:** None. **P. Lu:** None.

Poster

226. Spinal Cord Injury: Restorative Strategies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 226.03/H13

Topic: C.10. Trauma

Support: NIH Grant R24 HD050838

Shriners Grants SHC-85220

Shriners Grants SHC-85400

Title: Expression of the CSPG receptor, LAR, after spinal cord transection in the lamprey

Authors: *W. RODEMER[1], G. ZHANG[1], J. HU[1], M. SELZER[1,2];
[1]Shriners Hosp. Pediatric Res. Ctr., [2]Neurol., Temple Univ. Sch. of Med., Philadelphia, PA

Abstract: Traumatic spinal cord injury (SCI) results in devastating long-term neurological and musculoskeletal impairments arising from the failure of axon regeneration within the central nervous system. The chondroitin sulfate proteoglycans (CSPGs) are potent, extracellular inhibitors of regeneration that appear to create a sustained chemical barrier to regrowth through multiple mechanisms, some of which are mediated by members of the receptor protein tyrosine phosphatase family, including PTPσ and LAR. The anatomical complexity and extremely high barrier to regeneration set by the mammalian spinal cord make analysis of potential therapeutic strategies difficult. For this reason, we have used the larval sea lamprey as an experimental model. The lamprey recovers functionally following complete spinal cord transection. Individually identified large reticulospinal (RS) neurons regenerate their axons with varying but predictable probabilities, despite the presence of extracellular inhibitory molecules, including the CSPGs, as well as their receptors, PTPσ and LAR. Lamprey neurons that are “bad regenerators” also undergo a very delayed form of cell death, which is preceded by caspase activation and TUNEL positivity, suggesting that they die of apoptosis. Previous reports by this lab have demonstrated that PTPσ mRNA is expressed selectively in “bad” regenerating neurons and precedes caspase-activation induced by SCI. The present study examines LAR expression in the larval lamprey via *in situ* hybridization using a custom, antisense riboprobe targeting the region of mRNA encoding the extracellular Fibronectin III domain of the LAR protein. Similarly to PTPσ, LAR transcripts are preferentially expressed in “bad” regenerating RS neurons in the uninjured animal. However, the inverse correlation between mRNA expression and regeneration is stronger with PTPσ than LAR. Following complete spinal cord transection, the number of LAR mRNA-positive RS neurons increases, peaking at 7 weeks post-transection and returning to baseline after 10 weeks. Results from this study will guide future experiments to more directly address the role of CSPG receptors in inhibiting regeneration and promoting retrograde cell-death after SCI.

Disclosures: W. Rodemer: None. **G. Zhang:** None. **J. Hu:** None. **M. Selzer:** None.

Poster

226. Spinal Cord Injury: Restorative Strategies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 226.04/H14

Topic: C.10. Trauma

Support: Interdisciplinary Graduate School for Brain Research and Translational Neuroscience (iBrain)

German Research Foundation (DFG)

Title: Role of CXCR4 in mediating axon sprouting after intrathecal infusion of CXCL12 in a mouse model of spinal cord injury

Authors: *F. TUNDO[1,2,3], V. ESTRADA[1], A. STAHR[3], A. HESKAMP[2], M. HENDRICKS[1], A. HALLENBERGER[3], A. ANDREADAKI[2], C. VON GALL[3], D. FISCHER[2], H. W. MÜLLER[1]; [1]Mol Neurobiol Lab, Neurology, Heinrich Heine Univ., Düsseldorf, Germany; [2]Div. of Exptl. Neurology, Neurology, Heinrich Heine Univ., Düsseldorf, Germany; [3]Inst. of Anat. II, Heinrich Heine Univ., Düsseldorf, Germany

Abstract: Background: Regeneration of injured axons is impaired in the central nervous system (CNS). The chemotactic cytokine stromal cell-derived factor-1 (SDF-1/CXCL12) is disinhibitory towards CNS myelin and plays a key role during development of the nervous system. Recent studies revealed that CXCL12 enhances sprouting of dorsal corticospinal tract (dCST) axons rostral to the lesion in a rat model of spinal cord injury (SCI) (Opatz et al., 2009). CXCL12 reportedly interacts with two G protein-coupled receptors, CXCR4 and CXCR7. However, which receptor(s) is involved in the sprouting effect is still unknown. Using a mouse model and murine primary DRG cultures, we are investigating the functional role of CXCR4 in mediating the axon growth promoting and disinhibitory effects of CXCL12 after its intrathecal infusion. As this method is rarely used in mice due to the very thin dura mater and the narrow epidural space, we had to establish an intrathecal application (epidural catheterization) method for the treatment of thoracic SCI in this species. Methods: CXCR4 floxed mice were cross-bred with ROSA reporter mice (CXCR4flox/flox/Rosa/-). Stereotaxic injection of adeno-associated virus serotype 2 expressing Cre recombinase into the layer V of the sensorimotor cortex leads to the generation of a conditional knockout of CXCR4 in the transduced pyramidal neurons projecting into the CST which, at the same time, expresses the reporter gene red fluorescent protein. After a dorsal spinal cord hemisection CXCL12 was intrathecally infused via an osmotic minipump. The CXCL12-induced rostral sprouting of dCST axons was investigated. To analyze the catheter fixation and the delivery via the osmotic minipump, and to rule out catheter-induced compression, minipumps were filled with Evans Blue. Trichrome staining was chosen to visualize connective tissue in spinal cord slices. Results: Strong Evans Blue staining at the lesion site confirmed the local distribution as well as stable epidural catheter fixation over seven days, whereas trichrome staining revealed no catheter-induced spinal cord compression. CXCR4 was expressed in

pyramidal neurons of the somatosensory motor cortex and in dCST axons. Upon Cre transduction CXCR4 immunoreactivity in pyramidal neurons as well as in dCST axons disappeared. Rostral sprouting of dCST axons was quantified after SCI. Conclusions: The ROSA reporter mouse was suitable to visualize the lack of CXCR4 expression after conditional gene knockout. We will discuss the sprouting of dCST axons in a mouse model of SCI after intrathecal CXCL12 infusion and the functional role of CXCR4 in promoting CXCL12 induced axon growth.

Disclosures: F. Tundo: None. **V. Estrada:** None. **A. Stahr:** None. **A. Heskamp:** None. **M. Hendricks:** None. **A. Hallenberger:** None. **A. Andreadaki:** None. **C. von Gall:** None. **D. Fischer:** None. **H.W. Müller:** None.

Poster

226. Spinal Cord Injury: Restorative Strategies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 226.05/H15

Topic: C.10. Trauma

Support: NRF-2014M3A9B6034224

Title: Temperature sensitive injectable hydrogel bridges cystic cavities and supports functional recovery following contusive spinal cord injury

Authors: *H. A. LE;
Ajou Univ., Suwon, Korea, Republic of

Abstract: Cavity formation after spinal cord injury (SCI) is one of the major obstacles for axonal regeneration. Implanting artificial scaffolds into the cavity has been considered as a promising strategy, yet successful bridging with scaffolding biomaterials has not been convincingly demonstrated in clinically relevant contusive SCI model. Unpredictable and irregular geometry of cystic cavities formed in this model would necessitate the use of injectable hydrogel for this purpose. In the present study, we injected temperature sensitive poly(phosphazene) hydrogel, with a sol-gel transition behavior at 37°C, into the lesion epicenter in contusive rat SCI model at 1 week after injury. The hydrogel injection almost completely prevented cavity formation. In animals with the hydrogel injection, the lesion epicenter was replaced by fibronectin-enriched ECM by 4 weeks after the injection. Co-localization of FN and collagen-1α1 suggested that the majority of the newly formed ECM originates from perivascular fibroblasts. Intense activation of CD45+ macrophages indicated that the blood born macrophages were involved in the generation of new matrix. The fibronectin positive ECM was surrounded by GFAP positive glial scars with an interface laden with chondroitin sulfate proteoglycans (CSPG). Interestingly, zymography showed upregulation of MMP-9 activity in animals with the hydrogel

injection, and MMP-9 was highly expressed at the center of the fibronectin enriched ECM. The cellular source for the MMP-9 immunoreactivity was CD11b positive macrophages. Animals with hydrogel injection showed improvement in coordinated locomotion as evidenced by BBB test and Catwalk analysis. The improvement in locomotor function was accompanied by better preservation of myelinated white matter around the lesion epicenter. Our study establishes a proof of principle that the temperature-sensitive hydrogel can be used as a cavity-bridging therapy for contusive SCI. Considering the facility of hydrogel in a sol state incorporating various drugs and cells, this approach can also be utilized as a versatile platform for multifaceted combinatorial therapy.

Disclosures: H.A. Le: None.

Poster

226. Spinal Cord Injury: Restorative Strategies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 226.06/H16

Topic: C.10. Trauma

Support: Shriners Hospitals for Children Foundation, Grant #: 85210

Title: Protein synthetic machinery and mrna in regenerating tips of spinal cord axons in lamprey

Authors: *L.-Q. JIN[1], C. R. PENNISE[1], W. RODEMER[1], K. S. JAHN[2], M. E. SELZER[1]; [1]Shriners Hosp. Pediatric Res. Ctr., Temple Univ. Sch. of Med., Philadelphia, PA; [2]The Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Protein synthesis and mRNAs have been seen in growth cones of neurons *in vitro* and in regenerating peripheral nerve, but evidence for this is lacking in mammalian CNS. Based on work in the lamprey, we postulate that regeneration in the CNS does not involve conventional growth cones, so we asked whether regeneration in CNS axons involves local protein synthesis in the axon tips. Spinal cord axons were backlabeled by fluorescent tracers applied to a fresh transection. Two to three weeks later, the cords were re-exposed and the growth status (growing, static or retracting) of axons in the proximal stump determined over a 3hr interval. Then the axon tips were studied by one of two methods: 1) *In situ* hybridization for total mRNA (oligo(dT)) or individual transcripts, focusing on cytoskeletal proteins; or 2) microaspiration of axon tip cytosol followed by PCR amplification of mRNAs. Of 39 axon tips in 41 animals 14 tips were growing forward, 15 were static, and 10 were retracting. mRNAs were detected in axon tips but not in uninjured control axons. mRNAs were more abundant in regenerating than in static or retracting tips. Axoplasms were pooled from 6 growing and 6 static tips, and mRNAs were amplified by PCR. The cDNA products were used as templates for target-specific PCR. Lamprey NFL and β-tubulin mRNAs were abundant in growing tips, less so in static tips. β-actin and NF95 transcripts

were scarce in growing tips and not detected in static tips. NF180, NF132, and vimentin were not detected in the growing tips. EM showed polyribosomes, and in one tip, rough endoplasmic reticulum, in the distal-most regions of axon tips. Immunohistochemistry showed riboprotein S6 in a distribution similar to that of ribosomes. The results suggest that local proteins synthesis may participate in the mechanism of axon regeneration in the CNS.

Disclosures: L. Jin: None. **C.R. Pennise:** None. **W. Rodemer:** None. **K.S. Jahn:** None. **M.E. Selzer:** None.

Poster

226. Spinal Cord Injury: Restorative Strategies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 226.07/H17

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Support: VA RR&D Grant B733

VA RR&D Grant B9260L

National MS Society

CT Stem Cell Res Program 12-SCB-Yale05

Title: Blood-spinal cord barrier disruption after contusive spinal cord injury (SCI) rapidly recovers following intravenous infusion of bone marrow mesenchymal stem cells (MSCs) or MSC-derived exosomes

Authors: *K. L. LANKFORD[1], T. MATSUSHITA[1,2], E. J. ARROYO[1], P. W. ASKENASE[3], J. D. KOCSIS[1];
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Abstract: Traumatic spinal cord injury (SCI) damages the blood spinal cord barrier (BSCB) and allows immune cells and toxic molecules to infiltrate into the cord, contributing to the neuronal loss, axon severing, and demyelination that can lead to paralysis. Intravenous infusion of mesenchymal stem cells (MSCs) can reduce the severity of experimental spinal cord injury (SCI), but the mechanisms are not fully understood. In this study, adult male Sprague Dawley rats were subjected to a moderate contusive spinal cord injury (SCI) at the T9 level, i.v. infused with $1x10^6$ rat MSCs or media one week post-SCI, and assessed for functional recovery, BSCB permeability, or distribution of transplanted MSCs. Alternatively, a small number of rats were infused with exosomes isolated from MSC-conditioned media. Locomotor function was assessed using the Basso-Beattie-Bresnahan (B-B-B) rating scale. Spatial and temporal changes in BSCB

integrity were assessed by i.v. infusions of Evans blue (EvB) with *ex vivo* optical imaging and spectrophotometric quantitation of EvB leakage into the parenchyma. Distribution of DiR-labeled GFP-expressing MSCs was assessed by *in vivo* and *ex vivo* imaging of organs and fluorescence microscopy of frozen sections. The results showed that contusive SCI resulted in diffuse and persistent BSCB leakage but that post-SCI BSCB leakage in was reduced in MSC transplanted rats. Furthermore, locomotor function in contused rats was improved beginning 1 week post-MSC infusion. However, i.v infused MSCs were not detected within the spinal cord at any time point, but instead appeared to traffic transiently to the lungs. Our preliminary data also showed that exosomes isolated from MSC conditioned media also reduced BSCB permeability and improved locomotor functioning 1 week post-infusion. Taken together, this data indicated that infusions of either MSCs or MSC-derived exosomes one week after contusive SCI were effective in reducing BSCB permeability and improved functional recovery and suggest that the therapeutic effects of MSCs on SCI may be mediated by MSC-derived exosomes.

Disclosures: K.L. Lankford: None. **T. Matsushita:** None. **E.J. Arroyo:** None. **P.W. Askenase:** None. **J.D. Kocsis:** None.

Poster

226. Spinal Cord Injury: Restorative Strategies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 226.08/H18

Topic: C.10. Trauma

Support: Fapesp

Title: Neuroprotection and immunomodulation by mesenchymal stem cell and fibrin sealant treatment following intraspinal axotomy

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Abstract: Despite great efforts, treatment of spinal cord lesion is vastly inefficient, resulting in poor outcome. In this sense, new repair procedures are needed. We have recently demonstrated that the use of fibrin sealant (FS) for reimplantation of avulsed ventral roots improves motor recovery. The use of mesenchymal stem cells (MSC), that secrete neurotrophic and angiogenic factors, enhances survival and decreases glial reaction. Thus, the present study investigated the inflammatory environment, neuronal survival and synaptic preservation following intraspinal axotomy (IA) and treatment with FS and/or MSC. In this way, female Lewis rats were subjected to intraspinal section of the ventral funiculus, that axotomizes motoneurons within the central nervous system. The lesion was either untreated or stabilized with FS. In addition, the

combination of MSC and/or FS was evaluated. RT-qPCR, one week after injury demonstrated in the groups that received FS, coupled or not with MSC, increased mRNA levels of TNFα (50%), IL-4 (450%), IL-10 (600%) and IL-13 (100%), indicating a predominantly anti-inflammatory microenvironment acutely after lesion. IL-6 expression was similar in all groups. In the acute phase post lesion, BDNF (brain derived neurotrophic factor) mRNA levels were unchanged, and VEGF mRNA (vascular endothelial growth factor) was downregulated (-100%) in all lesioned groups. Hematoxylin/Eosin staining demonstrated strong cell infiltrate, with predominance of putative M2 macrophages (Iba1/arginase-1 positive). Two weeks after injury, neuronal survival was calculated in Nissl stained sections, revealing enhanced motoneuron preservation after treatment with MSC and/or FS. Such rescued neurons, which were evidenced by GAP-43 immunolabeling, presented significant preservation of pre-synaptic inputs, positive to synaptophysin. Synaptic circuit preservation, in the MSC treated groups, was coupled with decreased astrogliosis, which has been previously shown to actively contribute to the detachment of synapses from the motoneuron surface. Overall, the present results indicate that the combination of MSC and FS therapy results in neuroprotection. Importantly, FS modulates local inflammatory response towards a predominantly anti-inflammatory profile, thus contributing to a more efficient recovery of homeostasis.

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Poster

226. Spinal Cord Injury: Restorative Strategies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 226.09/H19

Topic: C.10. Trauma

Support: Commonwealth Universal Research Enhancement (CURE) Program Grants (MRD)

NIH NS055976 (JDH)

Title: Exercise induced changes in inflammation along the sensory neuroaxis after unilateral cervical spinal cord injury

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Abstract: Microglia and macrophages are activated by immune and neuronal cytokines and may play a critical role in the onset and modulation of chronic neuropathic pain after spinal cord injury (SCI). Exercise is widely used in the clinical SCI population, and we have previously

shown that early administration of exercise prevents neuropathic pain development after experimental SCI. Here, we determined whether the exercise-induced reduction in pain correlates with a change in the post-injury inflammatory response throughout the spinal cord and brain as well as systemically. Sprague-Dawley rats received a moderate unilateral C5 spinal cord contusion and were separated into SCI and SCI+Exercise groups. Starting at 5 days post injury (dpi), SCI+Exercise group was exercised on automated running wheels for 20 minutes/day, 5 days/week for 4 weeks. Tactile allodynia was assessed weekly using von Frey monofilaments and mechanical conflict avoidance operant testing assessed cognition of nociceptive tactile stimuli. Blood was collected, and serum separated weekly. At 31 dpi, rats were sacrificed and cervical spinal cord and dorsal root ganglia, the ventroposterolateral nucleus of the thalamus and somatosensory cortex were dissected. Serum and tissue homogenates were probed for pro- and anti-inflammtory cytokines including CCL2, TNFa, IL-12, IL-4 and IL-10 and 22 others via multiplex ELISA (Eve Technologies). A subset of tissue will be processed for immunohistochemical markers of the microglial/macrophage response (Iba-1, ED-1, Arginase-1, CD206). By examining the inflammatory response in the brain, spinal cord, and DRG we will better understand possible mechanisms for the onset of neuropathic pain and for the efficacy of exercise. Detection of changes in systemic inflammatory response after SCI and/or exercise over time will identify potential biomarkers or predictors of pain development that could be directly translated to clinical SCI. These biomarkers would also inform on potential pharmacologic targets, as well as the effectiveness of the exercise possibly leading to refinement of the rehabilitation protocol.

Disclosures: D. Quiros-Molina: None. **S. Chhaya:** None. **J.R. Bethea:** None. **J.D. Houle:** None. **M.R. Detloff:** None.

Poster

226. Spinal Cord Injury: Restorative Strategies

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Support: Commonwealth Universal Research Enhancement (CURE) Program Grants (MRD)

NIH NS055976 (JDH)

Title: The acute inflammatory response after spinal cord injury and its role in the development of spinal cord injury-induced chronic neuropathic pain

Authors: *S. CHHAYA[1], D. QUIROS-MOLINA[1], J. R. BETHEA[2], J. D. HOULE[1], M. R. DETLOFF[1];

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Abstract: Neuropathic pain that develops in a majority of patients with spinal cord injury (SCI) is often refractory to treatment. Early after SCI, nociceptive processing by primary sensory neurons in the dorsal root ganglion (DRG) is altered and pain afferent fibres in the dorsal horn of the spinal cord exhibit robust aberrant collateral sprouting. Studies from our lab showed that exercise beginning early after SCI prevented the onset of neuropathic pain; however exercise initiated once pain is established had no effect, demonstrating that the early time window post-SCI is critical in the development of pain. Acutely after SCI, there is a robust intraspinal inflammatory response at and distal to the lesion. Cytokines and chemokines activate, recruit and polarize microglia and macrophages in the spinal cord and DRG into a pro-inflammatory (M1) phenotype. The upregulation of inflammatory mediators influences neuronal plasticity and may alter nociceptive processing of DRG neurons, contributing to the development of chronic pain. Thus, we assessed the acute inflammatory microenvironment in the dorsal horn of the cervical spinal cord and associated DRGs for levels of macrophage attractant CCL2, pro-inflammatory cytokines TNF-α, IL-1β, IL-6, IL-12 relative to levels of anti-inflammatory cytokines such as IL-4 and IL-10, and how they correlate with 1) the development of SCI-induced neuropathic pain and 2) effects of rehabilitative exercise. Female Sprague Dawley rats received a C5 hemilaminectomy or a moderate, unilateral C5 spinal cord contusion (n=6/group). A subset of rats were exercised 20 minutes/day 5 days/week using a forced exercise wheel walking system beginning at 5 dpi through to 17 dpi. Rats were assessed for the development of neuropathic pain using von Frey and Hargreaves' tests pre-operatively and weekly after SCI. Serum was collected at 1 hour, 24 hours, 3, 5, 7, 14 and 17 days to assess circulating markers and systemic inflammation. Subsets of rats were sacrificed at each of these time points and cervical spinal cord and DRGs were processed for ELISA. Preliminary data show elevated levels of chemokines such as CCL2 one day post-injury in the dorsal horn of the spinal cord, DRGs and serum that remain elevated above baseline beyond 14 days, which marks the onset of neuropathic pain. Identification of mediators involved in the maladaptive pro-inflammatory response and their pathway of activation may provide potential therapeutic targets to contain this aberrant immune response and resultant pain co-morbidity after SCI, as they may modulate nociceptors in the DRG as well as affect higher order pain-inhibitory pathways in the spinal cord resulting in chronic pain.

Disclosures: S. Chhaya: None. **D. Quiros-Molina:** None. **J.R. Bethea:** None. **J.D. Houle:** None. **M.R. Detloff:** None.

Poster

226. Spinal Cord Injury: Restorative Strategies

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Craig H. Neilsen Foundation 313739 (JH)

Craig H. Neilsen Foundation 224308 (ARF)

Title: Initial limb unloading after spinal cord injury alters glutamatergic synaptic plasticity associated with impaired functional recovery

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Abstract: Sensory inputs from the lower extremities modulate neural circuits controlling gait. Loading-related sensory inputs are essential for functional recovery after spinal cord injury (SCI). Identifying relevant loading conditions and therapeutic time-windows for locomotor training will lead to advances in neurorehabilitation. Here we investigate the impact of initial limb unloading after SCI using adult female SD rats subjected to mild contusive injury (T9; 50 kdyn IH). Three days following injury, subjects were randomized to two experimental groups: 1) hindlimb unloading (HU) by tail suspension for 2 weeks followed by normal-loading for 6 weeks, or 2) normal-loading control for 8 weeks. Assessments included: 1) BBB and kinematic gait analysis; 2) electrophysiological H-reflex testing; 3) biomolecular and robotic confocal microscopy for plasticity-related changes in spinal motoneurons. Results demonstrated HU reduced coordinated forelimb-hindlimb stepping by kinematics and impaired long-term locomotor recovery (BBB = 12 for HU vs 17 for normal-loading controls). H-reflex testing of the plantaris muscle at 8 weeks showed frequency dependent H-reflex suppression was diminished in HU compared to controls, indicating motoneuron hyperexcitability. Furthermore, acute HU drove a chronic increase in glutamate AMPA receptors in post-synaptic membranes of spinal motoneurons as measured in biochemical and confocal microscopic studies, suggesting that HU induced maladaptive plasticity in the spinal cord. Our findings also suggest that initial limb unloading after SCI can induce maladaptive plasticity that persists, undermining long term

recovery, and supporting the need for early rehabilitation to reverse maladaptive plasticity after SCI.

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Poster

226. Spinal Cord Injury: Restorative Strategies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: C.10. Trauma

Support: DFG Research Training Group 1033

Interdisciplinary Graduate School for Brain Research and Translational Neuroscience (iBrain)

Research Commission of the Medical Faculty of the University Düsseldorf

Deutsche Gesetzliche Unfallversicherung (DGUV)

Title: A mechanical microconnector to improve regeneration after spinal cord injury: characterization of the therapeutic effect by tracing studies

Authors: *J. KREBBERS[1], N. BRAZDA[1], V. ESTRADA[1], C. VOSS[2,3], V. T. RIBAS[4], K. SEIDE[3], H. K. TRIEU[2], P. LINGOR[4], H. W. MÜLLER[1];
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Abstract: Traumatic injuries of the central nervous system (CNS) lead to irreversible damage and prolonged functional deficits. As a result of spinal cord injury (SCI), supraspinal neuronal connections are interrupted, thus intentional movement and sensory perception are permanently impaired. In contrast to the peripheral nervous system, neurons in the CNS are hardly able to regenerate. In this study the previously described mechanical microconnector system (mMS) [Brazda et al. (2013) Biomaterials 34 10056-10064] was further characterized for its potential to support supraspinal reconnectivity using anterograde and retrograde tract tracing methods in the adult rat after complete midthoracic spinal cord transection. Briefly, the mMS is a device for re-adaptation of separated spinal cord stumps after complete transection and is made out of polymethylmethacrylate (PMMA) with honey comb structured holes. As described earlier, the two spinal cord stumps are sucked into the mMS by applying negative pressure and remain in place after removal of the vacuum. Rats with complete midthoracic SCI and mMS implantation show improved locomotor function compared to injured control animals without mMS. Moreover increased axonal outgrowth, angiogenesis, and the invasion of Schwann cells, which myelinated regenerating axons, was seen [Estrada et al. (2012) SfN Abstract 123.03]. In this study, supraspinal reconnectivity following mMS implantation was further investigated and three putative mechanisms were tested: (1) Regeneration of supraspinal axon tracts, especially the corticospinal tract (CST), (2) regeneration of interneurons through the mMS, and (3) formation of additional synapses of CST axons on interneurons rostral and caudal to the lesion, establishing a novel interneuronal bridge passing through the mMS. To test these hypotheses tracing studies and synapse stainings were performed. To trace the CST, an adeno-associated virus (AAV) construct coding for red fluorescent protein (dsred) was used. Descending tracts and interneurons regenerating beyond the lesion site into caudal healthy tissue were traced by using the retrograde tracer fluorogold. These morphological investigations could reveal important mechanisms for the previously observed functional benefits of the mMS, which is currently fabricated using bioabsorbable materials. Those techniques can be used to investigate reasonable combinations of the mMS with other already approved neuroregenerative therapies, such as chondroitinase ABC to degrade axon growth inhibitors or the application of small electric fields (through gold-coated mMS) to enhance and guide axonal outgrowth.

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Poster

226. Spinal Cord Injury: Restorative Strategies

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Topic: C.10. Trauma

Support: NRF-2014R1A1A3052554

Title: clinical trials of stem cell therapy for spinal cord injury in south korea

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Abstract: There is no fundamental treatment to recover spinal cord injured (SCI) patients in the clinical setting, and various therapeutic strategies are developing to regenerate damaged spinal cord and make a hope for SCI patients. Since almost a decade ago, many clinical trials of stem cell transplantation (SCT) had been performed in many countries, albeit SCT is not yet approved as one of the treatment options of SCI in the clinical field. Herein we performed the survey for clinicians and SCI patients about SCT via multiple centers and individuals in South Korea, and also performed physical examination to stem cell-received SCI patients. We classified the subjects to four separated groups; non SCT-received SCI patients, SCT-received SCI patients, non SCT-performed clinicians, and SCT-performed clinicians. Expectation of moderate-to-complete functional improvement following SCT was the greatest in SCT-received patients (70%) than non SCT groups (27.4% for patients and 43.5% for clinicians). All participants worried about complications following SCT, and agreed with necessity of active rehabilitation program even after SCT. Among twenty SCT-received patients, stem cell types for transplantation were bone marrow mesenchymal stem cell (MSC) (65%), adipose MSC (25%), and unknown (10%). SCT were performed via intralesional (60%), intravenous (28%), and unknown (12%). Number of times to SCT was shown as once (35%), twice (10%), and more than 3 times (55%). Functional improvement following SCT was not obvious in SCT-received patients. About 15% of them felt some improvements following SCT, however physical examination revealed that neurological level of injury was not changed (60%) in most cases, but 20% cases showed even worsening. The proportion of bladder dysfunctions including frequency and incontinence was not different between non SCT-received and SCT-received patients. Some of SCT-received patients suffered from side effects following SCT, and neuropathic pain was obvious following SCT than non SCT-received patients (95% versus 63.1%). In conclusion, recognition to stem cell therapy was the same in all clinicians and SCI patients, and the greatest expectation group in stem cell therapy was SCT-received patients. Nevertheless we could not find the obvious neurological nor functional improvements in SCT-received patients while the neuropathic pain was increased following SCT.

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Poster

226. Spinal Cord Injury: Restorative Strategies

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Support: US Department of Defense CDMRP SCIRP W81XWH-10-1-1014 and W81XWH-10-1-1018

Title: Lack of effect of anti-integrin treatment in contusion models of rat spinal cord injury

Authors: N. M. GEREMIA[1], T. HRYCIW[1], F. BAO[1], F. STREIJGER[2], E. OKON[2], J. H. T. LEE[2], B. K. KWON[2], L. C. WEAVER[1], *A. BROWN[1], G. A. DEKABAN[1];
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Abstract: Neurological outcomes of rats and mice after clip compression spinal cord injury (SCI) are improved by acute treatment with a monoclonal antibody against an integrin expressed by neutrophils and monocyte/macrophages. However, the limited effects of this anti-integrin treatment reported by others suggest that severity and type of lesion may play an important role in the success of the treatment. For a comparison to the published efficacy of this treatment after clip compression SCI, we tested it in a moderately severe contusion models of SCI at the 8th (T8) and 12th (T12) thoracic spinal segments of rats. Dwell time and force in these contusion SCI models were adjusted to produce an injury severity comparable to that of our 35g clip compression SCI with respect to open field locomotor scores and tissue myeloperoxidase levels. Whereas the anti-integrin treatment lowered acute myeloperoxidase levels and improved locomotor recovery in the clip compression injury, no improvements in these measures were observed in the T8 and T12 contusion SCI models. Similarly the anti-integrin treatment did not improve neurological outcomes in a cervical hemi-contusion model of SCI. This suggests that the beneficial effect of the anti-integrin treatment depends on the type and severity of spinal cord injury being addressed.

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Poster

226. Spinal Cord Injury: Restorative Strategies

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Support: Morton Cure Paralysis Fund

Title: The role of fibronectin in BMSC survival and repair potential after spinal cord injury

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Abstract: Spinal cord injury (SCI) results in continuous loss of nervous tissue and poor functional recovery, which has devastating consequences for those that suffer damage to their spinal cord. Stem cell transplantation presents a unique opportunity for SCI treatment in part because the stem cells continuously secrete various growth factors that could halt secondary tissue loss and potentially induce regeneration/repair of damaged axons across the injury site. Adult mesenchymal stem cells exist in a variety of niches throughout the body, including bone marrow. These cells (best known as bone marrow stromal cells; BMSCs)) have previously been shown to improve tissue sparing (survival of healthy tissue) and, albeit to a limited degree, functional recovery when transplanted into rodent spinal cord following a contusive SCI. However, cell survival after transplantation is poor and we (and others) have previously shown that by two weeks post-transplantation the majority of the cells have died. BMSCs are susceptible to anoikis, or programmed death due to lack of adherence to a substrate, and this could be at least in part responsible for their poor survival after transplantation. Therefore,in order to address this problem we have studied whether fibronectin, an extracellular matrix protein that is important in attachment and anchoring of cells, when used as a transplant matrix would support BMSC survival. Fibronectin does not spontaneously polymerize in solution, which could potentially limiting cell binding motif availability. Others have shown that when fibronectin is mixed with the III-C fragment, it can be induced to self-polymerize in solution and when transplanted into the injured spinal cord, which does not lead to additional damage or side-effects. In order to maximize available binding motifs, we have considered multiple concentration and combinations of Fn and FnIII-C fragment to optimize BMSC survival *in vitro* and *in vivo* using an adult rat model of contusive SCI. By optimizing the transplant matrix for cell adhesion, we can decrease anoikis-driven apoptosis and enhance the repair potential of a BMSC transplant for treatment of SCI. A portion of this work was conducted at The University of Pittsburgh.

Disclosures: A.E. Haggerty: None. **M. Oudega:** None.

Poster

226. Spinal Cord Injury: Restorative Strategies

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Leona M & Harry B Helmsley Charitable Trust

Title: Task-specific training-based rehabilitation improves bladder outcomes following human spinal cord injury

Authors: *C. HUBSCHER[1,2], A. HERRITY[2,3], L. MONTGOMERY[1,2], A. WILLHITE[2,3,4], C. ANGELI[2,3,4], S. HARKEMA[2,3,4];
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Abstract: One of the major secondary challenges impacting overall health and quality of life following spinal cord injury (SCI) is the development of lower urinary tract dysfunction. Urological complications include bladder overactivity, uncoordinated contractions between the muscles of the bladder wall and bladder outlet, decreased bladder compliance and loss of continence. As a result, the ability to properly store and expel urine in a coordinated and timely manner becomes compromised. In an effort to ameliorate bladder outcomes post-SCI, management approaches include various catheterization techniques, pharmacological, surgical and electrical stimulation interventions. In addition, our previous study in a clinically relevant rodent contusion model indicates that rehabilitative methods introduced after SCI such as locomotor training (LT) positively influence micturition parameters (Ward P.J. et al., 2014. J Neurotrauma, 31: 819-833). Given the overlap of neural networks controlling bladder and locomotor function in the lumbosacral spinal cord, an interaction between these circuitries may be expected. We hypothesized that a vesico-somatic relationship is influenced by LT to improve bladder integrity and overall function. In this study, subjects who sustained a SCI (AIS: A or B) received 80 daily sessions of LT on a treadmill using body-weight support (one hour per session) or LT plus stand (weight bearing without stepping) training (one hour of each per day, separated by at least 3 hours). Urodynamic assessments were performed at pre-and post-training time points. Cystometry measurements demonstrated increases in bladder capacity and voiding efficiency post-training in the step and step/stand trained participants. These results suggest that an appropriate level of repetitive sensory information, in this instance, generated through task-specific stepping and/or loading provided to the spinal cord, appears to influence the neural circuitry controlling micturition. Experiments in progress include the impact of training-based rehabilitation without loading, using arm-crank exercise, and the effects of activity-dependent

plasticity induced by stand and step training on bladder outcomes in SCI subjects receiving epidural stimulation.

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Poster

226. Spinal Cord Injury: Restorative Strategies

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Topic: C.10. Trauma

Support: Chinese National Natural Science Foundation (No.81102646)

Title: Electroacupuncture combined with bone marrow mesenchymal stem cells transplantation attenuates inflammatory response and reactive astrogliosis in the transected spinal cord of rats

Authors: ***Y. DING**[1], R.-Y. ZHANG[2], Y.-S. ZENG[3];
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Abstract: It is known that the glial scar resulting from spinal cord injury (SCI) impedes axonal regeneration. Our previous study reported that Governor Vessel electroacupuncture (EA¬) combined with bone marrow mesenchymal stem cells (MSCs) treatment downregulated the expression of glial fibrillary acidic protein (GFAP) and chondroitin sulfate proteoglycans (CSPGs) after SCI. However, little is known about the mechanism of EA combined with MSCs downregulating GFAP and CSPGs expression. The present study investigated whether EA combined with MSCs (EA+MSCs) treatment could inhibit inflammatory response, and further attenuate the glial scar following SCI. The results showed that EA+MSCs treatment could decrease activation of microglia/macrophage, mRNA and protein expression of proinflammatory factors (TNF-α and IL-1β), and increase mRNA and protein expression of anti-inflammatory factor (IL-10) in injured spinal cords. Moreover, EA+MSCs treatment remarkably reduced reactive astrogliosis and accumulation of CSPGs after SCI. In addition, *in vitro* study demonstrated that TNF-α and IL-1β could increase astrocyte proliferation and mRNA transcription of GFAP and CSPGs. Therefore, the results of present study indicate that EA+MSCs treatment can inhibit inflammatory response, further attenuate the reactive astrogliosis and CSPGs deposition, and eventually inhibit glial scar formation following SCI. EA combined with MSCs treatment may be a potential therapeutic strategy for clinical treatment SCI.

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Poster

226. Spinal Cord Injury: Restorative Strategies

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Support: CIHR operating grant

CIHR post-doctoral fellowship

AIHS post-doctoral fellowship

Title: Automated robot system for high-throughput training, testing, and analysing skilled reaching performance in rodents before and after CNS injury

Authors: *K. K. FENRICH[1,2], A. TORRES-ESPIN[1,2], J. FORERO[1,2], Z. MAY[1,2], D. J. BENNETT[2,3], K. FOUAD[1,2];
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Abstract: The single pellet grasping (SPG) task is a skilled forelimb motor task commonly used to evaluate fine motor control in rodent models of CNS injury and disease. The task requires animals to obtain food pellets located on a presentation shelf beyond a slit at the front of an SPG task enclosure. The major down side of training and testing animals in this highly detailed task is that it is very labour intensive - to the point where students and/or technicians can go crazy and attempt a lab mutiny! To reduce the incidence of lab mutiny, we developed an automated SPG training protocol using an Automated Pellet Presentation (APP) robot system. This system allows animals to train ad libitum 24 hours a day, 7 days a week, and improves grasping success rates while reducing researcher time and outcome variability compared to manually trained animals. Here we show that the APP system can be used to rehabilitate reaching and grasping movements in rats with cervical spinal cord injuries that impair forelimb function. Additionally, we describe an updated version of the APP system (APP 2.0) that allows automatic quantification of the SPG task and animal identification. Each APP 2.0 system is attached to a home cage for full-time ad libitum training and testing, which greatly reduces animal handling and variability in task performance associated with repeated handling. Moreover, to identify animals each system is equipped with a radio frequency identification (RFID) module that can read transponder tags implanted into each rat prior to the start of training. Furthermore, the APP 2.0 system is equipped with optical sensors to track attempts and evaluate each attempt as a pass or fail. Preliminary testing shows that naïve rats can be trained using the updated system, and the system can accurately identify animals as they performed the task and correctly categorized attempts as a pass or fail. Importantly, along with improved task performance and reduced

outcome variability compared to manual training, the APP 2.0 system reduced researcher time commitments to less than 5 min/week/rat (manual training usually requires ~100 min/week/rat). Concomitant with our testing of the APP 2.0 system for rats, we are developing a reduced version for mice. Taken together, the updated APP system facilitates the training and testing of forelimb function in rodent models and can be simultaneously be used for rehabilitative training, a process needed to evaluate clinically relevant treatments.

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Poster

226. Spinal Cord Injury: Restorative Strategies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 226.19/H29

Topic: C.10. Trauma

Title: Development of research toolkits for locomotor functional recovery after spinal cord injury in common marmosets

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Abstract: Current preclinical researches on locomotor function after spinal cord injury (SCI) are mostly studied using rodent models. In terms of clinical trial, we have considered to test its compatibility in a newly established non-human SCI primate model of common marmosets. In the current study, we developed research toolkits for locomotion of marmoset including spinal contusion measure, a weight-support bipedal locomotion system, and a custom-made beam. Common marmosets (N = 2) received contusive SCI by a modified New York University (NYU) device (2.0 mm in diameter) by dropping the weight (10 or 20 g) from a height of 50 mm onto the dura mater at the T11 level. Manual bladder expression was carried out twice a day until voiding reflexes were reestablished. In addition, we tested bipedal locomotion on the treadmill with the body weight support in both the intact (N = 7) and SCI marmosets (N = 2). During bipedal locomotion, the reflective markers on the joints of marmosets were tracked by the motion capture system, and kinematics of the hindlimb movements was analyzed. The marmosets had little or no hindlimb movements until 3 weeks postoperative and then demonstrated a progressive recovery of the movements in next 2-3 weeks postoperative. In 8 weeks postoperative, a 20 g SCI model showed sweeping with hindlimbs during walking without

weight support, while 10 g SCI model showed frequent weight supported plantar stepping. Functional recovery of locomotion reached a plateau in both marmosets in 8-10 weeks postoperative. In a 20 g SCI model, luxol fast blue and cresyl violet staining showed 24 % of the spinal cord tissues were spared (bilateral anterior funiculus, 13 %; unilateral funiculus, 11 %) at the lesion site. In the both SCI models, the occasional hindlimb stepping was observed on the treadmill with the body weight support in 10 weeks postoperative. On the kinematics analysis, the maximum height of MTP joint position and length of time for swing phase were changed as compared to the intact of the same marmosets. Despite of gradual recovery of the hindlimb movements after SCI, the marmosets of the 20 g SCI model did not recover stepping movement with weight support even in 10 weeks postoperative. Previous studies showed that, remarkably small number of axons (a minimal amount 10%) could induce considerable recovery of stepping function in rats (Basso et al., Exp Neurol. 1996), but not in macaque monkeys (Eidelberg et al., Brain 1981). In marmosets, the recovery process of locomotor function after SCI was more similar to macaque monkeys than rats. This suggests primates may need more supraspinal inputs for stepping movements than rodents.

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Poster

226. Spinal Cord Injury: Restorative Strategies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 226.20/H30

Topic: C.10. Trauma

Title: Programmed freeze/thaw method dramatically improved cell viability of ips cell-derived neural stem cells for clinical application in spinal cord injury

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Abstract: Background: Recently, we have reported the effectiveness of transplanting human iPS cell-derived neural stem cells (iPS-NSCs) for subacute spinal cord injury (SCI) in mice as well as common marmosets. Because it takes 6 months to establish iPS-NSCs derived from patient's own somatic cells, at present, it is impossible to perform auto-graft of iPS-NSCs within an optimal therapeutic time window for SCI. To extend our results into clinical application, allogeneic transplantation is a realizable goal. However, there are still some concerns to overcome, such as iPS-NSCs storage and supply. It is especially critical to determine whether

freezing and thawing affects the viability and characters of cells since viability was extremely low when iPS-NSCs were cryopreserved in freezing container. **Purpose:** The purpose of this study is to improve the viability and assess the effects of cryopreservation on the characters of iPS-NSCs. **Materials and methods:** 201B7 iPS-NSCs, which are considered safe and non-tumorigenic as reported previously, were used in the present study. The iPS-NSCs were cryopreserved in STEM-CELLBANKER® by slow freezing method. We evaluated the cell viability to determine the timing of freezing (3 or 6 days after the last passage), the number of frozen cells (2 or 5 million/ml) and freezing method (programmed freezer or freezing container). Then proliferation, differentiation assays and microarray were performed under appropriate conditions in terms of cell viability. **Results:** The cell viability was highest when the iPS-NSCs were frozen on 6 days after the last passage at the concentration of 2 million cells/ml. Compared to the freezing container, the programmed freezer significantly increased the cell survival. Differentiation assay revealed that frozen-thawed (FT) cells dominantly differentiated into Tuj-1-positive neurons as same as non-frozen (NF) cells. There were no significant differences in proliferation and differentiation ability between FT and NF cells. Principal component analysis and hierarchical clustering revealed that the gene expression profile of FT cells was similar to NF cells. **Conclusion:** Towards clinical application of cell transplantation for SCI, cryopreservation of iPS-NSCs is essential in terms of cell viability. In this study, we succeeded in improving the viability of the iPS-NSCs by the programmed freezer. Furthermore, FT cells showed similar proliferation, differentiation ability as well as gene expression profile to NF cells, suggesting that our programmed freeze/thaw method would be useful for clinical application of cell therapy in SCI.

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Poster

226. Spinal Cord Injury: Restorative Strategies

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Program#/Poster#: 226.21/H31

Topic: C.10. Trauma

Title: Transplantation of human iPS cell-derived oligodendrocyte precursor cells enriched neural stem/progenitor cells in chronic and subacute spinal cord injury

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Abstract: Background: We have reported that there are limits to functional recovery by transplantation of neural stem/progenitor cells (NS/PCs) for chronic SCI. It was partly because of inadequate remyelination of surviving axons by transplanted cells. It is well known that remyelination of demyelinated axons could be a viable target in transplantation therapy for chronic SCI. Since human iPS cell-derived oligodendrocyte precursor cells (hiPSC-OPCs) enriched NS/PCs have potentials to differentiate into mature oligodendrocytes, these cells might be effective for the chronic SCI by remyelinating demyelinated axons in the injured spinal cord. In this study, we verified the effectiveness of transplanted hiPS-OPC enriched NS/PCs for mouse chronic SCI, then compared with the subacute transplantation. Methods: hiPSC-OPCs enriched NS/PCs were induced from pre-evaluated safe iPS cell line, and cytokine antibody array experiments were performed. Contusive SCI was induced in immunodeficiency mice and these cells were transplanted into the injured spinal cord 9 or 45 days after SCI (subacute and chronic transplantation group). Instead of cells, PBS was injected in each vehicle control group. For histological analyses, mice were intracardially perfused 12 weeks after transplantation. Locomotive motor functions were periodically assessed. Results: Cytokine antibody array analysis revealed that much more trophic factors were secreted from hiPSC-OPCs enriched NS/PCs than hiPSC-NS/PCs. Many grafted cells differentiated into MBP positive mature oligodendrocytes in both transplantation groups. Nodes of Ranvier were observed in the transplanted cells derived myelin sheathes. Furthermore transplanted cells promoted axonal growth and contributed to the synapse formation between grafted cells derived neurons and host neurons. Therefore there were many NF-H+ neuronal fibers in the both transplanted groups whereas a few NF-H+ axons were observed in the control group. The subacute transplantation group demonstrated significantly larger myelinated areas compared to control group, whereas myelinated areas did not significantly differ between the chronic transplantation group and chronic control group. Moreover, no significant motor function recovery was observed in the chronic transplantation group, compared to PBS group. Conclusion: The effectiveness of hiPSC-OPC enriched NS/PCs transplantation for chronic SCI was restricted compared to the transplantation for subacute SCI. Combination therapy of transplantation with debridement of glial scar formation or rehabilitation may be critical to achieve functional recovery for chronic SCI.

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Poster

226. Spinal Cord Injury: Restorative Strategies

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Title: Functional regeneration of descending propriospinal axons through and beyond a growth-promoting pathway constructed by transplanted Schwann cells overexpressing GDNF after a thoracic spinal cord transection in adult rats

Authors: *L. DENG[1], Y. SUN[2], Y. RUAN[3], M. WALKER[4], M. HAMILTON[5], W. QU[6], Y. WANG[6], G. M. SMITH[7], X.-M. XU[6];
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Abstract: Unsuccessful axonal regeneration in adult mammals following spinal cord transection is mainly attributed to the physical gap created between the two cord stumps, the glial scar formation, and the low intrinsic regenerating capacity of injured central nervous system (CNS) neurons. Schwann cells (SCs) can provide a permissive bridge for axonal regeneration across the lesion site. However, the reactive astrocytes surrounding the grafted SCs limit their integration into the host environment and therefore weaken the graft's efficiency. The segregation between grafted SCs and host astrocytes prevents regenerated axons inside the graft from growing back into the host cord, which is necessary for functional reconnection of neuronal circuit. We previously demonstrated that glial cell line-derived neurotrophic factor (GDNF) could modify interactions between SCs and astrocytes. In this study, we constructed an axonal growth permissive pathway following a thoracic spinal transection at T11 by transplanting SCs overexpressing GDNF (SCs-GDNF) into the lesion gap as well as into the caudal spinal cord. GDNF significantly improved the graft-host interface by promoting integration between SCs and

host astrocytes resulting in the migration of host astrocytes into the SCs-GDNF territory. The glial response within the caudal graft area was significantly attenuated. The astrocytes inside the grafted area were elongated with slim processes and bipolar orientation accompanied with dramatically reduced expression of glial fibrillary acidic protein. Remarkable number of descending propriospinal axons and supraspinal axons expressing serotonin and tyrosine hydroxylase regenerated across the lesion gap and grew back to the caudal spinal cord, which were otherwise hard to be seen in control groups. The regenerated axons were remyelinated and formed synapse with host neurons in the caudal spinal cord. The locomotor function in the group receiving SCs-GDNF-constructed growth-promoting pathway was also improved.

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Poster

226. Spinal Cord Injury: Restorative Strategies

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Title: Neurotrophin-3 normalises spinal reflexes after central nervous system injury

Authors: *C. KATHE[1], T. H. HUTSON[3], S. B. MCMAHON[2], L. D. F. MOON[2]; [2]Wolfson CARD, [1]King's Col. London, London, United Kingdom; [3]Ctr. for Restorative Neurosci., Imperial Col. London, London, United Kingdom

Abstract: Neurotrophin-3 (NT3) promotes the survival and neurite extension of specific neuronal populations including Ia proprioceptive fibers. We are investigating the mechanism by which NT3 modulates spinal reflexes mediated via this neuronal population and motor neurons *in vivo*. Firstly, we evaluated retrograde trafficking of NT-3 towards the spinal cord following AAV-NT3 overexpression in forelimb muscles. We have detected increased levels of NT3 protein in dorsal root ganglia connecting to the treated muscle groups. We are currently investigating transcriptional and post-translational changes of downstream molecules of its receptor trkC in dorsal root ganglia. Secondly, we use electrophysiology to functionally test the effects of NT3 overexpression in the muscle on the modulation of spinal reflexes involving proprioceptors and motor neurons. Rodents, like humans, develop spasticity after spinal cord

injury or stroke, caused by the hyper-excitability of the spinal reflex pathway. Thus, we developed an animal model, which allows for repeated electrophysiological assessment of this monosynaptic reflex. More specifically, we recorded the H-reflex from the abductor digiti quinti, a forepaw muscle, in rats every two weeks up to 10 weeks after a bilateral pyramidotomy. Rats were treated 24 hours post-injury by injection of an AAV-NT3 (or AAV-EGFP as a control treatment) into the forelimb flexor muscles on one side. Naive animals show a frequency-dependent depression of the H-wave at higher stimulation frequencies. After injury, this effect is reduced. However, neurotrophin-3 treated animals recover to baseline levels 6 weeks post-injury. Furthermore, rats treated with neurotrophin-3 have normalized polysynaptic reflexes and recover some motor function in their forelimbs whereas the control treated animals do not. Due to the profound effects observed after spinal cord injury following NT3 treatment, our data shows that spinal reflexes can be positively modulated after CNS injury with intramuscular neurotrophin-3 treatment.

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Poster

226. Spinal Cord Injury: Restorative Strategies

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International Spinal Research Trust

Title: Optogenetic evaluation of functional synaptic reconnection by corticospinal tract axons in the injured spinal cord

Authors: *N. JAYAPRAKASH, B. HOEYNCK, N. KRUEGER, Z. WANG, M. BLACKMORE;
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Abstract: Spinal cord injury results in partial or complete loss of neural communication across the site of injury. To restore function, axons must extend into denervated territory, and, critically, must form functional synapses with appropriate targets. We have previously shown that forced overexpression of transcription factors KLF7 or Sox11 promotes axon regeneration in corticospinal tract (CST) neurons, but that behavioral outcomes are modest or even negative. It is therefore unclear whether the newly sprouted axons are able to form functional synapses, or alternatively, whether functional synapses are formed in a mistargeted fashion. Here we use an optogenetic strategy to assess the ability of Sox11-stimulated axons to form functional synapses.

A pyramidotomy was performed in adult mice to deprive the left side of the spinal cord of CST input, and the right CST was treated with AAV-Sox11 or EBFP control, along with viral channelrhodopsin (rAAV9/CamKII-ChR2-EYFP). As we have shown previously, Sox11 treatment caused robust midline crossing of CST axons into the previously denervated left spinal cord. We then paired optogenetic stimulation of newly sprouted CST axon terminals in the left spinal cord with extracellular recordings of post-synaptic spinal neurons. We observed clear post-synaptic responses driven by optogenetic activation of Sox11-treated axons, demonstrating the ability to form functional synapses with target cells. Further, the use of multiunit electrodes enabled mapping of the pattern of spinal activity following light stimulation in different regions of the spinal cord. The presence of functional synapses without significant behavioral benefit suggests that new connections may be mistargeted, motivating ongoing experiments that incorporate rehabilitative training to refine the newly formed synapses. Overall these data illustrate the utility of an optogenetic approach to monitor and optimize functional reconnection by newly sprouted axons in the injured spinal cord.

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Poster

226. Spinal Cord Injury: Restorative Strategies

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The International Spinal Research Trust

Title: Combinatorial testing of transcriptional modification and chondroitinase treatment to promote axon regeneration and functional recovery after spinal cord injury

Authors: ***Z. WANG**, K. WINSOR, E. BALLE, P. POWERS, N. KRUEGER, M. BLACKMORE;
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Abstract: Axon regeneration in the central nervous system (CNS) is prevented by extracellular inhibitory cues, as well as a failure on the part of many injured CNS neurons to activate transcriptional programs needed to sustain axon growth. We have previously identified two transcription factors, KLF7 and Sox11, which enhance regenerative axon growth when overexpressed in adult corticospinal tract (CST) neurons. Here we explore the hypothesis that combining these transcriptional interventions with chondroitinase, an enzyme that degrades growth-inhibitory chondroitin sulfate proteoglycans, may lead to additional gains in axon

regeneration and/or behavioral recovery. We created a lentiviral vector to drive expression of chondroitinase in host cells (Lenti-Chase) and confirmed that Lenti-Chase injections caused CSPG degradation in the injured spinal cord using immunohistochemistry with 2B6, and anti-CSPG stub antibody. In one set of experiments, a thoracic crush experiment was performed in adult mice, and CST neuronal cell bodies were treated with transcriptional active KLF7 (AAV8-VP16-KLF7) while Lenti-Chase was delivered to the site of spinal injury. Compared to control viral treatments, KLF7-treated axons showed enhanced proximity to the proximal side of the injury, an effect that appeared to be elevated in the presence of Lenti-Chase. In contrast to previous results using partial spinal injury, however, KLF7-stimulated axons were not observed to regenerate distal to the complete crush injury. In a second experiment, animals were challenged with a cervical hemisection, and AAV-Sox11 treatment of CST cell bodies was combined with spinal lenti-Chase. Consistent with our previous results, Sox11 significantly enhanced CST axon growth distal to the injury site. Growth was not further increased by application of Lenti-Chase. Animals did, however, show a trend toward behavioral improvement when Sox11 and Chondroitinase were combined. Replicating this finding, and further combining these treatments with rehabilitative training, are areas of ongoing research

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Poster

226. Spinal Cord Injury: Restorative Strategies

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Topic: C.10. Trauma

Title: Mesoporous silica-supported lipid bilayer nanoparticles target gene transfer to motoneurons

Authors: *M. A. GONZALEZ[1,2], A. MUNIZ[4], J. BRINKER[4], G. C. SIECK[2,3], C. B. MANTILLA[2,3];
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Abstract: The potential for treating neurological diseases using gene therapy is vast but underachieved. At present there are no effective non-viral vectors that specifically target motoneurons. Nanoparticles are novel drug delivery systems with exceptional therapeutic potential. We hypothesized that mesoporous silica nanoparticles (MSNPs) can be engineered to specifically target motoneurons delivering various cargos and thus be useful for gene therapy. We used MSNPs coated with a lipid bilayer (protocells) which exhibit advantageous fusion characteristics including 1) increased surface area with larger loading capacity, 2) stability and 3)

improved biocompatibility. Fluorescently labeled protocells (130 nm in diameter) were loaded with GFP plasmid to evaluate transfection efficiency in cultured motoneurons *in vitro*. Protocells showed efficient motoneuron uptake quantified by measurement of intracellular protocell fluorescent intensity. Quantitative assessment of confocal images for co-localization of fluorescently labeled protocells and lysosomes revealed that protocells can escape endo-lysosomal degradation. Biocompatibility was determined by lack of cytotoxicity and unimpaired proliferation. Transfection of motoneuron-like NSC-34 cells was evident by ~20% of motoneurons expressing GFP. In conclusion, protocells are a promising, customizable non-viral approach for targeting and delivering genes to motoneurons *in vivo*.

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Poster

226. Spinal Cord Injury: Restorative Strategies

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Topic: C.10. Trauma

Title: Evaluation of immunological response in human iPS cell-derived neural stem cells using mixed lymphocyte reaction focusing HLA matching

Authors: *M. OZAKI[1,2], A. IWANAMI[1], J. KOHYAMA[2], G. ITAKURA[1], H. IWAI[1], M. MATSUMOTO[1], M. NAKAMURA[1], H. OKANO[2];
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Abstract: Background Recent studies demonstrated the effectiveness of transplantation of human induced pluripotent stem cell-derived neural stem cells (hiPSC-NSCs) for rodent as well as non-human primate spinal cord injury (SCI) model. Although human leukocyte antigen (HLA) matched allogeneic transplantation would be a realistic goal of first-in-human trial in taking advantage of iPS bank in Japan, the significance of HLA matching in hiPSC-NSCs transplantation for SCI has not been evaluated in detail. The purpose of this study is to determine the importance of HLA matching in allogeneic hiPSC-NSCs transplantation using mixed lymphocyte reaction (MLR). **Materials and Methods** Integration-free human iPS clone-derived NSCs were used in this study. Human peripheral blood mononuclear cells (PBMCs) which were purified from blood of healthy volunteers were co-cultured with irradiated HLA-mismatched hiPSC-NSCs and proliferative lymphocyte response was quantitatively measured by incorporation of ^{3}H-thymidine. As a control, human PBMCs were co-cultured with HLA-mismatched human fetal NSCs at the same time. Effects of passage number of hiPSC-NSCs on immune response were also evaluated. Then, PBMCs from same person who donated iPS cells

were co-cultured with autologous hiPSC-NSCs and autologous lymphocyte response to hiPSC-NSCs was compared with allogeneic lymphocyte response. **Results** The proliferation of lymphocytes co-cultured with HLA-mismatched allogeneic hiPSC-NSCs was significantly enhanced under the optimal condition. In allogeneic condition, proliferation of lymphocytes co-cultured with hiPSC-NSCs was similar to that of lymphocytes co-cultured with human fetal NSCs. Passages of hiPSC-NSCs less than 8 times did not affect the proliferation of allogeneic lymphocytes. Furthermore, the proliferation of lymphocytes stimulated by HLA-mismatched allogeneic hiPSC-NSCs significantly enhanced compared to that of lymphocytes co-cultured with autologous hiPSC-NSCs. **Conclusions** This study demonstrated that optimized MLR could predict immune responses in HLA-mismatched allogeneic hiPSC-NSCs transplantation. A higher response was observed in allogeneic MLR compared to autologous MLR, suggesting immune rejection was relieved by HLA matching in hiPSC-NSCs transplantation for SCI patients.

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Poster

226. Spinal Cord Injury: Restorative Strategies

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Title: 14-3-3 proteins targeted to promote axon regeneration

Authors: *A. KAPLAN[1], A. KRONER[1], S. LEONG[1], C. MADWAR[1], S. BANERJEE[2], I. RAMBALDI[1], N. BISSON[2], J. ANTEL[1], S. DAVID[1], A. FOURNIER[1];
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Abstract: Spinal cord injury (SCI) results in the damage and disconnection of long axonal tracts. The inability of severed axons to regenerate is responsible for sustained paralysis and loss of sensation in SCI patients. An important strategy to approach this unmet need is to develop drugs that promote regenerative axon growth. We have identified 14-3-3 proteins, a family of phospho-serine/threonine-binding cytosolic adaptor proteins, as important mediators of axon regeneration. Overexpression of 14-3-3 isoforms enhances axon regeneration in an *in vitro* cortical neuron scratch assay. Moreover, we have identified a compound that stimulates axon regeneration through a putative mechanism of stabilization of 14-3-3 protein-protein interactions (PPIs).

Treatment of rat cortical neurons and human fetal neurons with the 14-3-3 stabilizer compound enhances neurite outgrowth in a dose-dependent manner, indicating a conservation of activity from rodent to man. The 14-3-3 stabilizer compound is known to bind to a pocket created by the interface between the 14-3-3 binding groove and certain client proteins, forming a tri-partite complex that enhances the stability of the PPI. PPI stabilization is a unique concept in drug design that, unlike PPI inhibition, does not necessitate competition with a natural ligand for target binding. Current efforts are focused on elucidating the mechanism-of-action of the 14-3-3 stabilizer compound and testing its efficacy in stimulating corticospinal tract regeneration after dorsal hemisection SCI in mice. Compounds that stabilize 14-3-3 PPIs may be valuable neuroregenerative agents for use in SCI and other conditions characterized by axonal damage.

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Poster

226. Spinal Cord Injury: Restorative Strategies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 226.29/H39

Topic: C.10. Trauma

Support: TWU Department of Biology

TWU Research Enhancement Program

Southeast Missouri State University Department of Physics and Engineering Physics

Title: Dynamin inhibitors and their effect on endocytosis of surface functionalized nanospheres

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Abstract: Surface functionalized nanospheres (SFNPs) have the potential to target therapeutics to different subcellular destinations in damaged neurons. These SFNPs are entering the cells either through receptor mediated endocytosis or through adsorptive mediated endocytosis. Dynamin is a large GTPase involved in the fission of endocytic vesicles from plasma membrane in many pathways. The well known clathrin mediated endocytosis, caveolae mediated endocytosis, RhoA dependent endocytosis and some forms of macropynocytosis require dynamin for vesicle fission. We demonstrated that -COOH and -NH2 surface functionalized nanospheres can be endocytosed through caveolae and clathrin mediated mechanisms in B35

neuroblastoma cells and PC12 pheochromocytoma cells. Dynamin inhibitors can be used to block dynamin-dependent endocytic pathways to confirm whether SFNPs employ these pathways to enter the cells. In the present study, we treated B35 cells with dynamin inhibitors and tested their effect on endocytosis of SFNPs. Dynamin inhibitors can rapidly and reversibly block dynamin by targeting its specific domains. We tested the dynamin lipid binding (PH) domain targeting inhibitors MiTMAB and OcTMAb as well as GTPase allosteric site (GAS) domain targeting inhibitors dynoles and iminodyns. In future, we will be testing dynamin inhibitors in PC12 cells and rat cortical neurons to assess the mechanisms of endocytosis employed by SFNPs.

Disclosures: **S. Sebastian:** None. **R. Ammassam veettil:** None. **D. Hynds:** None. **T. Mcallister:** None. **S. Ghosh:** None.

Poster

226. Spinal Cord Injury: Restorative Strategies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 226.30/H40

Topic: C.10. Trauma

Title: Poly(ADP-ribose) polymerase 1 is a novel target to promote axonal regeneration

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Abstract: Therapeutic options for the restoration of neurological functions following acute axonal injury such as spinal cord injury are severely limited. In addition to limiting neuronal loss, effective treatments face the challenge of restoring axonal growth within an injury environment where inhibitory molecules from damaged myelin and activated astrocytes act as molecular and physical barriers. Overcoming these barriers to permit axon growth is critical for the development of any spinal cord injury repair strategy. Here, we identify Poly (ADP-ribose) polymerase 1 (PARP1) as a novel and critical mediator of multiple growth-inhibitory signals. We show that exposure of neurons to growth-limiting molecules such as myelin-associated glycoprotein and chondroitin sulfate proteoglycans activates PARP1, resulting in the accumulation of poly(ADP-ribose) (PAR) in the cell body and axon, and limited axonal growth. Accordingly, we find that pharmacological inhibition or genetic loss of PARP1 markedly facilitates axon regeneration over non-permissive substrates. Together, our findings provide critical novel insights into the molecular mechanisms of axon growth inhibition and identify PARP1 as an effective target to promote axon regeneration.

Disclosures: **C. Brochier:** None. **D. Willis:** None. **B. Langley:** None.

Poster

227. Psychosis: Mechanism

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Program#/Poster#: 227.01/H41

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: MH043784

MH084053

MH096985

MH100066

Nara Medical University

Title: Alterations in Regulator of G-protein Signaling 4 (RGS4) in schizophrenia

Authors: *S. KIMOTO[1,2], J. R. GLAUSIER[2], K. N. FISH[2], D. W. VOLK[2], H. H. BAZMI[2], D. ARION[2], D. DATTA[2], D. A. LEWIS[2,3];
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Abstract: NMDA receptor (NMDAR) hypofunction in the dorsolateral prefrontal cortex (DLPFC) has been implicated in the pathology of schizophrenia. Accumulating evidence suggests that NMDAR hypofunction may reflect dysregulation of NMDAR signaling. NMDAR activity can be negatively regulated by signaling of some G-protein coupled receptors (GPCRs), and the ability of these GPCRs to regulate NMDAR signaling is dependent upon Regulator of G-protein Signaling 4 (RGS4). Because RGS4 activity reduces GPCR signaling, lower levels of RGS4 enhance the ability of GPCRs to reduce NMDAR activity. RGS4 expression levels have been reported to be lower in the DLPFC in schizophrenia, suggesting a possible mechanism that could contribute to NMDAR hypofunction. To further investigate this relationship, we quantified the laminar- and cell-type specific changes in RGS4 mRNA and protein levels in the DLPFC. To investigate the possible molecular mechanisms underlying altered RGS4 expression, we quantified levels of small non-coding RNAs, known as microRNAs (miRs), which regulate RGS4 mRNA after transcription. RGS4 mRNA and protein levels were positively correlated in all subjects examined, and both were significantly lower in schizophrenia subjects relative to healthy comparison subjects. The RGS4 mRNA deficit in schizophrenia was present in DLPFC pyramidal neurons in layers 3 and 5. In contrast, levels of miR16 were significantly higher in the DLPFC of schizophrenia subjects, and higher miR16 levels predicted lower RGS4 mRNA levels. The present findings provide convergent evidence of lower RGS4 expression in schizophrenia that may result from increased expression of miR16. Lower RGS4 is predicted to augment the ability of GPCRs to decrease NMDAR signaling in DLPFC pyramidal cells in schizophrenia.

Disclosures: **S. Kimoto:** None. **J.R. Glausier:** None. **K.N. Fish:** None. **D.W. Volk:** None. **H.H. Bazmi:** None. **D. Arion:** None. **D. Datta:** None. **D.A. Lewis:** 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.; Bristol-Myers Squibb, Pfizer. F. Consulting Fees (e.g., advisory boards); Autifony, Bristol-Myers Squibb, Concert Pharmaceuticals, Sunovion.

Poster

227. Psychosis: Mechanism

Location: Hall A

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: 1 R21 MH100622 (Miller,PI)

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Winston & Maxine Wallin Neuroscience Discovery Fund (Miller, PI)

Fight for Sight

NSF CAREER award CBET 1135581

IGERT Neuromodulation training grant

Title: Signals recorded from the eye reveal disease states, including schizophrenia

Authors: ***R. F. MILLER**[1], A. MACDONALD, III[2], D. C. MILLER[3], M. S. LEE[4], C. G. SUMMERS[4], T. NETOFF[5], S. AMERI[2], T. TAKARA[4], D. C. MILLER[4], P. MOGHIMI[5]; [1]Neurosci., Dept. of Neuroscience, Univ. of Minnesota, Minneapolis, MN; [2]Dept. of Psychology, [3]Neurosci., [4]Dept. of Ophthalmology and Visual Neurosciences, [5]Biomed. Engin., Univ. of Minnesota, Minneapolis, MN

Abstract: We have used signals recorded from the eye, with conventional, non-invasive, ERG techniques to determine if patients diagnosed with schizophrenia (SZ) have differences when compared to a control group. Patients with SZ and demographically-similar controls were screened for diagnostic criteria and ophthalmologic contraindications before undergoing electroretinography (ERG). Our findings have revealed that signals from the eye have significant differences between the controls and the SZ population. Initially, we emphasized the response to patterns (pERG) and the Photopic Negative Response (PhNR) because they represent activity of retinal ganglion cells where NMDA receptors are prominently represented. We were surprised, however to find differences in almost every measurement we carried out with the exception of the Scotopic ERG. We found differences in most of the ERG recordings, including the photopic

ERG, the flicker response (30 Hz), and the oscillatory potentials. The main difference in these responses was a delay in the SZ population such that the peak was delayed by less than 1 ms, yet this difference is valid below the .02 ($p = < .02$). The flicker response amplitude is decreased in SZ patients, with a delay in the peak response. The most interesting differences were found in the pERG and the PhNR; recordings of the pERG reveal significant differences in the amplitude and peak delay with SZ patients showing reduced amplitude and a slower rise time to the stimulus. The PhNR reveals an absence of the PhNR especially to increased light stimulation (5 and 7 cd.s/M2). To determine if the pERG can be used to identify SZ patients and distinguish them from controls, we used a machine learning algorithm. Stimulus response waveforms were reduced to 6 dimensional features using principle component analysis. The principle components were used to train a support vector machine (SVM) using a subset of the SZ patients and controls. Then, the SVM was used to predict patients from controls in patients not used for the training. In this pilot sample, SVM was able to classify patients using the pERG with 75% accuracy ($p<0.01$). Combining the PhNR with the pERG should improve the accuracy of our prediction. Perceptual abnormalities in schizophrenia have often been interpreted as the result of dysfunctions in occipital cortex, however the present findings raise the possibility of degraded inputs even at the earliest levels of perception. In addition, PhNR and pERG signals may track with cortical abnormalities in NMDA receptor function and therefore serve to understand heterogeneity in patients' predispositions, course and treatment.

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Poster

227. Psychosis: Mechanism

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Schizophrenia research fellowship - Yale University / VA Connecticut Healthcare System

Title: Computer model of endocannabinoid effects in CA3

Authors: *M. A. SHERIF[1,3,4,8], P. SKOSNIK[1,3], M. HAJÓS[2], W. LYTTON[5,6,7,9]; [1]Psychiatry, [2]Comparative Med., Yale Univ., New Haven, CT; [3]Psychiatry, VA Connecticut Healthcare Syst., West Haven, CT; [4]Grad. program in biomedical engineering, [5]Neurol., [6]Physiol. & Pharmacol., [7]Biomed. Engin., SUNY Downstate Med. Ctr., Brooklyn, NY; [8]Ain Shams Univ. Inst. of Psychiatry, Cairo, Egypt; [9]Neurol., Kings County Hosp. Ctr., Brooklyn, NY

Abstract: Schizophrenia (SCZ) involves disturbances in neural function at different spatial and temporal scales. Theta (5-10 Hz) and gamma (30-100 Hz) oscillations have been shown to be abnormal in SCZ. In healthy volunteers, delta-9-tetrahydrocannabinol (THC), a cannabinoid-1 receptor (CB1R) partial agonist, induces symptoms similar to those reported in SCZ. CB1R agonism has been used to model some aspects of SCZ-related neural dysfunction in animals. Previous work has shown that CB1R agonism decreases theta (5-10 Hz), slow gamma (30-50 Hz), and fast gamma (60-90 Hz) power in CA3 region in rats. These converging data provide the impetus for developing a computer model of the CA3 region incorporating glutamatergic, GABAergic and endocannabinoid systems to investigate how CB1R agonism might explain the oscillatory changes that may in part underlie the various symptom domains of SCZ. The current model consisted of 2000 pyramidal cells, 100 parvalbumin-positive basket cells (PV cells), 100 cholecystokinin-positive basket cells (CCK cells) and 100 Oriens lacunosum Moleculare (OLM cells). Connectivity was based on the available literature and our previously published models. CB1R effects were implemented by varying GABA transmission at the synapses from CCK to pyramidal cells, as well as varying glutamatergic transmission at recurrent pyramidal connections. Experimentally, LFPs from CA3 region of 5 rats anaesthetized by intraperitoneal injection of chloral hydrate were obtained at baseline for model validation. Frequency bands within LFP signals were used to compare theta and low gamma oscillations with LFPs from the computer model. Model neurons were tuned to produce 8.3 ms membrane time constant for PV cells, 25 ms for CCK cells. In response to a depolarizing current of 100 pA, PV cells spiked at rate of 60 Hz, while CCK cells spiked at rate of 28 Hz. In response to a depolarizing current of 300 pA, PV cells spiked at rate of 132 Hz, while CCK cells spiked at rate of 52 Hz. In the LFPs from the model, emergent theta oscillations were seen at a frequency of 6 Hz, while slow gamma (high beta) oscillations were observed at 30 Hz, in agreement with rat controls from our experiments. These oscillation frequencies emerged from the much slower firing of pyramidal cell due to network interactions. Multiscale computer modeling is one suitable way to integrate findings from various modalities to reach a mechanistic understanding of the role the different elements play in the etiology and pathophysiology of brain disease. Such an understanding in SCZ can suggest potential treatments which would not be likely to be found through random ligand assessment.

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Poster

227. Psychosis: Mechanism

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 227.04/H44

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Chicago Biomedical Consortium with support from the Searle Funds at The Chicago Community Trust

UICentre for Drug Discovery at UIC

Title: Antipsychotic drugs, social interaction behavior and AMPA receptor RNA editing in mice

Authors: *E. NWABUISI-HEATH[1], E. DONG[2], A. GUIDOTTI[2], M. SODHI[3];
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Abstract: Glutamate receptors have been implicated in the pathophysiology of stress-related psychiatric disorders, including schizophrenia. The non-NMDA ionotropic receptors are modified by a process known as adenosine to inosine (A to I) RNA editing which has been shown to influence the alternative splicing, trafficking and function of these receptors. We have investigated the role of glutamate receptor RNA editing in adult mice subjected to prenatal restraint stress (PRS). C57BL6 male mice subjected to PRS and non-stressed (NS) controls were administered with clozapine (5mg/kg), haloperidol (1mg/kg) or saline (vehicle) at postnatal day 70 via repeated subcutaneous injections twice daily for 5 days. PRS mice exhibited reduced social interaction behavior. Clozapine administration reversed the deficit in social interaction behavior in PRS mice, and significantly increased hippocampal GRIA2 flop RNA editing in PRS mice. In contrast, haloperidol administration did not reverse deficits in social interaction behavior, but increased GRIA2 flop RNA editing in the hippocampus of PRS mice. NS mice administered with clozapine had lower levels of GRIA2 flop RNA editing in the hippocampus, while haloperidol was associated with lower levels of GRIA2 flip RNA editing in the prefrontal cortex. Analyses of the RNA editing enzymes, ADAR1, 2 and 3, revealed significantly lower ADAR3 mRNA levels in NS mice administered with clozapine ($F_{1,8}=12.4$, $p<0.01$) in the hippocampus, while higher ADAR3 mRNA levels were detected in the PRS mice administered with clozapine in the hippocampus ($F_{1,8}=11.7$, $p<0.01$), relative to vehicle controls. We are testing RNA editing of GRIA3, GRIA4, GRIK1 and GRIK2 to determine whether PRS and antipsychotic drugs alter glutamate receptor RNA editing in a generalized manner or if these effects are specific to GRIA2. Our results indicate that GRIA2 RNA editing in the hippocampus is associated with social interaction behavior and antipsychotic drug treatment. GRIA2 RNA editing is predicted to result in altered trafficking and reduced channel conductance of AMPA receptors, which is considered to be important for synaptic plasticity, learning and memory. Therefore RNA editing of GRIA2 may be a novel target for the development of psychotropic drugs.

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Poster

227. Psychosis: Mechanism

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 227.05/H45

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIMH MH60046

Title: Acoustic startle response in patients with bipolar disorder and schizophrenia

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Abstract: The overlapping symptoms found in bipolar I disorder (BPI), schizophrenia paranoid type, and schizoaffective disorder (SczA) can make the diagnoses difficult to differentiate. This may lead to ineffective treatments. The goal of this study was to investigate a possible biomarker derived from an electrooculogram called the acoustic startle response. Both sensorimotor gating of the acoustic startle response, measured using prepulse inhibition (PPI), as well as startle habituation, have been found to differ in patients with BPI or schizophrenia and control subjects. PPI describes the phenomenon in which a weaker, prepulse stimulus inhibits the subject's startle response to a stronger stimulus. Impaired PPI levels correspond to abnormalities in the inhibitory processing system. Startle habituation occurs when the acoustic startle response is diminished to trials at the end compared to the beginning of the trial sequence. Previous studies have investigated PPI and habituation in psychiatric disorders, however most lack analysis of specific subgroups within these disorders. The current study investigated differences between disorders as well as their subgroups to provide evidence regarding the specificity of these acoustic startle measures as biomarkers. There were seven different conditions to measure startle habituation and prepulse inhibition: Two conditions were used to measure percent startle habituation: (1) Startle Alone (white noise burst 115dB, 40ms tone duration) was presented six times in succession at the beginning of the sequence, and compared to (2) Startle Alone presented six times at the end of the sequence. Five different conditions were used to measure PPI: (3) Prepulse tone (78dB, 20ms duration) preceding Startle Alone by 60ms; (4) Prepulse tone (86dB, 20ms) preceding Startle Alone by 60ms; (5) Prepulse tone (78dB, 20ms) preceding Startle Alone by 120ms; and (6) Prepulse tone (86dB, 20ms) preceding Startle Alone by 120ms; used with (7) Startle Alone, randomly presented with the PPI trials to calculate percent PPI. Subjects' ocular motor response to each Startle Alone stimulus was measured. Using repeated measures analysis of variance (Group x Condition), for all the groups, there were significant differences among stimulus conditions for PPI and startle habituation. PPI showed significant differences between the groups; PPI latency was longest for the SczA group. PPI also showed differences between subgroups within SczA, with SczA BP type showing the least PPI. The results indicate that the use of PPI may provide an objective measure to help differentiate psychiatric disorders.

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Poster

227. Psychosis: Mechanism

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: University Hospital Foundation

Title: Perineuronal net deficits in the polyi:c model of schizophrenia

Authors: *J. W. PAYLOR[1], B. LINS[2], Q. GREBA[2], N. MOEN[1], J. HOWLAND[2], I. WINSHIP[1];
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Abstract: Schizophrenia (SCZ) is a psychiatric disorder that, despite a high prevalence (1% population worldwide) and burden of disease (1% global burden of disease), has a poorly understood etiology. While symptoms of SCZ typically manifest during adolescence and early adulthood, there is a substantial body of research linking prenatal events (e.g. early infection) to one's risk for developing SCZ. Human post-mortem analyses have suggested perineuronal nets (PNNs) as a potential biomarker for SCZ, as PNNs are deficient in a number of disease-specific regions of SCZ patients. PNNs, which surround parvalbumin positive (PV) inhibitory interneurons, typically mature during late adolescence and early adulthood when SCZ symptoms often first present. As such, we sought to examine whether a developmental PNN deficit is present in a maternal immune activation model of SCZ in rats. Long-Evans rat dams were treated at gestational day 15 with polyriboinosinic-polyribocytidilic acid (PolyI:C) to induce a strong maternal immune response. The litters were carried to birth and offspring allowed to develop to post-natal days 7 (PND7), 21, 35, and 90. We used immunohistochemistry to assess PNNs, staining with the lectin *Wisteria Floribunda Agglutinin,* and examined their integrity using epifluorescent and confocal microscopy across a number of brain regions: frontal association cortex, prelimbic cortex, reticular thalamic nucleus, primary auditory cortex, amygdala, and dorsal and ventral hippocampus. Additionally, we stained for microglia (anti-IBA1) and PV interneurons (anti-PV) to assess immune activation and PV assess neuronal integrity respectively. Our results indicate that PNNs are not present in postnatal day 7 rats but are significantly upregulated by PND21 and reach a mature state by PND35. From PND21 on, we found that PolyI:C treated offspring have widespread deficits in PNN development in the frontal lobe, temporal lobe, and thalamus. While we did not observe parallel deficits in PV interneurons in the adult stage, we did observe a deficit of these interneurons in the reticular thalamic nucleus at early developmental time points (PND7 & 21). PNNs are critical structures in the regulation of neuroplasticity and along with PV interneurons are crucial to critical windows of heightened plasticity during development. A deficit in PNNs could lead to a permanent pathological upregulation of structural plasticity into adulthood, as well as PV interneuron and cortical inhibitory network dysfunction. Our results not only further validate the PolyI:C model as an

effective model of SCZ, but provide a platform in which the role of PNNs in SCZ pathology can be further examined.

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Poster

227. Psychosis: Mechanism

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant U01MH103392

Title: PsychENCODE: cis-regulatory epigenome mapping in schizophrenia

Authors: ***M. KUNDAKOVIC**[1], Y. JIANG[1], V. POTHULA[1], L. BROWN[1], E. ZHAROVSKY[1], A. DINCER[1], R. JACOBOV[1], I. MAGRO[1], D. KAVANAGH[1,2], M. FROMER[1,2], M. PETERS[3], S. SIEBERTS[3], J. S. JOHNSON[1,2], P. SKLAR[1,2], S. AKBARIAN[1]; [1]Friedman Brain Institute, Dept. of Psychiatry, Icahn Sch. of Med. At Mount Sinai, New York, NY; [2]Dept. of Genet. and Genomic Sci., Icahn Sch. of Med. at Mount Sinai, New York, NY; [3]Sage Bionetworks, Seattle, WA

Abstract: The majority of genetic risk factors for psychiatric disorders reside in non-coding regions of the human genome which have largely unknown functions. Recently, the Encyclopedia of DNA Elements (ENCODE) project has characterized functionally relevant parts of the human genome in multiple cell lines and peripheral tissues, showing that gene regulatory regions are often cell- and tissue-specific. This led to the launch of an NIMH-funded PsychENCODE consortium aiming to identify non-coding functional genomic elements relevant to neurodevelopment and mental disorders. The goal of our PsychENCODE project is to construct detailed maps for multiple active (H3K4me1/me3, H3K27ac, and H3K36me3) and repressive (H3K27me3) chromatin modifications in human neurons and glia, and to subsequently assess the relationship of two of these marks (H3K4me3 and H3K27ac) to genetic risk factors for schizophrenia (SCZ) in the Common Mind Consortium cohort of 338 SCZ cases and 315 controls. We focus on post-mortem brain tissue including the prefrontal cortex and anterior cingulate cortex as the most likely sites of pathological changes related to SCZ. As the cellular milieu of the human brain is complex, our project includes epigenomic profiling of neurons and glia using FACS-based separation of neuronal and non-neuronal nuclei followed by ChIP-seq. To be able to perform cell-type specific epigenomic profiling in a large-scale study in an efficient and timely fashion, we have developed a very rigorous pipeline for the quality control of our data. Here, we will provide a detailed step-by-step procedure of all quality control steps

that include: control of the FACS-sorted material; testing of the efficiency of the micrococcal nuclease digestion; quality control of histone antibodies; quality control of ChIP DNA and ChIP-seq libraries as well as quality control of the sequencing data using a bioinformatics pipeline. We will show our first set of the ChIP-seq data that confirm and emphasize the importance of performing cell-type specific epigenomic profiling in the brain. Our H3K4me3 and H3K27ac ChIP-seq data from control brain specimens show sharper resolution when compared to the data derived from the whole cortical homogenates as well as consistent differences between neuronal and non-neuronal chromatin across different subjects and genomic regions. As the final step of this project, we intend to integrate the epigenomic profiles with DNA variation and RNA sequencing profiles from the same brain specimens. The findings of this project will provide an extraordinary opportunity to improve our understanding of gene regulation in the brain and schizophrenia-relevant pathology.

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Poster

227. Psychosis: Mechanism

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Intramural Research Program of the National Institute on Drug Abuse

Title: Schizophrenia susceptibility gene dysbindin maintains prefrontal function

Authors: *W. ZHANG, K. M. DALY, B. LIANG, L. ZHANG, Y. LI, D.-T. LIN;
Intramural Res. Program, Natl. Inst. On Drug Abuse, Baltimore, MD

Abstract: Schizophrenia is a chronic brain disorder that affects ~ 1% of general population. Genetic studies have identified substantial numbers of susceptibility genes, however the pathophysiology of schizophrenia is still unclear and current pharmacological treatments offered limited success, especially for improving negative and cognitive symptoms. Unraveling pathological mechanisms of schizophrenia is critical for developing effective therapeutic strategies. Dysbindin (DTNBP1) is one of the earliest identified susceptibility genes. Genetic variations of Dysbindin have been well replicated as genetic risks associated with schizophrenia, and reduced dysbindin expression has been found common in brain areas of schizophrenia patients. To illustrate the mechanism of Dysbindin on brain function, we injected AAV1-Cre-eGFP virus into the prefrontal cortex (PFC) of Dysbindin conditional knockout mice, a well-

established brain region for its involvement in cognition and schizophrenia. We found that Cre virus injection led to loss of Dysbindin expression in neurons of PFC. We then analyzed synaptic transmissions and behavior of mice injected with virus. We found that loss of Dysbindin significantly shifted excitation/inhibition balance toward hyperexcitation in PFC compared with that of wild-type mice. Deletion of Dysbindin in PFC also disrupted behavior of those mice. These results showed the important role of Dysbindin in synaptic activities and functions of PFC.

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Poster

227. Psychosis: Mechanism

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: An investigation of synapse deficits in 22q11.2 Schizophrenia using patient induced stem cell derived neurons

Authors: *E. DEBOER;
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Abstract: A growing body of research has demonstrated that schizophrenia (SZ) is a neurodevelopmental disorder characterized by deficiencies in cortical connectivity and signaling, where post-mortem studies of SZ patients have uncovered reductions in cortical synaptic spine density and callosal white matter that astroglia are critical to synapse formation and the fidelity of the cortical network connectivity deficits observed in SZ is a virtually unexplored. Historically, there have been several great challenges to investigating connectivity in schizophrenia. First, there is extreme heterogeneity in the causal background genetics between patients of schizophrenia, as demonstrated recently nature of psychotic disorders to human, mouse models of psychotic disorders are limited in scope not only from an evolutionary perspective, but also because they typically represent a single, rare genetic risk factor of many hundreds. Finally, given the nature of schizophrenia, patient tissue samples are rare and typically represent a lifetime's worth of pharmaceutical and other confounds which complicate experimental control. The most common genetic correlate to SZ is the 22q11.2 deletion syndrome (22qdel), in which 20-30% of those affected develop SZ by adulthood; an incidence rate 20x greater than that of the general population. We have combined the use of induced pluripotent stem cells (iPSCs) from this patient population together with a previously published protocol for the rapid generation of forebrain neurons to conduct a robust, reductive analysis of synaptic and signaling deficits in 22qdel neurons.

Disclosures: E. Deboer: None.

Poster

227. Psychosis: Mechanism

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 227.10/I2

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIMH Grant MH60046

NARSAD

Title: P200 activity patterns in bipolar, schizoaffective, and paranoid schizophrenia patients in response to gaps-in-noise

Authors: *R. B. BATINO[1], C. VILLEGAS[2], S. MONDAL[2];
[1]Univ. of California, Irvine Med. Ctr., Anaheim, CA; [2]Univ. of California, Irvine Med. Ctr., Orange, CA

Abstract: The overlap of bipolar I disorder (BPI) and two types of schziophrenia—paranoid schizophrenia (SczP) and schizoaffective disorder (SczA)—both genetically and clinically made it difficult to uniquely distinguish the three types of disorders. Identifying biomarkers or endophenotypes that distinguish these disorders more objectively than the clinical criteria defined in the DSM-V would aid in better understanding both the neurological and genetic underpinnings of the disorders. Temporal processing has been studied in psychiatric disorders, especially schizophrenia, showing that patients have temporal resolution deficits. This study focused on abnormal auditory temporal processing as a possible biomarker in BPI, SczP, and SczA. The main goal was to compare the brain response activity patterns in participants with BPI, SczA, and SczP to controls and to each other using a Gaps-in-Noise (GIN) paradigm. The GIN test is used to analyze auditory temporal processing by assessing subjects' ability to detect silent gaps in continuous white noise. Almost no studies have been done on whether there are differences between psychiatric groups using GIN testing to detect temporal processing deficits. In particular, event-related brain potentials (ERP) following the GIN can be used to reflect temporal resolution. One of the ERP components that reflect auditory temporal processing in the gap detection test is the P200. More studies are needed to clarify the significance of the P200 in healthy populations, schizophrenia, and BPI. However, P200 appears to be relevant to the study of schizophrenia, given that it has been linked to deficits in stimulus processing. This study evaluated ERP component P200 in BPI, SczA, SczP, and control patents in response to length of silent gaps in continuous noise (50, 20, 10, 5, and 2 ms). Specifically, there was a statistically significant interaction between gap length and diagnosis ($F= 2.77$, $p<.001$). To highlight, it was found that the larger the gap length, the larger the difference in peak-peak to amplitudes between

the four groups studied. There were significant differences between patient groups at 50, 20, and 10 ms silent gaps but not at 5 and 2 ms gaps. All groups showed significant effects of event type for latency. Differences between patient subgroups were also observed with the BPI manic and SAD Bipolar type subgroups showing the smallest amplitudes, again increasing as gap length increased. We can conclude that there is potential to use GIN testing as a better way to understand temporal processing in psychiatric disorders and to investigate its potential as a biomarker.

Disclosures: **R.B. Batino:** None. **C. Villegas:** None. **S. Mondal:** None.

Poster

227. Psychosis: Mechanism

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 227.11/I3

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Cortico-limbic circuit dysfunction in NMDA receptor hypofunction model of schizophrenia

Authors: *A. RAVIKRISHNAN, S. C. GUPTA, B. HILLMAN, S. M. DRAVID; Pharmacol., Creighton Univ., Omaha, NE

Abstract: Schizophrenia (SZ) is a neurodevelopmental disorder that occurs in approximately 1% of the population worldwide. Previous studies have demonstrated that pharmacological inhibition of NMDA receptors leads to schizophrenia-like phenotypes, and that genetic suppression of NMDAR function in inhibitory neurons of the cortico-limbic region enhances the likelihood of disease development and progression. Our previous behavioral analysis indicates that GluN2C knockout exhibits several behavioral phenotypes observed in schizophrenia including a deficit in prepulse inhibition. In addition lower GluN2C subunit expression is reported in cortex and thalamus of schizophrenia patients suggesting GluN2C knockout model exhibits both face and construct validity for schizophrenia. GluN2C subunit is enriched in the parvalbumin (PV) interneurons in the medial prefrontal cortex (mPFC) and reticular thalamus (nRT) that drive gamma oscillations which are thought to govern cognitive function and social behavior affected in schizophrenia. We observed a reduction in parvalbumin expression in the mPFC and reticular thalamus in GluN2C KO mice. Further electrophysiology and biochemical analysis demonstrated a reduction in excitatory tone as indicated by a reduction in mEPSC frequency in the mPFC which corroborated with a reduction in dendritic spine density. A contrasting increase in inhibitory tone on pyramidal neurons in the mPFC as indicated by an increase in mIPSC and perisomatic GAD67 puncta on pyramidal cells was observed. Overall, while some of our observations in the GluN2C KO mice parallel those seen in schizophrenic subjects, there also appear to be inconsistencies, particularly the shift towards greater inhibition, instead of cortical

overexcitation. We hypothesize that these changes represent early synaptic abnormalities during the progression of schizophrenia-like state. Ongoing studies are evaluating the developmental changes in cortical and thalamic E/I imbalance in the NMDA receptor hypofuncton model.

Disclosures: A. Ravikrishnan: None. **S.C. Gupta:** None. **B. Hillman:** None. **S.M. Dravid:** None.

Poster

227. Psychosis: Mechanism

Location: Hall A

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Program#/Poster#: 227.12/I4

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH F32HD078051-02

Title: Human neuropsychiatric disease modeling using conditional deletion reveals synaptic transmission defects caused by heterozygous mutations in Neurexin-1

Authors: *C. PAK[1], T. DANKO[1], Y. ZHANG[1], J. AOTO[1], G. ANDERSON[1], S. MAXEINER[2], F. YI[1], M. WERNIG[1], T. SUDHOF[1];
[1]Stanford Univ., Stanford, CA; [2]Universitätsklinikum Homburg, Univ. des Saarlandes, Homburg, Germany

Abstract: Heterozygous mutations of the *NRXN1* gene, which encodes the presynaptic cell-adhesion molecule neurexin-1, are repeatedly associated with autism and schizophrenia. However, diverse clinical presentations of *NRXN1* mutations in patients raise the question whether heterozygous *NRXN1* mutations alone directly impair synaptic function in human neurons. To address this question under conditions that precisely control for genetic background effects, we generated human embryonic stem (ES) cells with two different heterozygous conditional *NRXN1* mutations, and differentiated them into homogenous excitatory neurons using direct differentiation protocol. Upon analyzing two different types of isogenic control and *NRXN1*-mutant neurons derived from targeted ES cells, we found striking synaptic phenotypes associated with these mutations. Both heterozygous *NRXN1* mutations selectively impaired neurotransmitter release without changing neuronal differentiation or synapse formation. Our results show that unexpectedly, heterozygous inactivation of *NRXN1* directly impairs synaptic function in human neurons, and suggest novel avenues to selectively study the effects of disease-associated mutations independent of genetic background.

Disclosures: C. Pak: None. **T. Danko:** None. **Y. Zhang:** None. **J. Aoto:** None. **G. Anderson:** None. **S. Maxeiner:** None. **F. Yi:** None. **M. Wernig:** None. **T. Sudhof:** None.

Poster

227. Psychosis: Mechanism

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 227.13/I5

Topic: C.15. Schizophrenia and Bi-polar Disorder

Title: Understanding the neurobiology of early-onset schizophrenia using whole-brain computational modelling: a connectomics approach

Authors: *H. M. FERNANDES[1,2], J. CABRAL[1], T. V. HARTEVELT[1], T. CROW[3,1], A. C. JAMES[3,1], G. DECO[4,5], M. L. KRINGELBACK[1,2];
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Abstract: Early-onset schizophrenia is a severely disabling illness which is currently not well understood in terms of its underlying neurobiology. However, emerging evidence suggests that schizophrenia is strongly linked to disrupted cortical connectivity. Yet, it remains unclear how to link between this structural connectivity with the altered network topology in the large-scale resting-state functional connectivity (rsFC) in schizophrenia. Whole-brain computational modelling has proved to be a robust and valuable tool to simulate and predict the underlying network dynamics that arise spontaneously from intrinsic brain processes. Such models have successfully reproduced many details of the empirical neuroimaging data (rs-fMRI and rs-MEG). We compared the structural connectivity of a group of 20 adolescent patients with early-onset schizophrenia with a group of 20 age and gender-matched healthy controls, having constructed the structural connectomes using probabilistic tractography on diffusion tensor imaging data. We used a whole-brain computational model of spontaneous large-scale brain activity to characterize the dynamical shifts from the optimal healthy regime and explore the role of the structural connectivity in the large-scale dynamics of the brains of patients and healthy controls. Complex network analysis was used to characterize the different properties of the SC network dynamics for the two groups. We found significant changes in the structural connectivity in core brain areas associated with cognitive and emotional processing. The computational modelling revealed that the brain operates with lower global coupling strength, which shifts the dynamics from the optimal healthy regime to a point where functional networks appear subtly randomized and less small-world when compared to healthy participants.

Disclosures: H.M. Fernandes: None. **J. Cabral:** None. **T.V. Hartevelt:** None. **T. Crow:** None. **A.C. James:** None. **G. Deco:** None. **M.L. Kringelback:** None.

Poster

227. Psychosis: Mechanism

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 227.14/I6

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIMH RO1 MH 56528

Title: Altered gene expression of proinflammatory cytokines and their membrane-bound receptors in the lymphocytes of schizophrenia patients

Authors: *H. ZHANG, H. S. RIZAVI, X. REN, G. N. PANDEY;
Univ. Illinois at Chicago, Chicago, IL

Abstract: Abnormalities of the immune function have been observed to be associated with the pathophysiology of schizophrenia. The abnormalities of the immune function in SZ are based on both direct and indirect evidence. For example, the administration of cytokines such as interferon (IFN) to rats causes a constellation of symptoms known as "sickness behavior" that includes cognitive changes, slowed cognitive speed, diminished social interactions and reduced locomotor activity and executive functions. That inflammatory processes are also involved in SZ are based on the observation that proinflammatory cytokines, which are released from the immune cells as a result of inflammation or stress, are abnormal in the serum of patients with SZ. Whereas proinflammatory cytokines and their soluble receptors are studied in SZ, the membrane-bound receptors, which are involved in mediating the biological and functions effects of cytokines have not been studied in the blood of SZ patients. To examine if SZ is also associated with the abnormal gene expression of cytokines and their membrane-bound receptors, we studied mRNA expression of proinflammatory cytokines and their receptors in lymphocytes of SZ patients and normal control (NC) subjects. We determined the protein and mRNA expression of proinflammatory cytokines and mRNA expression of their receptors in lymphocytes from 30 SZ patients and 30 drug-free NC subjects. The subjects were diagnosed according to DSM-IV criteria. Protein levels of cytokines were determined by ELISA, and mRNA levels in lymphocytes were determined by the qPCR method. We found that the mRNA levels of IL-6, TNF-α, IL-1R1, TNFR1, and TNFR2, but not IL-1β, IL-1R2, IL-1RA, IL-6R, or GP130 were significantly increased in lymphocytes of SZ patients compared with NC subjects. We also found that the protein expression of IL-6 and TNF-α, but not IL-1β, was also significantly increased in SZ patients compared with NC subjects. These studies suggest that in addition to the reported abnormalities of proinflammatory cytokines and their soluble receptors in the plasma of SZ patients, an abnormal gene expression of these cytokines and their membrane-bound receptors may be involved in the pathogenesis of SZ.

Disclosures: H. Zhang: None. **H.S. Rizavi:** None. **X. Ren:** None. **G.N. Pandey:** None.

Poster

227. Psychosis: Mechanism

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 227.15/I7

Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: Graham Boeckh Foundation

Title: Brain volume changes following chronic antipsychotic treatment in animal models: MRI and histological study

Authors: *E. GUMA[1], J. ROCCHETTI[1], G. A. DEVENYI[2], J. LERCH[3], G. DAL BO[1], A. MATHIEU[2], B. COURCOT[2], M. M. CHAKRAVARTY[2], B. GIROS[1];
[1]Douglas Res. Ctr. - McGill Univ., Verdun, QC, Canada; [2]Brain Imaging Ctr. - Douglas Res. Ctr., Montreal, QC, Canada; [3]Mouse Imaging Ctr. Hosp. for Sick Children, Toronto, ON, Canada

Abstract: Over the past few decades, Magnetic Resonance Imagine (MRI) studies have shown that schizophrenic patients have specific brain abnormalities. However, these patients are generally treated with antipsychotic drugs (APD), making it difficult to determine whether the changes in brain structure are due to the pathology, to the effects of the medication, or to a combination of both. In order to address this confound further, we used animal models to longitudinally investigate the effects of APD on regional and global brain volume using chronically treated D3 dopamine receptor (D3R) knock out (D3KO), and D2 dopamine receptor (D2R) knock out (D2KO) mice with typical –haloperidol (HAL 1mg/kg/day) – or atypical – clozapine (CLZ 5mg/kg/day). Studying the effect of a typical and atypical AP medication on the brain volume in D2KO and D3KO mice allows us to further investigate if any observed neuroanatomical remodeling observed is the result of interactions with D2-like receptors, highly targeted by typical AP medication, or whether these changes involve alternate pathways. Animals were scanned using a Bruker 7T small animal MRI scanner before starting treatments, and then at 3, 6, and 9 weeks, generating T2/T1-weighted images with 140 μm isotropic voxels. The Multiple Automatically Generated Templates (MAGeT) brain algorithm was used to automatically segment the volumes of 62 regions of interest per hemisphere. We found treatment by genotype interaction in the left hippocampus of D2KO mice ($p<0.03$), a genotype effects in the striatum and globus pallidus of D2KO ($p<0.0001$) and D3KO ($p<0.05$) mice, as well as the basal forebrain for D2KO ($p<0.001$) and D3KO ($p<0.001$) mice. Voxel-wise deformation-based analysis confirmed that D2KO mice have a strikingly larger striatum compared to WT, following 9 weeks of HAL treatment, both D2KO and WT mice showed increases in striatal size (after 1% FDR correction). D3KO mice tend to have larger striatums than WT; following HAL treatment, both had reductions in the basal forebrain (after 5% FDR correction). To further understand the cellular basis of these volume changes, analysis of NeuN+ neuronal populations, Iba1+ microglia, and GFAP+ astrocytes was performed in the prefrontal cortex (PFC), striatum and

hippocampus of all the scanned animals to assess for cell density measures. Preliminary analysis shows a decrease in astrocyte density following APD treatment in the WT but not D3KO mice. In conclusion, this study helps to elucidate the region-specific structural effects of antipsychotics on mouse brain and the pathways implicated in these changes, which could allow for improvements in the design of treatments for psychotic disorders.

Disclosures: **E. Guma:** None. **J. Rocchetti:** None. **G.A. Devenyi:** None. **J. Lerch:** None. **G. Dal Bo:** None. **A. Mathieu:** None. **B. Courcot:** None. **M.M. Chakravarty:** None. **B. Giros:** None.

Poster

227. Psychosis: Mechanism

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: National Basic Research Program of China 973 program 2011CB707800

the Strategic Priority Research Program of the Chinese Academy of Sciences Grant No. XDB02030300

the Natural Science Foundation of China (91132301)

Title: Adolescent stress induced white matter changes revealed by diffusion mri in disc1 transgenic mice

Authors: *C. LIU, Y. LI, T. JIANG;
Queensland Brain Inst., ST LUCIA, Australia

Abstract: Environmental stress can exert long-lasting influences on brain maturation, but responses to stress also depend on genetic make-ups. Here, we utilised a recently described transgenic mouse model that combined genetic (a putative dominant-negative DISC1 under expression control of the prion protein promoter) and environmental (adolescent social isolation) factors (Niwa et al., 2013). Since DISC1 is a risk gene for various mental illnesses that are associated with white matter abnormalities, we mainly investigated the association between white matter impairments and behavioural abnormalities in the model by diffusion MRI and histological approaches. Four groups of mice were involved in this study, including group-housed wild types, group-housed DISC1 mice, social-isolated wild types, and social-isolated DISC1 mice. The social isolation began at P35 and lasted for three weeks. Compared with group-housed mice, social-isolated DISC1 mice showed significant behaviour abnormalities, including hyper-locomotion activities, and deficits in spatial working memory and fear conditioning. Based on high-resolution diffusion MRI, we observed a significant difference in

the mean diffusivity of the left fimbria across groups, which was significantly correlated with locomotion activities and fear conditioning. Targeting the left fimbria, we quantified the densities of total cells (DAPI+), oligodendrocyte lineage cells (OLIG2+), and mature oligodendrocytes (CC1+), as well as intensity of myelin (MBP+). Social-isolated mice had significantly higher densities of oligodendrocytes than group-housed mice. Importantly, the higher density of oligodendrocytes in the left fimbria was associated with the lower mean diffusivity of diffusion MRI, supporting the assumption that the diffusion of water molecules was hindered within abnormal white matter structures. Considering that hippocampus/fimbria plays a key role in the negative regulation of hypothalamic-pituitary-adrenal (HPA) axis, our findings support a possible mechanism in which structural impairment of the fimbria may impact the HPA axis and glucocorticoids following influences of genetic and environmental factors.

Disclosures: C. Liu: None. **Y. Li:** None. **T. Jiang:** None.

Poster

227. Psychosis: Mechanism

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: C.15. Schizophrenia and Bi-polar Disorder

Support: NIH Grant MH097997

Title: Human mutant DISC1 induced expansion of the hindbrain oligodendrocytes progenitors to the forebrain during early development - relevance to schizophrenia

Authors: *P. L. KATSEL[1], P. FAM[2], W. TAN[3], S. RUDCHENKO[4], M. PLETNIKOV[5], V. HAROUTUNIAN[6];
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Abstract: Strong evidence corroborates involvement of oligodendrocyte (OLG) dysfunction in the pathophysiology of schizophrenia (SZ). We previously showed that the forebrain-restricted expression of mutant human DISC1 (hDISC1) exerts a significant influence on oligodendrogenesis during early development evidenced by premature OLG differentiation and increased proliferation of their progenitors in forebrain regions. Concurrent reduction of OLG progenitor markers in hindbrain regions during fetal stage suggested expansion of hindbrain glial progenitors into the forebrain of hDISC1 mice. We tested this hypothesis by examining gene and protein expression of the molecular determinants of hindbrain OLG development (EGR2 and Nkx2-2) in samples from forebrain and hindbrain regions at E15 and P0 hDISC1 mice.

Additionally, in a postmortem study the gene expression of hindbrain OLG markers were measured by qPCR in the superior temporal cortex of persons with SZ (N=61) and compared to those of neuropsychiatrically normal controls (N=59). We found forebrain-restricted upregulation of gene and protein levels of the OLG hindbrain markers (EGR2 and Nkx2-2) at E15. Mutant hDISC1 and endogenous DISC1 are colocalized with Nkx2-2 and EGR2 in OLG progenitor cells. Proximity ligation assay between endogenous DISC1 and Nkx2-2 determined that these proteins interact with each other. Expression of mutant hDISC1 showed reduced interactions between DISC1 and Nkx2-2, which was associated with increased proliferation and differentiation of OLGs. These data suggest suppressive function of endogenous DISC1 for differentiation of Nkx2-2 positive OLG progenitors during early development. In parallel to animal study, several markers of hindbrain OLGs (PRX, LAMA1 and MPZ) were significantly upregulated in superior temporal cortex of persons with SZ. Our findings suggest a suppressive role of DISC1 in hindbrain OLG development, while expression of mutant hDISC1 interfered with endogenous DISC1 suppressive function and leads to expansion of Nkx2-2 positive OLG progenitors responsible for developmental positioning of OLG identity cells along the rostrocaudal axis and thus affecting cortical organization of the brain. Given the critical role of DISC1 in migration of neuronal and glial progenitors during brain development, our results provide new clues for the developmental mechanisms contributing to oligodendrocyte dysfunction in SZ.

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Poster

227. Psychosis: Mechanism

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 227.18/I10

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH062349

Title: Impulsive or indecisive: Impairments of decision making in a cortical circuit model of disrupted excitation-inhibition balance associated with schizophrenia

Authors: *J. D. MURRAY[1], T. BORDUQUI[1], J. HALLAK[2], A. ROQUE[3], X.-J. WANG[1]; [1]Ctr. for Neural Sci., New York Univ., New York, NY; [2]Neurosci. and Behaviour Dept., [3]Dept. of Physics, Univ. of São Paulo, São Paulo, Brazil

Abstract: There is an increasing recognition that, ultimately, behavioral disorders associated with mental illness need to be understood in terms of impairments of basic neural computations and their underlying circuit causes. However, rigorous studies to test this perspective are scarce.

In this study, using a biophysically-based model of an association cortical microcircuit (Wang, Neuron 2002), we investigated how circuit abnormalities may lead to impaired time integration underlying multiple cognitive deficits. Disruption in the balance between excitation and inhibition (E/I balance) in cortex is a leading hypothesis for pathophysiologies of neuropsychiatric disorders such as schizophrenia. Disorders such as schizophrenia are also associated with cognitive deficits, including impaired decision making. However, it is poorly understood how disruptions of E/I balance at the synaptic level propagate upward to the behavioral level leading to deficits in cognitive processes such as decision making. To link these levels of analysis, we studied the effects of synaptic disruption of E/I balance on neural activity and decision-making behavior in a cortical microcircuit model. In particular, we tested the effects of hypofunction of NMDA receptors at two key sites: on inhibitory interneurons (thereby elevating E/I ratio via disinhibition), versus on excitatory pyramidal neurons (thereby reducing E/I ratio). We found that disruptions to E/I balance in either direction can lead to impaired decision making performance as assessed by psychometric curves. Nonetheless, these two regimes make dissociable predictions that can be tested with fine-grained analyses of the time course of evidence accumulation. In the regime of elevated E/I ratio, behavior can be characterized as impulsive. In this regime, evidence early in time is weighted much more than evidence late in time, compared to a control circuit. In contrast, in the regime of reduced E/I ratio, behavior can be characterized as indecisive. In this regime, the circuit exhibits weakened evidence integration and reduced competition between options. This is consistent with a crucial role of NMDA receptors in slow integration. These regimes are further distinguished from the scenario of impaired upstream coding of sensory evidence. Our findings characterize a role of E/I balance in cognitive functions supported by cortical circuits. The model makes specific predictions for behavior and neural activity that can be tested in humans or animals under pharmacological manipulation or in disease states. We propose important features of task design for sensitive probing of altered evidence accumulation in decision making.

Disclosures: **J.D. Murray:** None. **T. Borduqui:** None. **J. Hallak:** None. **A. Roque:** None. **X. Wang:** None.

Poster

227. Psychosis: Mechanism

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 227.19/I11

Topic: F.02. Animal Cognition and Behavior

Support: Johns Hopkins Woodrow Wilson Fellowship

Title: Targeting hippocampal dysfunction improves cognition in a schizophrenia model

Authors: Y. SHAO, *M. KOH, M. GALLAGHER;
Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Recent clinical studies using neuroimaging and preclinical models of schizophrenia indicate that aberrant hippocampal excitability may contribute to cognitive impairment and augment psychotic symptoms due to disinhibition of dopaminergic neurons. Thus treatment with compounds targeting hippocampal overactivity to normalize this condition might improve hippocampal-dependent memory and reduce dopamine hyperactivation via functional pathways connecting the hippocampus and ventral tegmental area. Here, we tested this therapeutic approach to assess both cognitive impairment and a potential benefit on the positive symptoms of schizophrenia associated with dopamine dysregulation. In a well-established ketamine model, rats were tested with the antiepileptic medication, levetiracetam (LEV), which mediates its effect via synaptic vesicle glycoprotein 2A (SV2A) that prime vesicles for activity-dependent transmitter release. For preparation of the preclinical model of schizophrenia, we injected male Long-Evans rats at 2-mo of age with ketamine, a non-competitive NMDA receptor antagonist (30 mg/kg; twice a day), or vehicle saline for two weeks, and behaviorally tested them at adulthood (4-6 mo-old). Rats were tested for hippocampal-dependent spatial memory in an eight-arm radial maze task using an extended delay to assess retention. Ketamine-exposed rats committed more memory errors than control rats at a 3-hr delay between study and test phases. When treated with varying doses of LEV, ketamine-exposed rats showed a dose-dependent memory improvement with significantly fewer errors at 10 mg/kg compared with vehicle. Rats were also tested for amphetamine-induced locomotor hyperactivity, a standard assay used to assess the efficacy of antipsychotics to attenuate positive symptoms in preclinical models of schizophrenia. Amphetamine (0.5 mg/kg), a dopamine agonist, was given with or without LEV (10 mg/kg) treatment in independent assessments on different days. When comparing LEV treatment to vehicle, ketamine-exposed rats showed an attenuation of the increased locomotor activity induced by amphetamine. In contrast, control rats that had not received ketamine exposure showed no effect of LEV administration on their response to the amphetamine challenge. Together, these findings indicate that LEV may be useful for normalizing hippocampal activity in schizophrenia-related cognitive dysfunction, as well as moderating a positive symptom of schizophrenia due to dopaminergic dysfunction.

Disclosures: Y. Shao: None. **M. Koh:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Johns Hopkins University. **M. Gallagher:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Johns Hopkins University.

Poster

228. Stroke Imaging and Diagnostic Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 228.01/I12

Topic: C.21.Stroke Recovery

Support: National Health and Medical Research Council NHMRC

New South Wales Office of Science and Medical Research

Title: Measuring the efficacy of therapy in chronic stroke, active and passive range-of-motion do not reflect improvements in upper-limb motor-function

Authors: *P. A. MCNULTY[1,2], T. TRINH[1,2], P. LEE[1], G. LIN[1], A. G. THOMPSON-BUTEL[1], C. T. SHINER[1], S. G. FAUX[3,2];
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Abstract: Quantification of upper-limb motor-function is important to identify the status of functional ability after stroke, measure the efficacy of rehabilitation interventions, and provide motivation for stroke survivors to continue therapeutic activities. Range-of-motion is commonly used as an outcome measure in clinical trials. Active and passive range-of-motion were measured as part of a suite of upper-limb assessments for i) a randomised controlled trial of a dose-matched 14-day program of Wii-based Movement Therapy (WMT) or modified-constraint therapy (mCIMT) (n=42); and ii) with additional kinematic data that were quantified using wireless electrogoniometry from 12 stroke survivors during early (day 2) and late (day 14) WMT. Functional assessments included the Wolf Motor Function Test-timed tasks (WMFT-tt), upper-limb motor Fugl-Meyer Assessment (FMA), and Motor Activity Log Quality of Movement scale (MALQOM). There was no clear pattern of change for either active or passive range-of-motion in the randomised controlled trial data after the WMT or mCIMT group, and no difference between groups. Motor-function improved on the WMFT-tt ($p<0.001$), FMA ($p<0.001$) and MALQOM ($p<0.001$) with no differences between groups. For the kinematic study there was no change in either active or passive range-of-motion after WMT, whereas improvements were seen for the WMFT-tt ($p=0.02$), FMA ($p=0.004$) and MALQOM ($p<0.001$). Significant changes were seen for shoulder but not elbow excursion, and peak acceleration and deceleration were faster for all movements. There were no correlations between active or passive range-of-motion and kinematics (joint excursion, velocity, acceleration or deceleration), game performance, WMFT timed-tasks or FMA scores. Significant correlations were found only for active range-of-motion and MALQOM but only at the shoulder: flexion ($r2=0.17$, $p=0.04$); extension ($r2=0.37$, $p=0.002$); and abduction ($r2=0.50$, $p<0.001$). The results of these two studies suggest that neither active nor passive range-of-motion are sufficient outcome measures in chronic stroke. Kinematic data provide greater resolution but participants experiences were better reflected in MALQOM scores which suggested significantly improved independence in activities of daily living.

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Poster

228. Stroke Imaging and Diagnostic Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 228.02/I13

Topic: C.21.Stroke Recovery

Support: MOP-106651

Title: A constrained motor connectome can predict function of fine motor tasks of the non-paretic hand after stroke

Authors: *S. PETERS, K. P. WADDEN, J. L. NEVA, L. A. BOYD;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Objective: The nonparetic hand may compensate for reduced motor function of the paretic arm after a unilateral stroke. Animal models of stroke have shown that white matter damage distant from the lesion may impact function. Thus, structural reorganization of both hemispheres after a stroke may contribute to the resulting altered function of both paretic and nonparetic hands. The purpose of this study was to examine whether motor function of the nonparetic hand could be predicted from a constrained motor connectome (CMC). Methods: Participants underwent diffusion imaging scanning at a 3T MRI research centre. A previously published bilateral sensorimotor network mask, created from a functional MRI connectivity analysis that demonstrated a connected motor network in healthy participants during motor performance, was used to extract CMC information (Wadden 2015). This functional motor network was shown to correlate with paretic hand performance post-stroke. To quantify motor performance for this study, the Wolf Motor Function (WMF) and Fugl-Meyer (FM) upper extremity tests were assessed. For the WMF, movement time to complete 15 items with the paretic and nonparetic arms was determined. A task rate was calculated as 60seconds/performance time (s) with a score of '0' given if a participant was unable to perform the task. The WMF was also divided into fine (WMF-f) and gross (WMF-g) motor tasks with separate task rates calculated. Pearson's correlations were computed for the WMF, WMF-f, and WMF-g, with diffusion measures (apparent diffusion coefficient (ADC), fractional anisotrophy (FA)). A regression followed for the non-paretic WMF-f with age, post-stroke duration (PSD), and ADC as predictors. Results: Twenty-six participants (age 66.2±11.8 years, PSD 62.0±57.5 months) were scanned. On average, participants were moderately impaired (FM 48.0±16.8). The nonparetic WMF-f was correlated with ADC ($r=-0.41$, $p=0.04$), and not with FA ($r=0.27$, $p=0.18$). No additional correlations were found. Multiple hierarchical regression analysis revealed that lower ADC within the CMC predicted faster fine motor task rates of the nonparetic hand ($r2=0.36$, $p=0.05$) and accounted for an additional 12% of the variance after age/PSD. Conclusions: A CMC can predict nonparetic fine motor hand performance after stroke. The diffusion metric ADC, characterized motor performance better than FA, consistent with other

work (Boespflug 2014). Application of this connectome to the post-stroke brain, may provide a comprehensive whole-brain biomarker for post-stroke sensorimotor function, and lends support to the theory that both hemispheres participate in reorganization after stroke.

Disclosures: **S. Peters:** None. **K.P. Wadden:** None. **J.L. Neva:** None. **L.A. Boyd:** None.

Poster

228. Stroke Imaging and Diagnostic Studies

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Program#/Poster#: 228.03/I14

Topic: C.21.Stroke Recovery

Support: HSFC Postdoctoral Fellowship

NIH Grant 5R24HD050821-11

Title: Increased interhemispheric coherence during transcallosal inhibition assessment in chronic stroke: a preliminary TMS-EEG investigation

Authors: ***M. R. BORICH**[1], L. A. WHEATON[2], B. LAKHANI[3], S. M. BRODIE[3], L. A. BOYD[3];
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Abstract: Introduction: Reorganization and remodeling of motor network connections contributes to recovery of arm function after stroke. Changes in effective network connectivity in humans after stroke can be studied using concurrent transcranial magnetic stimulation (TMS)-electroencephalography (EEG). The primary objective of this study was to use imaginary coherence (IC) analysis of TMS-evoked EEG responses to directly characterize interhemispheric interactions between the primary motor cortices (M1s) in individuals with stroke. Methods: Ten participants with chronic ischemic stroke in the right (n=5) or left (n=5) hemisphere and four matched controls were tested. Standard TMS procedures were conducted bilaterally. Transcallosal inhibition (TCI) was evaluated by delivering single suprathreshold (150% resting motor threshold) TMS pulses over M1 while performing an ipsilateral grip force contraction (50% maximum). Suprathreshold TMS pulses were also delivered at rest. 64-channel EEG recordings were collected concurrently during TMS assessments. All standard data pre-processing steps were performed in EEGLAB. Epochs were extracted for each participant and concatenated within each group for IC analysis. Post-TMS (0-300ms) IC values between electrodes overlying M1 (C3, C4) bilaterally were calculated within the beta frequency band (15-30Hz) as the primary dependent measure of interhemispheric IC. Secondary analyses subdivided the stroke group based on lesion hemisphere. Level of physical impairment was evaluated using the upper extremity portion of the Fugl-Meyer (FM) Assessment. Results: Individuals with

chronic stroke showed greater TMS-evoked interhemispheric IC compared to controls (p=.017) during TCI assessment. No differences were seen during the rest condition. Greater interhemispheric beta IC during TCI was observed in participants with lesions in the right hemisphere regardless of stimulation site (p=.002). Qualitatively, participants with lesions in the left hemisphere exhibited greater arm impairment (median FM score: 16) compared to individuals with right hemispheric lesions (median FM score: 57). Discussion: Preliminary findings suggest increased interhemispheric interactions between M1s during an active motor state are present in chronic stroke that may be behaviorally relevant to persisting disability.

Disclosures: M.R. Borich: None. **L.A. Wheaton:** None. **B. Lakhani:** None. **S.M. Brodie:** None. **L.A. Boyd:** None.

Poster

228. Stroke Imaging and Diagnostic Studies

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Program#/Poster#: 228.04/I15

Topic: C.21.Stroke Recovery

Support: NIH Grant R01NS080839

Title: A preliminary examination of electrical impedance myography in stroke

Authors: *H. SHIN, X. LI, S. LI, P. ZHOU;
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Abstract: Electrical Impedance Myography (EIM) was used to assess changes in muscle architecture and composition after a stroke. Eight subjects who suffered from chronic hemiparetic impairments (ages 47-70 years) participated in the study. The EIM was measured bilaterally from the biceps brachii muscles using high-frequency, low-intensity alternating current. Three major EIM parameters, resistance, reactance and phase angles, were calculated from the recorded voltage, representing tissue resistivity, membrane permittivity and membrane oscillation properties of the muscle, respectively. Electrical anisotropy of the muscle was also examined as the ratio between transverse and longitudinal impedance parameters. Comparison of muscle impedance variables between the paretic and contralateral side disclosed a pronounced decrease of values in muscle reactance and phase angle on the paretic biceps at 50 kHz frequency. The muscle tissue resistance, however, did not show significant difference between the two sides. Results from muscle anisotropy estimation indicated a lower value of the resistive anisotropy on the paretic biceps compared with the contralateral side. Such observance may reflect muscle structural changes induced by loss of muscle fibers and fat infiltration or quality changes of cell membranes post stroke.

Disclosures: H. Shin: None. **X. Li:** None. **S. Li:** None. **P. Zhou:** None.

Poster

228. Stroke Imaging and Diagnostic Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 228.05/I16

Topic: C.21.Stroke Recovery

Title: Imbalance between frontal and parietal cortex during choice reaction task in patient with spatial neglect

Authors: *Y. TAKAMURA[1,2], S. OHMATSU[1,2], M. IMANISHI[2], M. OSAKA[2], T. TOMINAGA[2], S. MORIOKA[3], N. KAWASHIMA[4];
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Abstract: Introduction Dorsal and ventral attention network is well known as distinct attentional processes such as top-down controlled attentional selection and the detection of unexpected stimuli, respectively. In this study, we attempted to clarify the relationship between two attention systems in patients with unilateral spatial neglect (USN) based on the results of electroencephalographic (EEG) recordings during choice reaction task. Method 21 patients with right hemisphere lesion participated in this study. The patients were divided into two subgroups based on the score of the behavioral inattention test (BIT) : spatial neglect: (USN, n=13), and non- USN right hemisphere damage group (RHD, n=8). Participants seated at the chair in front of eye tracker mounted PC display (Tobii TX60 Tobii inc., Sweden) and asked to perform eye pursuit-based choice reaction task. The task consists of 150 trials of eye pursuit motion toward a one of five randomly flashed circular objects after .5 second of auditory cue those are horizontally located on the display. In order to discuss neural mechanisms underlying behavioral results, EEG (19ch) was recorded during the task. We mainly focused on phase synchronization in the frontal and parietal lobes at before and after auditory cue. Result and discussion Our results clearly demonstrated that most of patients in USN group showed leftward gaze shift before the onset of eye movement. This phenomenon might reflect a compensatory strategy to overcome neglect symptom. While USN group showed strong synchronization in frontal lobe between before and after beep alert, RHD group contrastingly showed strong synchronization in parietal lobe. Interestingly, an extent of imbalance of phase synchronization between frontal-parietal lobes was clearly significantly correlated with the extent of leftward gaze shift in USN group, suggesting that those who could not activate bottom-up attention due to stroke tended to show greater frontal activity. Our result gives one of insight with regard to the compensational strategy if the bottom-up attention was disrupted due to brain damage.

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Poster

228. Stroke Imaging and Diagnostic Studies

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Topic: C.21.Stroke Recovery

Support: Spencer T. Olin Graduate Fellowship

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Title: Mapping brain function at the bedside during acute stroke recovery using High-Density Diffuse Optical Tomography

Authors: ***K. M. BERGONZI**[1,2], A. T. EGGEBRECHT[3], A. K. FISHELL[4], J.-M. LEE[5], J. P. CULVER[3,2,6];
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Abstract: Introduction: Ischemic stroke, the major stroke subtype, typically begins with the occlusion of an artery in the brain. During the first hours after onset, brain injury evolves rapidly. MRI and CT provide clinicians with snapshots of brain structural health, while behavior is quantified via the patient's NIH Stroke Scale (NIHSS). An imaging modality that continuously measures brain function at the bedside could provide more frequent assays to better understand the acute progression of stroke and potentially inform clinical decisions. We have developed a portable high-density DOT system (Fig. 1a) that can measure changes in cortical blood oxygenation at the bedside. Herein, we demonstrate that HD-DOT is sensitive to altered brain function brought about by ischemic stroke as measured in the first 72 hrs since last known normal. Methods: The HD-DOT system contains 48 sources, each with both 750nm and 850nm light for spectroscopy, and 34 detectors. The imaging cap covers sensory, motor and cognitive areas (Fig. 1b). A total of 32 patients have been scanned; 21 were excluded due to poor data quality. Functional data obtained includes up to 50 minutes of resting quietly. Scanning did not

interrupt standard clinical care. Functional connectivity (fcDOT) analysis was performed on the [HbO] data. The fc results were summarized by a within hemisphere similarity metric which, for given seed or group of seeds, calculates the spatial correlation between a subject of interest and a group map of healthy controls. Concurrent with the DOT scan, we acquired the NIH Stroke Scale as a behavioral metric of stroke-induced functional deficit. Results: The presence of an infarct appears to disrupt typical bilateral connectivity patterns (Fig. 1d,f,h). . Across a group of stroke subjects (n=11) all had statistically significant lower within hemisphere similarity scores ($p<0.001$). Conclusions: Functional imaging using High-Density fcDOT can differentiate between a healthy and injured hemisphere and may have the potential to serve as a continuous longitudinal surrogate to behavioral exams.

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Poster

228. Stroke Imaging and Diagnostic Studies

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Topic: C.21.Stroke Recovery

Support: Toyota Motor Corporation grant

Title: EEG phase synchrony of sensorimotor area reflects limb functions after stroke

Authors: *T. KAWANO[1], N. HATTORI[1], Y. UNO[2], K. KITAJO[2], M. HATAKENAKA[1], H. YAGURA[1], H. FUJIMOTO[1], T. YOSIOKA[1], M. NAGASAKO[1], I. MIYAI[1];
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Abstract: Objective: Focal brain lesions due to stroke can cause remote effects on neural networks. We previously reported a significant relationship between interhemispheric phase synchrony index (IHPS) and the Functional Independence Measure (FIM), a standard clinical scale for activity of daily living (ADL). The aim of this study is to investigate the association between the Phase Synchrony index (PSI) of a single electrode pair C3-C4, placed near the sensorimotor area symmetrically, and the motor scores of the Fugl-Meyer Assessment (FMA), a standard clinical scale for motor impairment after stroke. Methods: Forty-three patients with postacute supratentrial ischemic stroke (mean age 66.0 ± 13.7 years, 10 women, average duration was 40.6 ± 16.5 days after onset) admitted for inpatient rehabilitation were enrolled. After 2.5 minute measurement of eye-closed EEG, noisy epochs were eliminated by automatic processing.

The PSI of four frequency bands (alpha (α) (8-12Hz), beta (β)1 (13-18Hz), β2 (19−30Hz), and gamma (γ) (31-45Hz)) were computed, and correlations of the PSIs with the upper- and lower-limb motor scores of the FMA were investigated. In addition, EEG and the FMA scores were measured at discharge as well as on admission with average interval of 96.0±51.1 days in 19 subjects and index for the improvement in the upper-limb FMA scores (FMA index) and the increase in the PSI (PSI ratio) were calculated to evaluate longitudinal changes. For the statistical analysis, Spearman's correlation coefficient ρ was used with Bonferroni correction. Results: The PSI significantly correlated with upper and lower FMA scores in the β2 band ($\rho = 0.43$, $p = 0.0045$ and $\rho = 0.44$, $p = 0.0030$, respectively) and in the γ band ($\rho = 0.45$, $p = 0.0023$ and $\rho = 0.44$, $p = 0.0031$, respectively). No significant correlation was observed with the PSIs of adjacent electrode pairs (F3-F4 and P3-P4). In the longitudinal analysis, both the upper- and lower-limb FMA scores significantly improved at discharge. The upper-limb FMA indices and the PSI ratios significantly correlated only in the γ band ($\rho = 0.60$, $p = 0.0069$) Conclusion: The PSI near the sensorimotor area significantly correlated with the FMA scores representing upper and lower limb motor functions in a frequency-specific manner. In addition, the improvement of upper limb function significantly correlated with the increase of the PSI in the γ band in the relevant area. Thus, C3-C4 PSI can be a useful biomarker for the assessment of motor impairment after ischemic stroke.

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Poster

228. Stroke Imaging and Diagnostic Studies

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Topic: C.21.Stroke Recovery

Support: Clinical and Translational Science Awards program of the National Center for Research Resources, NIH/NCATS Grant 1UL1RR025011 (VP)

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American Heart Association Mid-West Affiliate Postdoctoral Fellowship (VAN)

Foundation of ASNR (VP)

Title: Correspondence of breath-hold and resting-state vascular reactivity measures in stroke patients

Authors: *R. V. RAUT, V. A. NAIR, C. LA, V. PRABHAKARAN;
Univ. of Wisconsin-Madison, Madison, WI

Abstract: Due to its vascular nature, stroke can lead to considerable changes in cerebrovascular reactivity (CVR) that could confound fMRI data. Measures of CVR may be of great utility in stroke patients for evaluating CVR and predicting regions of neurovascular uncoupling, as well as scaling task-fMRI data - which is dependent on intact neurovascular coupling - to increase its validity; however, they have thus far seen little use in the stroke population. Resting-state fluctuation amplitude (RSFA) is uniquely free of task-related confounds and may be best-suited for these purposes, though its correspondence with well-validated CVR measures has only been assessed in healthy populations, and in just a handful of studies (e.g., Kannurpatti & Biswal, 2008). We aimed to confirm voxel-wise agreement of RSFA and breath-hold CVR mapping in both young (N = 23, mean = 22 years) and old (N = 22, mean = 59 years) healthy subjects, and to determine this correspondence in acute stroke patients (N = 22, mean = 59 years). All participants completed a 10-minute resting-state and a 3-minute, 20-second-interval breath-holding scan. High average RSFA to breath-hold correlations ($r > .70$, $p < .01$) were observed for all three groups, with no significant between-group differences. This is the first study to assess the validity of RSFA in a clinical population. Our findings suggest that RSFA, which can be obtained from a short resting-state scan, may be a viable alternative to breath-hold CVR mapping and could be useful for the evaluation of CVR and task-fMRI scaling in stroke patients.

Disclosures: **R.V. Raut:** None. **V.A. Nair:** None. **C. La:** None. **V. Prabhakaran:** None.

Poster

228. Stroke Imaging and Diagnostic Studies

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Topic: C.21.Stroke Recovery

Support: Study supported by Project LS 10-32, NFB Austria

Title: Prolactin alteration after repetitive transcranial magnetic stimulation

Authors: *B. KEPPLINGER[1], B. SEDLNITZKY-SEMLER[2], C. KRONSTEINER[2], H. BARAN[2];
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Abstract: Background: Repetitive transcranial magnetic stimulation (rTMS), a non-invasive brain stimulation technique has been suggested to be effective for the treatment of stroke patients. This study was designed to evaluate clinical outcome, EEG and changes of clinical markers prolactin and creatine-phosphokinase (CK). rTMS was performed using a Magstim Rapid 2 stimulator with a frequency up to 100 Hz. Coil position was determined by Brainsight Navigator®. The treatment was performed with a total number of ten sessions per patient, each with a train of 1000 stimuli. Intensity ranged at 80 % of resting motor threshold. The therapeutic effects were measured by NIHSS, Barthel-index and Ashworth scale. Prolactin is secreted from the pituitary gland in response to eating, mating, estrogen treatment, ovulation and nursing. Prolactin and CK level raise following epileptic seizures therefore, measurement of prolactin and CK are used as indicators for safe application of rTMS. Methods: Stroke patients with an age between 52-70 years (2F/11M) and an age between 71-89 years (4F/17M), 6 months after stroke were involved in the study. EEG and blood sampling were performed before, after the 5th and after the 10th stimulation session. Serum was coded to make the investigation anonymous and the study was carried out according to the ethical regulations of Lower Austria. Prolactin and CK were determined by Cobas 6000, Roche Diagnostics using Electro-chemiluminescence immunoassay (ECLIA) and UV-Spectrophotometric method, respectively. The one-way ANOVA and Student's t test was applied. Results: Stroke patients reported positive response after rTMS application. Prolactin and CK levels in the serum of stroke patients were in normal range, however we could observe a decrease of prolactin levels in female's patients with progressed age, while in males prolactin levels was increased. After rTMS treatment prolactin levels in male stroke patients were affected moderately, while in female stroke patients prolactin levels were changed significantly in the serum. In serum of female stroke patients younger than 70 years, prolactin levels were reduced significantly after 10th rTMS application (55 %, P = 0.0260) comparing with concentration before the 1st rTMS. CK concentration was not changed in stroke patients after rTMS. Conclusion: A significant enhancement of motor performance and a reduction of spasticity could be observed after rTMS. Positive clinical response in stroke patients, particularly in women could be due to prolactin changes in the periphery and probably also in the CNS after rTMS. Revealed data strongly suggest an advantage of rTMS also in patients with Parkinson symptoms.

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Poster

228. Stroke Imaging and Diagnostic Studies

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Topic: C.21.Stroke Recovery

Support: Health Research Council of New Zealand

Stroke Foundation Northern Region Inc.

Julius Brendel Trust

Title: Proportional resolution of upper limb impairment after stroke depends on corticomotor integrity

Authors: *C. M. STINEAR[1], W. D. BYBLOW[1], A. BARBER[1], M. A. PETOE[2], S. J. ACKERLEY[1];
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Abstract: Recovery of motor function reaches a plateau within the first 3-6 months after stroke. Within this time patients also exhibit a resolution of upper limb impairment that is about 70% of the maximum possible, termed the "70% rule". A caveat is that the rule is upheld only for patients without severe initial impairment, but the boundary score has been difficult to identify a priori. Our aim was to determine if classifying patients based on functional and structural integrity of the corticomotor pathway would yield more accurate predictions about who will fit the rule. Using transcranial magnetic stimulation and MRI we tested the hypothesis that patients with a functional corticomotor pathway would fit the 70% rule, whereas those without would not. Resolution of upper limb impairment was examined in 93 patients in two cohorts. The first cohort of 48 patients received 4 weeks of standardised upper limb therapy. The Fugl-Meyer upper extremity assessment, motor evoked potentials and motor threshold in the paretic extensor carpi radialis, and fractional anisotropy asymmetry index between the posterior limbs of the internal capsule were determined. Initial impairment score, the presence of motor evoked potential and fractional anisotropy asymmetry were the only predictors of impairment change, indicating a key role for corticomotor tract function in recovery. For patients with motor evoked potentials at 5 days, initial impairment was the sole predictor of impairment change (impairment change = 0.45 × initial impairment, adjusted r2 = 0.88; 0.64 × initial impairment, adjusted r2 = 0.88; 0.70 × initial impairment, adjusted r2 = 0.95, at 6, 12 and 26 weeks respectively). The 70% rule was upheld for patients with motor evoked potentials irrespective of their initial impairment. The neural mechanisms underlying the rule are not known. The present study indicated that ipsilesional resting motor threshold decreased to a similar extent and time course to the upper limb impairment resolution. Another cohort (45 patients, all with motor evoked potentials at 5 days) was similarly examined in a rehabilitation setting. This cohort had a lower upper limb therapy dose than the previous cohort ($p < 0.001$). By 12 weeks impairment change was 0.68 × initial impairment (adjusted r2 = 0.92) and was insensitive to upper limb therapy dose ($p = 0.55$). These findings indicate that proportional resolution of impairment may occur within the ipsilesional corticomotor pathway, and may be insensitive to conventional upper limb physical therapy.

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Poster

228. Stroke Imaging and Diagnostic Studies

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Topic: C.21.Stroke Recovery

Support: CIHR

Heart and Stroke

Title: Stroke destabilizes the afferent and efferent connectivity of VIP expressing interneurons in the peri-infarct cortex: insights from *in vitro* imaging

Authors: *K. A. GERROW[1], N. LIANG[2], C. BROWN[2];
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Abstract: During stroke recovery, excitatory neurons in the peri-infarct cortex undergo structural re-arrangements, at both the level of dendritic branches and spines. In contrast, very little is known about how stroke affects the morphology of cortical interneurons. This knowledge gap should be addressed because most interneurons release GABA and regulate cortical excitability, which is profoundly disrupted after stroke. Interneurons expressing vasoactive intestinal peptide (VIP) specialize in inhibiting other classes of inhibitory cortical neurons, such as those expressing parvalbumin (PV) and somatostatin (SOM). We utilized longitudinal *in vivo* two photon imaging and Cre-dependent transgenic mouse lines to investigate how stroke alters the growth and stability of dendritic arbors, spines and axons of VIP cortical interneurons. Our data shows that like their pyramidal neuron counterparts, VIP neurons in the peri-infarct cortex lose a significant number of dendritic spines in the first week after stroke. The initial loss of spines was followed by a protracted period of dendritic spine instability. The dendritic arbors of these neurons were stable before stroke, but showed a significant and balanced increase in branch tip extensions and retractions (>5μm in length) for up to 3 weeks after stroke. Stroke also increased the turnover ratio of peri-infarct en passant and terminal axonal boutons in a spatially restricted manner. Thus, afferent and efferent connectivity of VIP interneurons becomes highly unstable after stroke which may permit excessive inhibitory drive (from downstream PV and SOM interneurons) in the recovering cortex. The functional consequences of stroke related changes in VIP interneuron connectivity are currently being explored.

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Poster

228. Stroke Imaging and Diagnostic Studies

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Topic: C.21.Stroke Recovery

Support: NIH Grant HD047520

Title: Dorsal damage - ventral compensation: structural reorganization of left superior temporal and bilateral fusiform gyri induced by perinatal infarct in occipito-parietal cortex

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Abstract: Brain lesion studies provide insights to the functional specificity of the focal region of injury and the compensatory neural mechanisms that may occur in lesion patients. However, little is known about the neurodevelopmental trajectory of such case studies when compared to gender and age matched longitudinal control samples. Here we report a longitudinal case of a female at age 9 and 10 with a perinatal infarct in the left occipito-parietal cortex, leading to aberrant cortical development of dorsal Brodmann area 19. In the absence of any neurological or psychiatric disorders, or learning disabilities, our goal was to identify compensatory mechanisms at the neuroanatomical level. We examined cortical morphometry using surfaced-based measures such as cortical thickness, local volume, and gyrification using FreeSurfer and computed the rate of change between the two time points. We then compared these measures at each vertex to the ones obtained from a longitudinal control group of females (n=19, age at Time 1 *M*= 8.5, *SD*=0.57 and Time 2 *M*= 9.5, *SD*=0.60) to obtain z-scores at each vertex, following previous approach suggested for automatic identification of focal dysplasia (Thesen et al., 2011). A cognitive profile was also compared against the same group of controls utilizing standardized measures of IQ (WASI) math and reading (WIAT-III & WJ-III). As expected, all cortical metrics above captured the localization of the lesion at baseline (z-score<-2). Further decreases in cortical thickness was observed in the left superior temporal gyrus (STG). Increased volume and area were identified in bilateral lingual/fusiform gyrus, right anterior temporal pole and left inferior temporal gyrus (z-score>2). Increased gyrification was found in the left inferior temporal gyrus. Patterns of thickness and volume changes were similar over a one year period, including less cortical thickness and volume decreases in the left superior temporal sulcus, supramarginal gyrus, bilateral dorsomedial prefrontal cortex, and right lingual gyrus (z-score<-2). This was accompanied by a fairly typical behavioral profile with the exception of superior nonverbal intelligence scores on standardized cognitive measures relative to controls, indicating potential compensatory mechanisms. Our study demonstrates the feasibility of automated extraction of cortical lesion, along with the delineation of associated compensatory changes from a longitudinal perspective. Results from the surface-based morphometry analysis suggest that

damage to the dorsal stream leads to structural reorganization within the ventral stream that may be associated with nonverbal visuospatial processing.

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Poster

228. Stroke Imaging and Diagnostic Studies

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Topic: C.21.Stroke Recovery

Support: CIHR Operating Grant

Heart and Stroke Foundation of Alberta, Nunavut, and Northwest Territories grant-in-aid

Title: Lesion location is associated with kinaesthetic impairment post-stroke

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Abstract: Objective: Kinaesthesia, our sense of limb movement, is impaired in over 60% of individuals after stroke. Kinaesthetic impairments correlate with decreased motor recovery and decreased functional independence post-stroke. However, these impairments often are not identified clinically due to a lack of sensitive and reliable measurement techniques. Methods: To evaluate kinaesthesia, we used a mirror matching task of the upper limbs on a KINARM robotic exoskeleton. During this task passive movements of the stroke affected arm were made by the robot and subjects used their unaffected arm to mirror match the movements. We recorded measures of movement direction, speed, amplitude and response time to the passive movement. Subjects also underwent brain MRI or CT imaging within 10 days post-stroke (mean = 2 ± 2 days) to identify the location of lesion. Voxel-based Lesion Symptom Mapping (VLSM) and statistical region of interest (sROI) analyses were used to compare lesion location with kinaesthetic performance. Results: A total of 136 subjects (93 M, 43 F) were assessed within 30 days post-stroke (mean = 9 ± 6 days). Compared to healthy controls, 64.7% of subjects had impairments in movement direction perception, 31.6% in movement speed, 44.1% in movement amplitude, and 41.9% in response time. VLSM and sROI analyses identified discrete lesion locations associated with these different aspects of impaired kinaesthesia. Impaired perception of speed and direction of movement were significantly associated with lesions to primary somatosensory cortex, supramarginal gyrus, and the superior parietal lobule. Impaired perception of speed was also significantly associated with lesions to the middle frontal gyrus. Conversely,

impaired perception of arm path length was significantly associated with lesions to the caudate head, anterior striatum, and internal capsule. Increased response latency was significantly associated with lesions to the insula, subcortical white matter, superior temporal lobe, inferior and middle frontal lobes, and parietal lobe. Performance on all but one kinaesthetic measure was significantly correlated with clinical measures of functional independence and motor ability. Conclusions: These data suggest that different cortical and subcortical areas are required for processing different aspects of kinaesthesia. In addition, we provide further evidence for the importance of intact kinaesthesia in functional independence post-stroke.

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Poster

228. Stroke Imaging and Diagnostic Studies

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Support: Magellan Grant 11530-14-36220

Title: Lesion Symptom Mapping of brain regions involved in action selection and specification during Trail Making

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Abstract: Introduction: Approximately one million Americans live with chronic post-stroke disabilities including limitations performing activities of daily living. Many daily activities, such as driving, involve continuous interactions between perceptual, cognitive and motor systems. Performance of these activities inherently involves neural networks that are broadly distributed across cortical and subcortical brain regions. A recent theoretical framework, the affordance competition hypothesis, proposes that action selection (choosing among alternative actions) is mediated by prefrontal-basal ganglia networks and action specification (planning spatial and temporal aspects of movements) is mediated by parietofrontal networks. Mapping patterns of brain damage to chronic behavioral impairments following stroke may improve our ability to identify and select rehabilitation interventions that minimize long-term disability. Here we use

Lesion Symptom Mapping (LSM) to identify brain regions that mediate performance of the Trail Making Test (TMT), a neuropsychological assessment that is predictive of post-stroke performance on driving tests. Methods: High-resolution anatomical neuroimaging was collected from 48 stroke survivors between 37 and 80 years of age. Subjects used a robotic device (KINARM Endpoint Lab, BKIN Technologies, Kingston, Canada) to perform two TMT tasks with their less affected hand. In the numeric task (TMT-A), subjects connected numbers (1, 2, ⋯ 25) and in the alphanumeric task (TMT-B) they connected numbers and letters (1, A, 2, B, ⋯ 13) in the shortest possible time. LSM was performed using the nii_stat tools in SPM12 (http://www.nitrc.org/projects/niistat), which mapped impairments in TMT-A and TMT-B to lesioned brain regions of the Johns Hopkins University (JHU) atlas. Results: Robotic measures of impairments on TMT-A were correlated with lesions of the precentral gyrus, postcentral gyrus, and middle frontal gyrus. Additional impairments observed in TMT-B were associated with lesions of the middle frontal gyrus and basal ganglia. Conclusions: These findings provide evidence that the parietofrontal and prefrontal-basal ganglia networks mediate action selection and specification during the TMT. They also suggest that neuroimaging may be used to provide prognostic information that can improve stroke outcomes through better optimization of rehabilitation interventions.

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Poster

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CIHR Operating Grant

Heart and Stroke Foundation of Alberta, Nunavut and Northwest Territories

Title: Ischemic versus Hemorrhagic Stroke: Differences in motor recovery rates

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Abstract: Stroke is a leading cause of disability that typically requires lengthy rehabilitation. Emerging evidence indicates that stroke subtypes have differing recovery patterns. Rehabilitation

outcome studies indicate that patients with hemorrhagic stroke show greater initial impairment, but greater improvement at discharge compared to those with ischemic stroke. These findings are based on broad clinical measures such as the Functional Independence Measure or the Rankin Scale. However, these measures do not directly relate to impairments targeted with rehabilitation interventions. This study aims to determine whether motor recovery profiles differ between ischemic and hemorrhagic stroke. Using a robotic exoskeleton, motor function was assessed using a standard 8-target center-out reaching task. Subjects (N = 115; 96 ischemic, 19 hemorrhagic) were assessed at 1, 6, 12, and 26 weeks post-stroke. Motor performance was quantified according to performance on several spatial (e.g. direction error) and temporal (e.g. movement time) parameters. Determination of task and parameter impairment was based on healthy control normative ranges. Statistical Region of Interest analyses were used to compare motor performance with stroke lesion location. Preliminary results suggest that ischemic (IS) and hemorrhagic (HS) strokes demonstrate significantly different motor performance across the course of recovery. At 1-week post-stroke, IS subjects had significantly better motor performance than HS subjects on four parameters of the reaching task (movement time; initial direction error; number of speed peaks; and max speed, unpaired t-tests, $p < 0.05$). At 26-weeks post-stroke, IS subjects performed significantly better on all four motor parameters ($p < 0.05$). This trend persisted when we considered overall task performance across all parameters. At 1-week post-stroke, 24% of IS subjects demonstrated normal performance. In contrast, no subjects in the HS group performed normally at 1-week post-stroke. At 26-weeks post-stroke, 64% of the ischemic sample performed within normal limits, compared to 32% in the hemorrhagic group. Thalamic lesions were present in 63% of those with hemorrhagic stroke who demonstrated poor performance at 26 weeks. Preliminary analyses suggest that ischemic and hemorrhagic strokes may have significantly different motor recovery profiles. Subjects with hemorrhagic stroke were more impaired initially and at 26 weeks post-stroke compared to those with ischemic stroke. Additionally, thalamic hemorrhages may negatively impact motor recovery. These findings may provide prognostic information to guide rehabilitation practitioners.

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Poster

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Support: Hotchkiss Brain Institute

Heart and Stroke Foundation of Alberta

NWT and Nunavut

CIHR

AI-HS

Title: Neuroanatomical correlates of visually guided reaching after stroke

Authors: J. E. TAPPER[1], S. E. FINDLATER[1], J. M. KENZIE[1], M. M. WANG[1], J. A. DESAI[1], A. Y. X. YU[1], T. M. HERTER[3], S. H. SCOTT[4], *S. P. DUKELOW[2];
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Abstract: Upper limb dysfunction occurs in many individuals after acute stroke. Understanding the neurological impairments that result from a given stroke lesion is fundamental to the development of targeted rehabilitation interventions to improve functional outcomes after stroke. This study invokes robotic technology and neuroimaging based voxel-wise lesion statistical analysis to investigate these structure-function relationships. We hypothesized that neuroanatomical correlates exist for performance deficits on visually guided reaching after stroke. We studied 141 subjects after acute stroke. Stroke lesions were identified on MR brain images (T2 FLAIR, DWI/ADC) or CT images at 2-10 days post stroke. Brain images were transformed to MNI stereotaxic coordinates for comparison across individuals. Movement of the stroke-affected arm during a visually guided reaching task was measured with the KINARM exoskeleton within 4 weeks of stroke. Nine behavioral variables were derived from the movement data (posture speed, reaction time, initial direction error, initial distance ratio, speed maxima count, min-max speed difference, movement time, path length ratio, maximum speed) and transformed to age and sex matched z-scores. Principal components were calculated from those z-scores. Voxel-based lesion symptom mapping was used to identify brain regions for which the presence of a lesion correlated with a performance deficit on the 9 behavioral variables and the 9 principal components. Statistical analysis was performed within voxels lesioned in >10% of subjects using t-tests with false discovery rate correction. Brain regions damaged by stroke in >10% of subjects were located primarily within the middle cerebral artery territory (80% of subjects) affecting large regions of the insular, parietal and temporal lobes (96% insula), white matter tracts (81% external/extreme capsule), and basal ganglia (75% putamen). Neuroanatomical correlates of reaction time, initial distance ratio, and speed maxima count were identified primarily within the insula, rolandic operculum and supramarginal gyrus (up to 56%). Neuroanatomical correlates of initial direction error, movement time, and maximum speed were identified within the superior corona radiata (up to 20%). Neuroanatomical correlates of the 1st principal component were found within white matter tracts (superior corona radiata 39%, superior longitudinal fasciculus 19% and external/extreme capsule 17%) and cortical regions (rolandic operculum 33%, supramarginal gyrus 22%, insula 25%). In conclusion, neuroanatomical correlates exist for performance deficits in visually guided reaching after stroke.

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Poster

228. Stroke Imaging and Diagnostic Studies

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Program#/Poster#: 228.17/I28

Topic: C.21.Stroke Recovery

Title: Preservation of parietal area 5 is associated with improved motor recovery and functional connectivity following MCA stroke in non-human primates

Authors: *J. Y. NASHED[1], J. Z. WANG[1], C. HERNANDEZ-CASTILLO[3], J. GALLIVAN[1], J. FERNANDEZ-RUIZ[4], D. J. COOK[1,2];
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Abstract: Stroke is a leading cause of death and disability worldwide. There is high variability in degree and rate of recovery following stroke. No single determinant of stroke recovery has been identified; however, stroke volume, anatomical location and patient demographics and comorbidities are likely contributors. We hypothesize that preservation of cortical areas that support functional remapping after stroke may enhance recovery. Parietal Area 5 (PA5), an integrator of sensorimotor information related to reaching and grasp, is at the edge of the ischemic penumbra and inconsistently infarcted following MCA occlusion (MCAO) in the cynomolgus macaque. We hypothesize that preservation of PA5 following MCAO is associated with improved post-stroke recovery and increased connectivity following MCAO. To test this hypothesis 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 in premotor (PM), primary sensory cortex (S1), primary motor cortex (M1), all Parietal regions including Parietal Area 5 (PA5) and White Matter were quantified. 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 and 30 days (both P>0.05). PA5 stroke volume was equivalent at 48h, but significantly decreased at 30 days (4.1mL vs.5.2mL, P=0.02) in the good versus bad recovery

group. Functional connectivity maps generated 30 days post-stroke confirmed stronger connections between contra- and ipsilateral M1, S1, and PA5 nodes in animals with good recovery. These data suggest that PA5 plays a key role in motor recovery following MCA stroke. Future mechanistic research focused on PA5-dependent motor recovery may lead to novel neurorestorative and/or neuromodulatory therapies.

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Poster

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Support: CIHR Research Operating Grant

Heart and Stroke Foundation of Alberta, Nunavut and Northwest Territories

AIHS Postdoctoral Felloship

Title: Quantifying motor recovery of the ipsilesional arm after stroke

Authors: ***J. A. SEMRAU**[1], 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: After stroke, functional recovery is thought to be highly dependent on the motor abilities of the stroke-affected arm. However, recent studies have suggested that there may be prolonged ipsilesional motor deficits related to stroke that commonly go unrecognized and untreated. Recent work in our lab has used robotics to quantify sensorimotor behaviour after stroke. Here we aimed to determine the frequency and recovery patterns of ipsilesional motor deficits over the first 6 months post-stroke. One hundred sixteen subjects with first time, unilateral stroke performed a visually guided reaching task (N = 64 Left-affected (LA), N = 52 Right-affected (RA)) with both the affected and unaffected arms at four timepoints post-stroke (1, 6, 12 and 26 weeks). For the task, subjects sat in the robotic exoskeleton with their arms supported against gravity. Subjects viewed their fingertip as a one cm diameter white cursor, and were instructed to hold at a center target and move to a peripheral target as quickly and accurately as possible when it appeared. Subjects made 10 reaches to each of 8 targets (80 movements total) in pseudorandom order. We evaluated spatial and temporal aspects of movement (9 parameters total) and made comparisons to a healthy control reference range. Based on control performance, overall task failure was considered to be impaired performance

on 3 or more parameters. At one week post-stroke, we found that 34% of subjects (N = 40 total, N = 27 (LA), N = 13 (RA)) had significantly impaired motor function in the ipsilesional arm and 80% (N = 93 total, N = 51 (LA), N = 42 (RA)) had significantly impaired motor function in the contralesional arm. Fifty-three percent of subjects with ipsilesional deficits still had significantly impaired motor function of the contralesional arm at 26 weeks post-stroke. In comparison by 6 weeks post-stroke, the number of subjects with ipsilesional deficits dropped to 15% (N = 17). By 26 weeks post-stroke, the number of subjects with ipsilesional deficits was only 3% (N = 3). Our results suggest that while damage to either hemisphere may result in ipsilesional motor deficits, recovery of the ipsilesional arm occurs at a faster rate than that of the contralateral arm.

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Poster

228. Stroke Imaging and Diagnostic Studies

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Support: ASPIRE Grant 11530-13-33191

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Title: Visuomotor learning is attenuated by impairments of visual search following stroke

Authors: *T. M. HERTER[1], K. FUSS[1], T. SINGH[1], C. PERRY[1], K. GOINS[1], B. MAREBWA[1,3], J. FRIDRIKSSON[2], S. FRITZ[1];
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Abstract: Visual search is used to gather visual information by actively scanning the visual environment with eye movements and peripheral vision. Visual search improves with practice and efficient visual search is linked with expertise in motor skills, suggesting that improvements in visual search may contribute to visuomotor learning. Impairments of visual search are seen in many stroke survivors, including many without spatial neglect. We predict that stroke survivors with impairments of visual search may experience poor motor outcomes. Here we examine the extent to which post-stroke impairments of visual search attenuate visuomotor learning.
Methods: Eight stroke survivors (38-81 years old) practiced a bimanual visuomotor task (Object

Hit and Avoid Task) using an upper-limb robotic device (KINARM Endpoint Lab, BKIN Technologies, Kingston, Canada). In this task, objects (eight distinct geometric shapes) moved towards the subjects who used virtual paddles attached to each hand to hit away two objects (Targets; n = 200), and avoid hitting the other six objects (Distractors; n = 100). Subjects completed six repetitions of the task once a week for six weeks. Object shape, location, and speed were randomly varied to ensure that every task repetition was distinct. Eye and hand movements were recorded to investigate the influence of visual search on task performance. Results: Four subjects (Selective Hitters) demonstrated improvements in task performance, reflecting increases in targets hit and distractors avoided. The other four subjects (Nonselective Hitters) showed increases in targets hit but decreases in distractors avoided, resulting in negligible improvements in task performance. Differences between Selective and Nonselective Hitters were linked to differential changes in several measures of visual search and eye-hand coordination. Measures of limb-motor control did not explain differences between Selective and Nonselective Hitters. Conclusions: These results provide evidence that impairments of visual search may contribute to poor motor outcomes following stroke. Interventions designed to improve visual search may enhance motor outcomes following stroke.

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Poster

228. Stroke Imaging and Diagnostic Studies

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Support: NS062097

R01 NS058710

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Title: Cell survival and regeneration in the ischemic core following focal cerebral ischemia in mice

Authors: *M. Q. JIANG[1], W. CAO[2], Z. Z. WEI[2], X. GU[2], L. WEI[2], S. YU[2], S. YU[2]; [1]Neurosci., [2]Anesthesiol., Emory Univ., Atlanta, GA

Abstract: Cerebral ischemia typically induces an ischemic core in the supplying territory of the occluded artery. The conventional definition of the ischemic core is that of “a volume of tissue with which all cells (neuronal and glial), blood vessels (arteries, veins and capillaries) and nerve fibers (myelinated and non-myelinated) have undergone necrosis” (Nedergaard M, 1988). So far,

research efforts have been heavily concentrated on the peri-infarct region due to the current consent that cells in the penumbra are savable. On the other hand, the fate and mechanisms of neuronal and vascular cells inside the core are largely unknown except the common belief that cells inside the core all die due to severe ischemia. In the present investigation, we tested the hypothesis that, inside the ischemic core, some neuronal and vascular cells could survive the initial ischemic insult and regenerative niches could exist many days after stroke. Adult male mice were subjected to focal cerebral ischemia. The ischemic insult uniformly reduced the local cerebral blood flow (LCBF) by about 90%. Massive cell death and a significant infarction were cultivated in the ischemic cortex 1-3 days later. Nevertheless, significant levels of trophic/growth factors BDNF and VEGF remained in the core tissue, some NeuN-positive and Glut-1/College IV-positive cells still resided inside the core 7-14 days post stroke. Using electron microscopy 7 days after stroke, we observed some surviving cells with neuronal features including a large nucleus with an intact plasma membrane and numerous cellular organelles in the cytoplasm. Some neuronal cells still maintained synaptic contacts between cells, composed of intact synapses with pre- and post-synaptic features such as presynaptic vesicles and a postsynaptic density (PSD). Myelinated axons were also identified in the core region. Many vessel like structures composed endothelial cells and astrocytes/pericytes that resembled the neurovascular unit existed in the core. *In vivo* and cultured cells from the ischemic core tissue also revealed regenerative activities several days after stroke. These data suggest that the ischemic core remains an actively regulated brain region with residual but viable neuronal and vascular cells acutely and chronically after stroke. The work advocates that the ischemic core should be an important target for neuroprotective as well as regenerative stroke therapies.

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Poster

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Topic: C.21.Stroke Recovery

Support: CIHR Grant MOP-106651

Title: Microstructural properties of a constrained motor connectome in individuals with stroke predicts motor learning

Authors: *K. P. WADDEN[1], S. PETERS[2], L. BOYD[2];
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Abstract: INTRODUCTION Diffusion tensor imaging (DTI) is a reliable method to quantify white matter microstructural properties in the chronic stage following stroke (Mang et al. 2014). The aim of this study was to utilize a multimodal neuroimaging approach to study the underlying white matter properties of a previously identified functionally connected motor network (Wadden et al. 2015). This approach generated data characterizing a constrained motor connectome of white matter structures that was then used to predict change in motor performance when skilled motor practice was paired with *continuous theta burst stimulation* (cTBS) stimulation over the contralesional sensorimotor. METHODS Thirty-six individuals in the chronic phase of stroke were recruited. Participants were randomized to one of two stimulation groups: contralesional sensorimotor (SM1c; 25), or sham (11). DTI was collected on individuals with stroke as well as seventeen healthy controls. Over a 5-day intervention, motor practice of a serial tracking task (STT) followed contralesional cTBS. A movement time change score was calculated to quantify improvements in the STT. A bilateral sensorimotor network mask was created from a prior fMRI connectivity analysis (Wadden et al. 2015) that demonstrated a connected motor network during motor learning. The clusters within the network were used as seed regions to create a constrained motor connectome. Fractional anisotropy (FA) was extracted and was used as a measure of white matter properties. RESULTS Constrained motor connectome FA was significantly reduced in the stroke compared to the healthy group ($F = 24.95$, $p < 0.001$). In the SM1c group, in a linear regression model, motor network FA significantly accounted for 32.4% of the variance in change in motor performance ($p = 0.011$), however, this relationship did not exist in the sham group ($p > 0.05$). CONCLUSION Combining fMRI and DTI may provide a neurophysiological method, known as a constrained connectome, to determine the microstructural properties of an isolated network of regions to predict motor performance following stroke. Similar to the blood oxygenated response (Wadden et al. 2015), the diffusivity of the water molecules along the fiber tracts within this motor network was significantly different between healthy individuals and individuals after stroke. Interestingly, our results demonstrate that dysfunction in the underlying constrained motor connectome following stroke is an important variable in predicting response to cTBS stimulation over the contralesional sensorimotor cortex. Wadden et al. 2015 Behav Brain Res; Mang et al. 2014 Clinph

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Poster

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Support: The Integrative Aging Research Center of Gwangju Institute of Science and Technology

Title: Metabolic pattern in capsular infarct model: ssm/pca and fdg-micropet based approach

Authors: *D. KIM[1], H.-I. KIM[2], S. JUN[1];
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Abstract: Objective: The voxel-wise stat which has been used widely has a limitation in analyzing static PET data due to a large inner-group and small inter-group variance of PET data. As alternative of this problem, scaled subprofile method with principal component analysis (SSM/PCA) which is a multivariate method to classify two groups and obtains representative pattern of group could be an answer. The method has been successfully applied in neurodegenerative disease (Parkinson's and Alzheimer's disease). The objective of this study is to verify SSM/PCA in stroke study. For this, we performed microFDG-PET scanning of capsular infarct model then investigated the effect of stroke as natural recovery using SSM/PCA method. Methods: The forty sprary-dawley rats were used for this experiment then divided into four groups determining whether performing the surgery destructing internal capsule, behavioral task, or returning their motor skill: moderately recovered group (MRG, n=10), poorly recovered group (PRG, n=10), sham-operated group (SOG, n=10). All rats received microFDG-PET scanning before surgery (baseline scan), after 7, 14, and 21 days. The static PET scanning was performed during 25 minutes. After reconstruction, each PET data were normalized and co-registrated on histological template. Based on this, we applied SSM/PCA method Results: The obtained pattern of each group (the pattern of 7, 14, and 21 days compared with baseline for each group) well separated the baseline group ($p < 4.7x10-3$). In analysis of MRG data with time, the pattern showed deactivation of ipsi-lesional motor cortex, sensory cortex, and thalamus which is comparable with the previous study using voxel-wise stat (Figure 1). However, the activation of cingulate cortex, retrosplenial cortex was not seen in the previous result. The other groups showed similar result with voxel-wise analysis. However, there were certain differences. Conclusion: We showed SSM/PCA method could be used in micro FDG-PET analysis of capsular infarct model and it is suitable for obtaining a representative pattern of specific group.

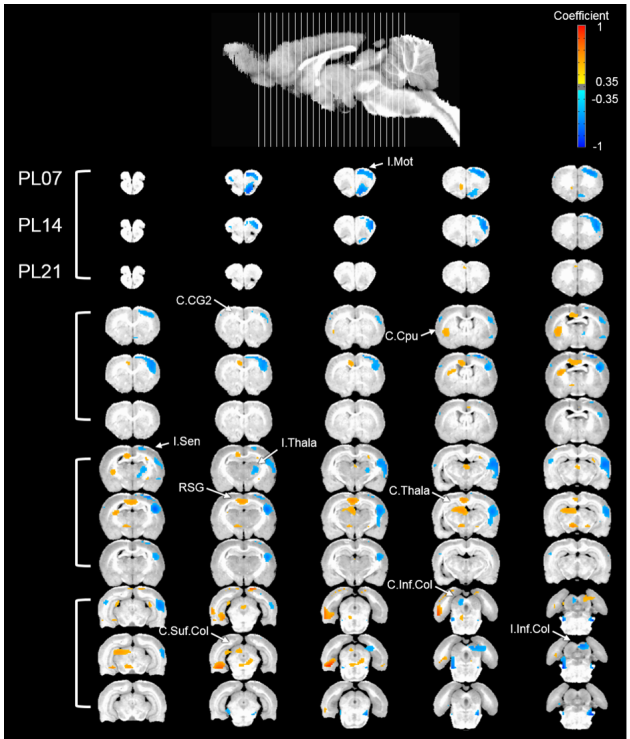


Figure 1. time-dependent metabolic pattern of MRG were depicted on the histological template. On the top, histological template with slice line. According to the line coronal view was shown. Positive value represents increase activity in post stroke and negative value represents decrease activity in post stroke. MRG, moderately recovered group; Mot, motor cortex; CG, cingulate gyrus; Cpu, caudate putamen; Sen, sensory cortex; Thala, thalamus; RSG, retrosplenial granular cortex; Inf.Col, inferior colliculus; Suf.Col, superior colliculus; PL, postlesion.

Disclosures: **D. Kim:** None. **H. Kim:** None. **S. Jun:** None.

Poster

228. Stroke Imaging and Diagnostic Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 228.23/I34

Topic: C.21.Stroke Recovery

Title: Differential sympto-diagnostic features of ischemic stroke, and tools for the measurement of tPA outcome

Authors: M. J. CHAVES[1], E. S. FULMER[2], T. J. COCHRAN[1], D. BLACKHURST[3], S. STERNBERG[3], R. LEACOCK[3], *T. I. NATHANIEL[1];
[1]Biomed. Sci., Univ. of South Carolina Sch. of Med. At Greenville, Greenville, SC; [2]Univ. of South Carolina Sch. of Med., Greenville, SC; [3]Greenville Hlth. Syst., Greenville, SC

Abstract: This study focuses on the differential symptoms, clinical course, and outcomes of ischemic stroke following treatments with tPA. Data were obtained from Greenville Health

System's stroke registry on patients presenting with ischemic stroke between January 1st, 2010 and June 30th, 2013. Patients with initial NIH stroke scale > 25 or who were ineligible for tPA were excluded. Patients were grouped according to the presenting symptoms including weakness, aphasia, and/or altered level of consciousness. Three levels of recovery were assessed for 505 patients following treatment with tPA. Patient's original ambulatory status was "completely recovered" (patient returned to his/her pre-stroke baseline), patient's original ambulatory status was "partially recovered" (but patient did not return to baseline), patient's original ambulatory status "was not recovered". 167 (33%) were administered tPA, while 338 (67%) did not receive tPA. Most patients with altered level of consciousness (with or without weakness) did not show improvement when administered with tPA as compared to the untreated patients. However, patients with weakness (with or without aphasia or with all 3 symptoms combined) showed improvement when compared to the control group. The current study indicates that there are differential symptoms and clinical features that, in addition to the initial examination, may help in understanding cortical ischemic stroke. These clinical features may be important for both diagnostic and therapeutic approaches to acute stroke.

Disclosures: M.J. Chaves: None. **E.S. Fulmer:** None. **T.J. Cochran:** None. **D. Blackhurst:** None. **S. Sternberg:** None. **R. Leacock:** None. **T.I. Nathaniel:** None.

Poster

228. Stroke Imaging and Diagnostic Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 228.24/I35

Topic: C.21.Stroke Recovery

Support: NIH Grant 1K01HD69504-01

RPC 2011-1006

Title: Employing patient's individual characteristics to derive personalized brain stimulation therapies

Authors: *V. SANKARASUBRAMANIAN[1], N. VARNERIN[1], D. CUNNINGHAM[1], K. POTTER-BAKER[1], K. SAKAIE[1], A. CONFORTO[2], A. MACHADO[1], E. PLOW[1]; [1]Cleveland Clin., Cleveland, OH; [2]Sao Paulo Univ., Sao Paulo, Brazil

Abstract: Background: Non-invasive brain stimulation is one of the most well studied advances in stroke rehabilitation. The classical approach focuses on facilitating adaptive plasticity of the damaged hemisphere, particularly the primary motor cortex (M1). However, the promise of stimulation becomes inconsistent when studies include patients with serious damage and disability. In such cases, pathways from M1 are damaged frequently. And, instead, plasticity of alternate substrates in both damaged and intact hemispheres can contribute to recovery. But, how

does one identify which substrate to stimulate to precisely affect recovery? **Objective:** Since individual substrates offering plasticity vary with damage and disability, we premise outcomes of stimulating these substrates will similarly vary across the range of damage and disability. Comparing their variances will help derive patient-specific substrates for most consistent brain stimulation therapies. **Methods:** In a repeated measures crossover design, patients with chronic stroke ranging from mild to severe upper limb impairment received stimulation to facilitate damaged M1 and other damaged and intact substrates, besides sham. Patient characteristics including baseline disability and damage were quantified using scores of impairment and corticospinal integrity. Outcomes were indexed as change in functional reaching ability, and individual expressions of plasticity measured using transcranial magnetic stimulation. **Results:** Stimulation rather than sham modulated plasticity and outcomes of paretic upper limb. As predicted, outcomes and plasticity of stimulating different substrates varied differently with baseline impairment and damage. While outcomes of stimulating damaged M1 were robust in patients with mild impairment ($r=0.64$), the more seriously impaired best responded to stimulation of intact motor cortices ($r=-0.812$) with cut-offs at mild-to-moderate damage and disability. **Significance:** Our findings are consistent with the hypothesis that outcomes of stimulating different substrates vary differently with individual characteristics. The diametrically opposite relationships witnessed between outcomes of stimulating M1 and other substrates could help identify intersection or cut-offs along the range of damage and disability. Cut-offs can stratify patients in which range will benefit from stimulating damaged M1 vs. other substrates for precisely maximizing rehabilitative recovery.

Disclosures: V. Sankarasubramanian: None. **N. Varnerin:** None. **D. Cunningham:** None. **K. Potter-Baker:** None. **K. Sakaie:** None. **A. Conforto:** None. **A. Machado:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ATI, Cardionomics and Enspire. F. Consulting Fees (e.g., advisory boards); Functional Neuromodulation and Spinal Modulation. **E. Plow:** None.

Poster

228. Stroke Imaging and Diagnostic Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 228.25/I36

Topic: C.21.Stroke Recovery

Support: VA CSR&D Merit Award CX000586-01

Title: Assessment of post-stroke white matter change with HARDI track density imaging

Authors: *A. U. TURKEN[1], N. F. DRONKERS[1,2];
[1]Res. Service, Veterans Affairs Northern California Hlth. Care S, Martinez, CA; [2]Neurol. Dept, UC Davis Med. Sch., University of California, Davis, CA

Abstract: Accurate characterization of white matter integrity is critical for the diagnosis and treatment neurological patients who have suffered stroke. Here, we employed high angular resolution diffusion imaging (HARDI) and streamline tractography to visualize brain white matter pathways in chronic stroke, and track density imaging (TDI) to quantify their structural integrity. The study group included 38 stroke patients with unilateral injury (left or right hemisphere) in middle cerebral artery (MCA) territory. In order to ensure that the acute effects of infarction (edema and mass effects) had stabilized, MRI scanning was performed in the chronic stage (> 6 months). The study group also included 40 healthy controls. High-resolution anatomical images (T1w, T2w, FLAIR) and high angular resolution diffusion imaging data (b = 2000 s/mm2, 64 directions) were acquired on a 3T Siemens Verio scanner. The patient participants also underwent comprehensive neuropsychological assessments to characterize their cognitive abilities, and to correlate stroke-related behavioral deficits with white matter integrity as assessed with TDI. To ensure accurate anatomical registration, the anatomical scans were transformed to MNI152 space with a modified version of SPM8's unified segmentation normalization technique (Crinion et al., 2007) and an age-specific healthy brain template. Diffusion MRI data were pre-processed with FSL's EDDY function for movement and distortion correction. MRTrix was used for HARDI processing (Tournier et al., 2012). Fiber orientation distribution (FOD) functions were estimated at each voxel with constrained spherical deconvolution (CSD). Probabilistic streamline tractography, (iFOD2 algorithm), was used to generate 10 million streamlines (10-200 mm long) in each brain. TDI maps were generated for each dataset with MRTrix, quantifying fiber track density and mean fiber track length at each voxel (Calamante et al., 2010; Pannek et al., 2011). A trained expert manually delineated brain lesions on each stroke patient's structural scans for comparison with the HARDI TDI analysis of stroke-related white matter change. We observed that TDI can detect subtle changes in white matter integrity even in brain regions outside the lesion sites, and that TDI can also provide information on surviving white matter within lesioned areas. Finally, we found that damage to white matter regions that are characterized by high fiber track density in the healthy control group, such as the extreme/external capsule and postero-lateral temporal lobe white matter (Turken & Dronkers, 2011) predicted severe behavioral deficits in the patient group.

Disclosures: A.U. Turken: None. **N.F. Dronkers:** None.

Poster

228. Stroke Imaging and Diagnostic Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

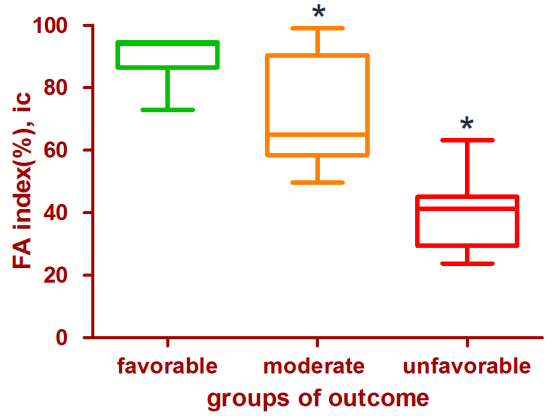
Program#/Poster#: 228.26/I37

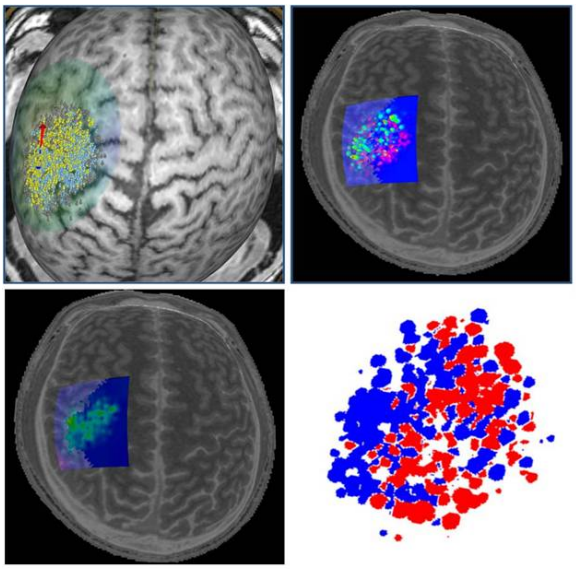
Topic: C.21.Stroke Recovery

Title: Multi-muscle navigated TMS mapping combined with corticospinal tract assessment in chronic ischemic stroke patients

Authors: *M. NAZAROVA[1], M. A. PIRADOV[2], P. NOVIKOV[3], D. POZDEEVA[1], E. BLAGOVECHTCHENSKI[1,4], V. NIKULIN[1,5];
[1]Natl. Res. Univ. - Higher Sch. of Ec, Moscow, Russian Federation; [2]Res. Ctr. of Neurol., Moscow, Russian Federation; [3]Bauman Moscow State Tech. Univ., Moscow, Russian Federation; [4]Lab. of translational neuroscience and molecular pharmacology, Saint-Petersburg State University,, Saint-Petersburg, Russian Federation; [5]Dept. of Neurology, Campus Benjamin Franklin, Charité Univ. Med., Berlin, Germany

Abstract: Hand motor recovery prognosis in stroke patients is crucial to develop a realistic individual rehabilitation program. The aim of the work was to compare predictive role of corticospinal tract integrity based on diffusion tensor imaging and functional state of both affected and unaffected (UH) motor cortex based on navigated TMS (nTMS) assessment including nTMS mapping considering relationship between extrinsic and intrinsic hand muscle`s cortical representations. Total of 30 patients with the only chronic supratentorial ischemic stroke and various severity of hand paresis were enrolled (12 females,50,0±8,0 y.o.). Ten healthy volunteers (36,6±15,2 y.o.) underwent nTMS study. A software for multi-muscle nTMS mapping for automatic calculation of areas, volumes, overlaps and centers of gravities was developed. A significant difference between group of unfavorable outcome versus other groups based on fractional anisotropy (FA) in internal capsule and cerebral peduncle let to consider it FA an easy available clinical measurement for hand motor prognosis. Significantly higher disinhibition in the UH in well recovered and normal SICI in poorly recovered patients was demonstrated. For ICF in UH no significant difference between groups of motor outcome was found, at the same significant negative correlation between ICF and percentage of muscles cortical maps overlap was shown.





Disclosures: **M. Nazarova:** A. Employment/Salary (full or part-time):; Centre for Cognition and Decision Making, National Research University Higher School of Economics, Russian Federation, Research Centre of Neurology, Moscow, Russian Federation. **M.A. Piradov:** None. **P. Novikov:** None. **D. Pozdeeva:** None. **E. Blagovechtchenski:** None. **V. Nikulin:** A. Employment/Salary (full or part-time):; Department of Neurology, Campus Benjamin Franklin, Charité University Medicine Berlin, Germany.

Poster

228. Stroke Imaging and Diagnostic Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 228.27/I38

Topic: C.21.Stroke Recovery

Support: NIH T32-HD07418 (Perreault)

Title: Tracking changes in passive muscle stiffness after acute hemispheric stroke

Authors: ***A. LAI**[1,2], X. HU[2], N. SURESH[2], W. RYMER[2];
[2]Physiol., [1]Northwestern Univ., Chicago, IL

Abstract: Following stroke, skeletal muscles often resist lengthening. Such abnormal resistance to lengthening may be explained in part by an increase in passive muscle stiffness. However, the cellular and molecular mechanisms responsible for increased muscle stiffness are unclear. This is partially due to lack of data in the acute phase of stroke; without such knowledge, it is not possible to determine if proposed mechanisms of impairment follow a time course that is

consistent with scientific observations. Thus, the objective of this study was to track the changes of muscle stiffness in the acute stage of recovery. For three stroke survivors, we estimated muscle stiffness by measuring the velocity of shear wave propagation. To evaluate the relative changes in the two sides (paretic - contralateral), we calculated the difference in shear wave velocity (SWV) on each side in each of the 6 locations, and expressed the change as a percent of the contralateral SWV. Preliminary results suggest that over a period of 2-3 months, the shear wave velocity on the paretic side increases relative to the contralateral side, culminating in large differences in the chronic stage. This may be indicative of slow changes in the properties intrinsic to the muscle, such as the type and spatial distribution of collagen content, the amount of fat infiltration within fibers, the number of serial sarcomeres, and potentially sarcomere resting length.

Disclosures: **A. Lai:** None. **X. Hu:** None. **N. Suresh:** None. **W. Rymer:** None.

Poster

228. Stroke Imaging and Diagnostic Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 228.28/I39

Topic: C.21.Stroke Recovery

Support: NIH Grant HD065438

Title: Brain sensorimotor structural network difference between two hemispheres in chronic stroke

Authors: ***B. KIM**[1], Y. OH[1,2], R. M. LEAHY[3,4], J. P. HALDAR[3,4], N. SCHWEIGHOFER[1,2], C. J. WINSTEIN[1];
[1]Div. of Biokinesiology and Physical Therapy, [2]Neurosci. Grad. Program, [3]Ming Hsieh Dept. of Electrical Engin., [4]Brain and Creativity Inst., USC, Los Angeles, CA

Abstract: Brain network among sensorimotor areas in both hemispheres is affected by hemispheric stroke. Brain structural network analysis was introduced to identify adaptive changes across the brain network after cerebral stroke, and to understand the relationship between changes in the network and motor behavior. This study aims to determine whether brain sensorimotor structural network analysis can be used to investigate the relationship between brain sensorimotor networks and motor behavior. This study is part of a longitudinal Phase-I clinical trial of rehabilitation in chronic stroke (ClinicalTrials.gov ID: NCT 01749358). Individuals with mild to moderate motor impairment after stroke participated (N=24, average chronicity= 3.04 years). Structural brain images (T1-weighted MRI and DTI) were acquired. Imaging data were processed using BrainSuite14a (http://brainsuite.org/). A total of twenty four cortical or subcortical sensorimotor areas (Twelve areas in each hemisphere) were chosen to

construct a structural connectivity network. Fractional anisotropy (FA) of the pathway (tractography between each pair of regions of interest [ROIs]) was calculated and a 24 X 24 FA matrix generated. We applied a threshold, optimized for each patient, to produce an undirected weighted graph and a binary adjacency matrix. Communicability between each pair of ROIs was calculated. Wolf Motor Function Test (WMFT) and Motor Activity Log (MAL) were performed to assess motor performance and the amount of the paretic arm use, respectively. For each ROI, the mean communicability was calculated, and a Wilcoxon signed-rank test was performed to compare the mean communicability between the homologous ROIs in the two hemispheres. Finally, Pearson correlation analysis was used to determine if there is a relationship between the brain sensorimotor network (communicability asymmetric index of each ROI and motor behavior (WMFT, MAL). The significance level was 0.05, and Bonferroni correction was applied for the Pearson correlation (alpha = 0.05/24 = 0.002). There was significantly lower communicability in ipsilesional superior parietal gyrus, caudate nucleus, putamen, and globus pallidus compared to communicability metrics of these same ROIs in contralesional hemisphere. However, any communicability metric was not significantly correlated with WMFT Time score or MAL score. These results show the potential use of sensorimotor tracts FA metrics to capture the brain sensorimotor structural network difference between two hemispheres in chronic stroke, although the asymmetry in communicability between hemispheres was not associated with the level of motor deficits.

Disclosures: B. Kim: None. **Y. Oh:** None. **R.M. Leahy:** None. **J.P. Haldar:** None. **N. Schweighofer:** None. **C.J. Winstein:** None.

Poster

228. Stroke Imaging and Diagnostic Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 228.29/I40

Topic: C.21.Stroke Recovery

Support: NIH NINDS R21 NS082894

Title: Optogenetic stimulation of cerebellar dentate nucleus reduces pCREB expression in the contralesional motor cortex after stroke

Authors: *M. Y. CHENG, S. ISHIZAKA, A. M. SHAH, E. H. WANG, A. R. BAUTISTA, G. SUN, G. K. STEINBERG;
Neurosurg., Stanford Univ., Stanford, CA

Abstract: Functional recovery after stroke has been observed in both human and animal studies. Post-stroke brain stimulations are promising neurorestorative techniques as they allow direct manipulation of the target area's excitability. Recently we have demonstrated that optogenetic

neuronal stimulation of the contralesional cerebellar dentate nucleus (cLCN) post-stroke promotes persistent recovery. In this study we investigate the effects of repeated cLCN stimulation on brain activation patterns during stroke recovery. In particular we examined phosphorylation of CREB (pCREB) as an indicator of neuronal activation and excitability. We hypothesize that stimulation of cLCN will cause a widespread change of excitability, as it sends excitatory outputs to multiple motor, premotor and sensory areas. Thy-1-ChR2-YFP line-18 transgenic male mice were used. Mice underwent stereotaxic surgery to implant a fiber cannula in cLCN, followed by an intraluminal middle cerebral artery suture occlusion. Three groups of mice were used: control non-stimulated stroke mice, short stim stimulated stroke mice (day5-14 post-stroke) and long stim stimulated stroke mice (day5-28 post-stroke). Sensorimotor behavior tests (rotating beam tests) were used to assess their recovery at day 0, 4, 7, 10, 14, 21 and 28 post-stroke. Animals were sacrificed at day29 and brains were processed for immunohistochemistry with antibodies targeting pCREB and CREB. Our data showed that cLCN stimulated stroke mice recovered quickly, with significant improvement in distance traveled as early as day7 ($p<0.05$), and faster speed at day14 post-stroke ($p<0.001$). The effect of stimulation was persistent, as stimulated stroke mice continued to recover after day14 without further stimulations and maintained similar functional performance at day21. Analysis of pCREB activation showed that cLCN stimulation activates the dentatothalamocortical pathway. Interestingly, cLCN stimulated stroke mice exhibited reduced pCREB expression in the contralesional hemisphere, most prominently in motor and sensory cortices. Our data suggest that cLCN stimulations post-stroke can promote persistent functional recovery, and stimulation leads to a reduced pCREB activation. Current studies examine the mechanisms of cLCN-induced recovery, including the contribution of pCREB and its signaling pathways.

Disclosures: **M.Y. Cheng:** None. **S. Ishizaka:** None. **A.M. Shah:** None. **E.H. Wang:** None. **A.R. Bautista:** None. **G. Sun:** None. **G.K. Steinberg:** None.

Poster

228. Stroke Imaging and Diagnostic Studies

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 228.30/I41

Topic: C.21.Stroke Recovery

Support: NIH grants AT007429, NS046400

Title: Deletion of the hemopexin or heme oxygenase-2 gene aggravates brain injury following stroma-free hemoglobin-induced intracerebral hemorrhage

Authors: ***B. MA**[1,2], J. DAY[1,2], E. TOLOSANO[4], S. DORE[1,2,3];
[1]Dept. of Anesthesiol., [2]Ctr. for Translational Res. in Neurodegenerative Dis., [3]Departments of

Neurology, Psychiatry, Pharmaceutics, and Neurosci., Univ. of Florida, Col. of Med., Gainesville, FL; [4]Departments of Mol. Biotech. and Hlth. Sci., Univ. of Torino, Torino, Italy

Abstract: The breakdown of hemoglobin released after intracerebral hemorrhage (ICH) causes brain injury. Hemopexin (HPX) has the highest binding affinity to heme, and heme oxygenase 2 (HO2) is the rate-limiting enzyme for the degradation of heme. Microglia are resident macrophages in the brain; however, the significance and role of HO2 and HPX on microglial clearance of hemoglobin after ICH still remain understudied. Intracerebral injection of stroma-free hemoglobin (SFHb) was used to induce ICH. HPX or HO2 knockout mice were injected with 10μL of SFHb in the striatum. After injection, behavioral/functional tests were performed along with anatomical analysis. Iron deposition and neuronal degeneration were shown by Perl's staining and Fluoro-Jade B staining, respectively. Immunohistochemistry with anti-Iba1 was used to demonstrate activation of microglial cells around the injured site. The study results showed that deleting HPX or HO2 aggravated SFHb-induced brain injury and revealed hemoglobin clearance by microglia after SFHb injection. Compared to wildtype littermates, larger lesion volumes were observed in HPX-/- and HO2-/- mice, which also bore more degenerating neurons in the perilesion area 24h post-injection. Fewer Iba1-positive microglial cells were detected at the peri-lesion area in HPX-/- and HO2-/- mice; which was associated with markedly increased iron-positive microglial cells. Moreover, the Iba1-positive microglial cells increased from 24 to 72h postinjection and were accompanied with improved neurologic deficits in HPX -/- and HO2-/- mice. These results suggested that activated microglial cells could engulf the extracellular SFHb and provide protective effects after ICH. We then treated primary microglial cells with SFHb. The results showed that microglial cells were activated to take up the extracellular SFHb. Moreover, we found that iron overload in microglia significantly reduced the Iba1 expression level and inhibited microglial phagocytosis. Together, this study reveals that microglial phagocytosis contributed to hemoglobin clearance after ICH, and that deleting HPX or HO2 will compromise this ability by reducing the Iba1 expression level induced by iron overload.

Disclosures: **B. Ma:** None. **J. Day:** None. **E. Tolosano:** None. **S. Dore:** None.

Poster

229. Olfaction: Olfactory Bulb

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 229.01/I42

Topic: D.01. Chemical Senses

Support: NIH 1R01DC013797

Title: Primacy coding of odor identity across concentrations

Authors: *C. WILSON[1], G. SERRANO[1], A. KOULAKOV[2], D. RINBERG[1];
[1]Neurosci., NYU Langone Med. Ctr., New York, NY; [2]Cold Spring Harbor Lab., Cold Spring Harbor Laboratory, NY

Abstract: The olfactory system supports identification of odorants regardless of concentration. This occurs despite the concentration driven variability of the input to the olfactory bulb from the nose. The mechanisms underlying the transformation of unstable sensory input into stable perceptual features is a fundamental question in sensory systems neuroscience. We proposed the Primacy Coding model for fast, concentration-invariant coding of olfactory stimuli. In this model, sensory neurons with the highest affinity for a given odorant encode identity across all perceptible concentrations. We hypothesize that this sensitive set can be determined using relative timing, with highly sensitive neurons responding earliest within the population regardless of concentration. One prediction of this model is that odor identification should be possible using information only from this early responding subpopulation. Through our optogenetic masking behavioral paradigm, we have identified that odor discrimination can be accomplished using sensory information arriving within the first 80 ms of inhalation. We hypothesize that information contained within this first 80 ms represents a primal set that supports odor identity. To test the stability of neural activity occurring within this time window, we recorded the mitral/tufted cell population response to odors across a range of concentrations.

Disclosures: **C. Wilson:** None. **G. Serrano:** None. **A. Koulakov:** None. **D. Rinberg:** None.

Poster

229. Olfaction: Olfactory Bulb

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 229.02/I43

Topic: D.01. Chemical Senses

Support: EA holds a PEW latin american fellowship in the biomedical sciences.

1R01DC013797

1R01DC014366

Whitehall foundation

Title: Transformation of odor information from defined olfactory receptor inputs

Authors: *E. M. ARNEODO[1], K. PENIKIS[2,3], N. RABINOWITZ[3,4], T. BOZZA[5], D. RINBERG[2];
[1]Physics, New York Univ. Neurosci. Inst., New York, NY; [2]Neurosci. Insitute, New York Univ. Langone Med. Ctr., New York, NY; [3]Ctr. for Neural Sci., New York Univ., New York, NY;

[4]Howard Hughes Med. Inst., New York, NY; [5]Dept. of Neurobio., Northwestern Univ., Evanston, IL

Abstract: Circuits within the olfactory bulb transform the odor representations of the sensory periphery. The logic behind this transformation remains elusive, because a) it has been difficult to record from olfactory bulb neurons while knowing what their inputs are, and b) recordings should be done in awake animals as network dynamics change drastically with wakefulness. In order to understand this coding transformation, we have developed a technique to optogenetically identify and then record from olfactory bulb neurons that receive input from genetically-identified inputs in awake animals. Olfactory sensory neurons (OSNs) that express the same odorant receptor gene send axons that converge to form glomeruli, where they provide excitatory input to a small number of mitral/tufted (M/T) cells. The peripheral representation of incoming odors is modified in the M/T layer via an extensive inhibitory network before odor information is relayed to cortex. Using a gene-targeted mouse line that expresses Channelrhodopsin-2 in a genetically-defined subpopulation of OSNs -- cells that express a specific receptor (M72) -- we can identify the subpopulation of M/T cells that are functionally coupled to this same glomerulus. Light stimulation of the M72 glomerulus elicits a reliable, short latency response in these “M72” M/T cells. This technique gives us the opportunity to characterize how a specific subpopulation of “sister” M/T cells transforms their shared input. We recorded the activity of M72 M/T cells from awake, head-fixed, freely-breathing mice while we presented a range of odorants, for which the M72 OSN responses have been characterized. We found that, for most odors, there was a surprising amount of variability in responses among sister M/T cells, despite their common excitatory input. This response diversity was comparable to that seen across the greater population of M/T cells. However, a single odor stimulus -- the strongest known ligand of M72 OSNs -- provided an exception to this response heterogeneity, as it evoked a temporal pattern of firing that was highly consistent across M72 M/T cells. This response stereotypy was not seen in the larger M/T population. These data raise the possibility that response stereotypy and diversity within specific neural subpopulations could form a substrate for sensory coding within the olfactory bulb.

Disclosures: E.M. Arneodo: None. **K. Penikis:** None. **N. Rabinowitz:** None. **T. Bozza:** None. **D. Rinberg:** None.

Poster

229. Olfaction: Olfactory Bulb

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 229.03/I44

Topic: D.01. Chemical Senses

Support: NIH Grant DC011184

Pennsylvania Department of Health's Commonwealth Universal Research Enhancement Program

Title: Effects of odorant exposure on the M72 glomerular module

Authors: *A. LIU[1,2], N. N. URBAN[3,2,1];
[1]Ctr. for Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; [2]Ctr. for the Neural Basis of Cognition, [3]Biol. Sci., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: The mechanisms regulating organization and wiring patterns of olfactory bulb (OB) circuitry are largely unknown. Olfactory sensory neurons (OSNs) located in the olfactory epithelium project axons to the OB that coalesce into spherical structures called glomeruli. These glomeruli and their post-synaptic targets (including mitral and tufted projection neurons and periglomerular cells) form networks called glomerular modules, which comprise the basic odor-coding units of the OB. The identity of the odorant receptor expressed by OSNs plays a crucial role in reliable targeting of OSN axons to specific locations within the OB, as well as the stabilization of glomerular location during the early postnatal period. Odorant experience accelerates the maturation of glomerular targeting by OSN axons. In addition, early prenatal and postnatal odorant conditioning leads to enlargement of activated glomeruli and alters odorant preference. Our experiments investigate 1) the stability of anatomical and behavioral changes caused by early odorant conditioning, and 2) the organization of the glomerular module's post-synaptic targets following prenatal and early postnatal odorant conditioning. We focus on the development of one specific, genetically-identified glomerulus, the dorsolateral M72 glomerulus. We use an odorant conditioning paradigm involving food odorized with methylsalicylate, a specific M72 odorant ligand. Consistent with previously published work (Todrank et al, 2007), we show that early prenatal odorant experience causes a significant increase M72 glomerular volume (control vs. conditioned, 110367 ± 14920 μm3 vs. 189106 ± 10655 μm3, n=10, p=0.003). Concomitant behavioral changes are also observed, with conditioned mice demonstrating a preference for methysalicylate-scented food over control food. However, our data demonstrate that while the increase in glomerular volume persists well beyond the early postnatal odorant exposure period (glomeruli of conditioned mice weaned onto control vs. methylsalicylate-scented food sacrificed at P63, 248183 ± 20573 μm3 vs. 244611 ± 12167 μm3, n=16, p=0.8833, n.s.), the effect on odorant preference is transient and dependent on continued odorant exposure, suggesting that experience-dependent changes in glomerular volume are irreversible beyond an early critical period, while alternative circuit mechanisms underlie flexible experience-dependent behavioral changes. We are currently using *in vivo* dye electroporation to discover odorant exposure dependent changes to the organization of mitral and tufted cells associated with the M72 glomerular module.

Disclosures: A. Liu: None. **N.N. Urban:** None.

Poster

229. Olfaction: Olfactory Bulb

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Topic: D.01. Chemical Senses

Support: NIH Grant R01 DC006640

Title: Ultrastructural analyses of mitral and external tufted cell dendrites in rat olfactory bulb glomeruli

Authors: *J. N. BOURNE, N. E. SCHOPPA;
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Abstract: Recent physiological studies in the olfactory bulb have provided surprising evidence that olfactory sensory neurons (OSNs) can signal to output mitral cells (MCs) through two parallel paths: a direct OSN-to-MC path and a multi-step path that is partly mediated by external tufted cells (eTCs; OSN-to-eTC-to-MC) and appears to be more dominant under a number of experimental conditions. To examine the morphological basis for these signaling mechanisms, we performed ultrastructural studies of the apical dendrites of MCs and eTCs in young rats (P9-14). MCs and eTCs (n = 2 for each) in bulb slices were filled with biocytin and the slices then fixed and incubated with an avidin-biotin complex and 3,3'-diaminobenzidine (DAB) to form an electron dense substrate within labeled cells. Three-dimensional analyses of DAB-labeled dendrites imaged on an electron microscope revealed that MCs have a significantly lower density of presumed OSN-synapses than eTCs (MCs: 0.32 + 0.06 syn/μm; eTCs: 0.59 + 0.12 syn/μm, $p<0.05$), although the densities of inhibitory dendrodendritic synapses were similar. OSN synapses on MCs, but not eTCs, also clustered on the 2 μm distal-most ends of the apical dendrites (MCs 2 μm: 0.71 + 0.19 syn/μm, MCs 4-8 μm: 0.22 + 0.08 syn/μm, $p<0.05$). Analysis of unlabeled dendrites within glomeruli indicated that some dendrites displayed both OSN synapses and gap junctions. In the gap junction-rich dendrites, which most likely reflected MCs (Gire et al., 2012), OSN synapses tended to cluster in distal regions (from distal end: 2-4 μm: 0.63 + 0.12 syn/μm, 6-10 μm: 0.14 + 0.05 syn/μm, $p<0.01$), but the gap junctions were evenly distributed (from distal end: 2-4 μm: 0.28 + 0.07 gj/μm, 6-10 μm: 0.28 + 0.05 gj/μm, n.s.). In addition, the gap junctions were positioned close to dendritic presynaptic release sites, suggesting a role in synchronizing signals in MC apical dendrites that result from dendritic glutamate release. Preliminary modeling of MCs provides evidence that an even distribution of gap junctions on the apical dendritic tuft can attenuate excitatory postsynaptic potentials originating in the distal apical dendrite by ~80%. Together, these data suggest that multiple mechanisms could favor a multi-step OSN-to-eTC-to-MC signaling over direct OSN-to-MC signaling, including a higher density of OSN synapses on eTCs versus MCs, a distal location for OSN synapses on MC dendrites, and more proximally-located gap junctions that could act to shunt direct OSN signals.

Disclosures: J.N. Bourne: None. **N.E. Schoppa:** None.

Poster

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WCI 2009-003

Yale University James Hudson Brown - Alexander Brown Coxe Fellowship

Title: *In vivo* imaging of mitral/tufted cell activity in the mouse olfactory bulb

Authors: *D. A. STORACE[1], L. B. COHEN[1,2];
[1]Cell. and Mol. Physiol., Yale Univ., New Haven, CT; [2]Ctr. for Functional Connectomics, Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of

Abstract: Genetically encoded protein sensors can be used to optically monitor neural activity from distinct populations of neurons. The goal of the present study was to genetically target the voltage and calcium sensors ArcLight or GCaMP6f to mitral/tufted cells in the mouse olfactory bulb, and measure their population response to odorant presentation. Cre-dependent AAVs expressing either ArcLight or GCaMP6f were injected into Pcdh21-Cre transgenic mice, which resulted in expression restricted to mitral/tufted cells. Signals were measured using widefield epifluorescence imaging in anesthetized mice in response to odorants. We also measured the input to the bulb using calcium dye loaded in olfactory receptor neurons in order to compare input and output activity patterns with respect to response timing and concentration invariance.

Disclosures: D.A. Storace: None. **L.B. Cohen:** None.

Poster

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Support: PEW Biomedical Scholars Program

NIH Grant DC013779

Title: Olfactory bulb mitral cells exhibit distinct temporal response profiles during prolonged odor stimulation

Authors: *M. C. OGG, M. BENDAHMANE, M. L. FLETCHER;
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Abstract: Mitral cells (MC), the primary output cells of the olfactory bulb (OB), receive olfactory sensory input via their apical dendrites in the glomerular layer and transmit odor information to multiple cortical regions. Processing by multiple interneuronal circuits within the OB can shape both the magnitude and time course of MC output responses. While an increasing number of studies have demonstrated that MC responses to brief odor stimulation show complex dynamics, comparatively little is known about how individual MC respond to prolonged odor input at timescales relevant to behavioral adaptation/habituation. To explore this question, we used *in vivo* widefield and two-photon calcium imaging and investigated the response profiles of mitral cells in the mouse OB before, during, and after a 30s odor presentation. We recorded activity of MC dendrites at the glomerular layer and MC soma at the MC layer. We found that glomerular responses tend to follow a stereotypical pattern of strong initial activity at odor onset followed by a decaying response. However, MC soma responses to the same odors exhibit a broad range of temporal patterns throughout and after the odor presentation. Using cluster analysis, we classified these soma response patterns into statistically significant response profiles. These profiles indicate that there are MCs that reflect the slow decay of the glomerular response pattern; that come on and go off rapidly; that maintain their response through the entire odor stimulation; and even some that initially do not respond very strongly but ramp up their activity as the odor presentation progresses. Since these response profiles seem to correspond to relevant odor events (e.g. odor onset, duration, odor offset) current work is underway exploring the consequences of these diverse patterns on OB odor coding. Ongoing experiments are also probing the mechanisms that underlie these varying temporal response patterns and their behavioral relevance in terms of odor perception and habituation.

Disclosures: **M.C. Ogg:** None. **M. Bendahmane:** None. **M.L. Fletcher:** None.

Poster

229. Olfaction: Olfactory Bulb

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Support: NIH Grant NS26494 (GLW)

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Title: Parallel synaptic processing of afferent sensory information in the olfactory bulb

Authors: *C. E. VAAGA, G. L. WESTBROOK;
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Abstract: In order to understand how a circuit transforms afferent synaptic information, it is critical to appreciate the synaptic properties and firing patterns of principal neurons embedded within the circuit. In the olfactory bulb, primary afferents from the olfactory nerve converge on synapse-rich glomeruli, however, the connectivity and synaptic properties between primary afferents and the principal neuron subtypes remains controversial. Here we examined the responses of two principal neuron populations in the olfactory bulb, mitral cells and external tufted cells, to afferent stimulation of olfactory nerve axons. Using small-bore theta electrode stimulation, we selectively activated afferent inputs to a single glomerulus, which revealed that both principal neurons receive a fast, monosynaptic input from primary afferents. In paired whole-cell recordings at maximal stimulation intensity, the fast, monosynaptic peak EPSC was 4.1±1.68 (n=6 pairs) fold larger in external tufted cells. However, despite a smaller fast monosynaptic peak, the total charge transfer in mitral cells was much larger (5.1±0.92 fold larger (n=6 pairs)). The larger synaptic charge was the result of slow feedforward excitation specific to mitral cells, resulting from the dendritic release and subsequent spillover of glutamate. Importantly, the slower synaptic response in mitral cells led to repetitive action potential firing in mitral cells in response to brief afferent stimulation, compared to one or a few spikes in external tufted cells. The input-output (I/O) relationship, as measured in number of spikes, suggested a larger dynamic range for mitral cells such that they are likely more efficient at or near the threshold for odor detection. In contrast, the brief response of external tufted cells may be better suited for the tuning of inhibition within the circuit. Our results indicate that both principal neurons receive monosynaptic input from primary afferents; however, cell-type specific synaptic transformations endow these parallel input paths with discrete functions.

Disclosures: C.E. Vaaga: None. **G.L. Westbrook:** None.

Poster

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Support: NIH NRSA 1F31NS066612

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CNRS

Title: Coordinated persistent granule cell and mitral/tufted cell structural plasticity in the olfactory bulb

Authors: *K. A. SAILOR[1], M. T. VALLEY[1], M. T. WIECHERT[1], G. J. SUN[2], W. ADAMS[3], C. DENNIS[3], H. RIECKE[3], G.-L. MING[2], H. SONG[2], P.-M. LLEDO[1];
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Abstract: Throughout adulthood the mouse olfactory bulb is repopulated with new granule cell (GC) neurons. These GCs have been shown to exhibit enhanced structural plasticity during their growth and integration. It is unknown whether these dynamics are retained throughout the life of the GC, or whether this structural plasticity is unique to adult born GCs, or instead, is common to all olfactory bulb GCs. In this study, we tracked the dendritic and spine development of adult-born GCs using 2-photon *in vivo* imaging. Overall, adult born GC dendritic structure stabilized at one month. In contrast, adult born GC apical dendritic spine dynamics plateaued at two months, but maintained a highly dynamic, 20% daily turnover. We compared the spine dynamics of the adult born GCs with early post-natal day 14 born GCs and found matching spine turnover. To test whether the GC spine dynamics correlated with synapse turnover of their synaptic partners, mitral/tufted cells (MC), we labeled mitral/tufted cells with fluorescently labeled gephyrin, which marks the GABA receptor-containing post-synaptic sites that are synaptically opposed to GC spines. *In vivo* imaging of gephyrin puncta demonstrated similar dynamics to GC spines with 14% daily turnover. Using a computational model of GC-MC structural plasticity, we found that these dynamics enable the network to rapidly optimize its output in response to changes in the odor environment. Surprisingly, odor representations quickly settled into a steady state despite continued rapid remodeling of the circuit. GC to mitral/tufted cell synapses have so far been demonstrated to lack synaptic plasticity, therefore the olfactory bulb appears to be unique in the adult brain, where persistent structural dynamics may be its dominant form of plasticity.

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Poster

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Support: NIH Grant DC014367

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Title: A hybrid coupled-oscillator network synchronizes gamma-band field oscillations in olfactory bulb slices

Authors: *S. T. PEACE[1], B. C. JOHNSON[2], A. C. MOLNAR[2], T. A. CLELAND[3]; [1]Neurobio. and Behavior, [2]Electrical and Computer Engin., [3]Psychology, Cornell Univ., Ithaca, NY

Abstract: In the rodent olfactory bulb (OB), coherent gamma oscillations (40-100 Hz) in the local field potential (LFP) are readily observed during active odor investigation, and have been attributed to reciprocal synaptic interactions between mitral and granule cells that synchronize neuronal activity across the OB. To investigate the mechanisms underlying this synchronous activity in the OB, we recorded from 300 μm thick OB slices taken from young adult (P28-P42) transgenic mice expressing channelrhodopsin-2 (ChR2) under the control of the olfactory marker protein (OMP) promoter, hence limiting ChR2 expression to olfactory sensory neuron (OSN) axonal arbors within OB glomeruli. To enable large area recordings across the OB slice, we used a 60-electrode planar microelectrode array (MEA; 200 μm pitch, 30 μm diameter, 30-50 kΩ impedance, 1.4 x 1.4 mm area) to record spike and local field activity. Metabotropic glutamate receptor agonists (ACPD or DHPG), the acetylcholine receptor agonist carbachol, or a brief 4 Hz blue light stimulus were used to induce long-lasting (>5 min) gamma oscillations (20-55 Hz in slice) that are coherent across ranges of up to 300 μm across the OB slice. The limited ranges of coherence are likely to be attributable to reductions in lateral dendritic connectivity arising from the slicing procedure itself; in support of this hypothesis, thicker slices exhibited larger pockets of coherence. Interestingly, the blockade of GABA(a) receptors in the OB slice (using BMI or gabazine) did not block induced gamma oscillations; rather, it reduced their frequency and limited the spatial extent of coherent regions to an area consistent with the field of a single glomerular column. We interpret these results as supportive of a hybrid coupled oscillator model of bulbar oscillogenesis, in which lateral GABAergic feedback via granule cells couples and synchronizes independent intracolumnar oscillators that rely on non-GABAergic coordination mechanisms (e.g., mitral cell subthreshold oscillations, electrical coupling).

Disclosures: S.T. Peace: None. **B.C. Johnson:** None. **A.C. Molnar:** None. **T.A. Cleland:** None.

Poster

229. Olfaction: Olfactory Bulb

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Topic: D.01. Chemical Senses

Support: ANR-12-JSV4-006-01

Title: Functional characterization of calretinin-expressing periglomerular cells in the olfactory bulb

Authors: *N. BENITO, A. SANZ, D. DE SAINT JAN;
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Abstract: Periglomerular (PG) cells expressing calretinin (CR) form the largest population of interneurons in the glomerular layer of the olfactory bulb. Anatomical studies have shown that these cells establish dendro-dendritic synapses with mitral and tufted cells, the principal neurons of the bulb. However, their functional properties and their impact on principal neurons remain unknown. We have examined this question in a transgenic mouse expressing EGFP under the control of the CR promoter (Caputi et al., EJN 2009). In this mouse, only 63 ± 10.6 % of CR-immunopositive PG cells expressed EGFP (n=14 mice, 10 days to 7 month old). EGFP(+) cells filled with neurobiotin had beaded dendrites restricted to one glomerulus and apparently lacked an axon (n=6). Using patch-clamp recording in horizontal slices at different postnatal ages (P15-P60), we found that EGFP(+) PG cells receive fewer (1.8 ± 1.9 Hz) and smaller (13.9 ± 6.3 pA, n=18) spontaneous excitatory synaptic inputs than unlabeled PG cells (17.5 ± 8.9 Hz and 50 ± 23 pA, respectively, n=24). Their average polysynaptic response to the stimulation of the olfactory nerve was also smaller (33 ± 34 pA, n=17) than in other type 2 PG cells (424 ± 302 pA, n=31). In current-clamp experiments EGFP(+) PG cells responded to depolarizing current steps with a single and often small action potential (average amplitude 26 ± 20 mV, range 0-80 mV, n=62 cells). Consistent with this, EGFP(+) PG cells expressed smaller voltage-dependent sodium currents (0.4 ± 0.4 nA, n=28) compared with unlabeled PG cells (1.8 ± 1 nA n=12). Because these properties are reminiscent of those of postnatally generated immature neurons in the hippocampus or in the olfactory bulb, we tested the hypothesis that CR-EGFP(+) PG cells might be newborn neurons. However, only 6.2 ± 1.7 % of EGFP(+) cells colocalized with doublecortin, an immunohistochemical markers of immature neurons. Moreover, we used nestin-CreERT2 inducible transgenic mice crossed with Cre-dependent TdTomato reporter mice to study the lineage of nestin-expressing cells generated in the sub-ventricular zone. As expected, the olfactory bulb of mice sacrificed at P57 (i.e. 34 days after tamoxifen administration) contained a large number of postnatally born labeled cells in the granule cell layer and in the glomerular layer. However, only 22 ± 10 % of the Tdtomato-labeled PG cells expressed CR and doubled labeled PG cells represented only 6 ± 2 % of all CR-expressing cells (n=3 mice). Thus, although CR-EGFP(+) PG cells are poorly connected to the glomerular network only a small fraction might be immature newborn neurons.

Disclosures: **N. Benito:** None. **A. Sanz:** None. **D. de Saint Jan:** None.

Poster

229. Olfaction: Olfactory Bulb

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Topic: D.01. Chemical Senses

Support: ANR-12-JSV4-006-01

Title: Functional mapping of circuits mediating inhibition of olfactory bulb periglomerular cells

Authors: ***A. SANZ DÍEZ**, N. BENITO, D. DE SAINT JAN;
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Abstract: The olfactory bulb (OB) is the first relay station in the brain for odor processing. It receives sensory afferents from olfactory sensory neurons (OSN). This information is transmitted to mitral and tufted cells, the principal output neurons of the bulb, within anatomical structures called glomeruli. Each glomerulus is surrounded by periglomerular (PG) cells that mediate intraglomerular inhibition. We recently characterized a specific PG cell subtype that mediates OSN-evoked intraglomerular inhibition of mitral and tufted cells (Najac et al. JNeurosci 2015). This PG cell subtype expresses EYFP in Kv3.1-EYFP transgenic mice represents 20-30% of all PG cells, is apparently axonless, projects its dendrites into a single glomerulus and receives input exclusively from mitral and tufted cells but not from the OSN defining it as a type 2 PG cell. EYFP(+) PG cells, like other PG cell subtypes, also receive inhibitory synaptic inputs (IPSCs) but the circuit mediating this inhibition is not known. We examined this question using whole-cell voltage-clamp patch-clamp recordings in horizontal OB slices from Kv3.1-EYFP transgenic mice. We found that stimulation of OSN producing excitatory postsynaptic currents (EPSC) in EYFP(+) PG cells (holding potential Vh = -75mV) did not evoke any IPSCs when the cells where clamped around the reversal potential for excitation (Vh = 0mV, n=14). In contrast, stimulation within distant (>200µm) glomeruli (n=13) or in the internal plexiform layer (n=5) produced a gabazine-sensitive monosynaptic IPSC (average amplitude 99.02 ± 83.38 pA; 20-80% rise time 0.73 ± 0.54 ms, decay time 19.08 ± 11.98 ms in the presence of NBQX and D-AP5) that were similar to those of spontaneous IPSCs (0.43 ± 0.14 ms and 20.23 ± 5.36 ms, respectively). These results suggested that inhibitory inputs onto EYFP(+) PG cells are not generated by the glomerular network but provided by deep short axon (dSA) cells with axons running within the glomerular layer. Interestingly, we found that 32% of the type 2 PG cells (n = 8/25) recorded in a transgenic mouse line expressing channelrhodopsin-2 (ChR2) under the control of the thy-1 promoter (Thy1-ChR2-YFP, Jackson Laboratory stock number 007612) responded with a monosynaptic IPSC to a short (1-10 ms) light (490 nm) stimulation (average amplitude 177.87 ± 207.01 pA (range 17-659 pA); rise-time 0.83 ± 0.29 ms and decay time 10.94 ± 6.44 ms, in the presence of NBQX and D-AP5). In contrast, light pulses did not evoke monosynaptic inhibition on type 1 PG cells (n = 4) nor on external tufted cells (n=7). Thus, this mouse line provides a valuable tool to map and evaluate the impact of the inhibitory circuits mediated by dSA cells in the olfactory bulb.

Disclosures: **A. Sanz Díez:** None. **N. Benito:** None. **D. De Saint Jan:** None.

Poster

229. Olfaction: Olfactory Bulb

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Title: Non-linear interactions between GABAergic inhibition and extrasynaptic glutamate gate output of olfactory bulb glomeruli

Authors: *J. D. ZAK, D. H. GIRE, N. E. SCHOPPA;
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Abstract: Recent studies have implicated GABAergic periglomerular (PG) cells as an important contributor to odor-evoked suppression of spiking in olfactory bulb mitral cells (MCs; Fukunaga et al., 2014). To investigate the mechanisms of PG cell-mediated suppression, we measured inhibition (I) and excitation (E) in external tufted cells (eTCs) during patch-clamp recordings in olfactory bulb slices. eTCs are an intermediary cell between olfactory sensory neurons (OSNs) and MCs, and likely regulate MC activation based on the balance between PG cell-to-eTC inhibition and excitation. Using first a protocol in which inhibitory and excitatory currents in eTCs were elicited by electrical stimulation of OSNs, we found dramatic changes in the I/E balance as a function of stimulus intensity. Weak stimuli (Stimthresh = 1-10 μA) produced far more inhibition (I/E = 11.8 ± 2.7, n = 24, p = 0.001; I and E estimated from integrated charge at Vhold = 0 mV and -70 mV, respectively) while stronger stimuli produced more excitation (I/E = 0.51±0.10 at Stimthresh + 30 μA, n = 15, p < 0.0001). Thus, PG cell-mediated inhibition is dominant only when OSN inputs into a glomerulus are weak. We next considered that a potential contributor to the shifting I/E balance was the unusual extrasynaptic nature of recurrent excitation in a glomerulus (Gire et al., 2012). Accumulation of extrasynaptic glutamate could produce supralinear increases in recurrent excitation, making excitation relatively large with respect to inhibition under strong input conditions. Indeed, in pair-cell recordings, single spikes in eTCs produced very small glutamatergic currents in other eTCs and MCs (-35±4 pA*ms; n = 16), but currents rose supralinearly with increasing spike number (F = 3.5±1.1, n = 5, p = 0.022; where F = E due to N spikes/E due to one spike x N). In contrast, single spikes in eTCs produced large excitatory synaptic currents in PG cells (-196±24 pA*ms; n = 9), and excitation rose sublinearly (F = 0.53±0.02, n = 5, p = 0.0024). The shifting I/E balance in eTCs did not appear to reflect the intrinsic properties of PG cells versus eTCs/MCs, since PG cells and eTCs displayed similar threshold intensities at which OSN stimulation generated spikes during pair-cell

recordings (n = 10; p = 0.30). Taken together, our results suggest that non-linearities in GABAergic inhibition and extrasynaptic glutamate-mediated excitation contribute to condition-specific suppression of glomerular output.

Disclosures: **J.D. Zak:** None. **D.H. Gire:** None. **N.E. Schoppa:** None.

Poster

229. Olfaction: Olfactory Bulb

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Topic: D.01. Chemical Senses

Title: Mosaic representation of odors in the output layer of the mouse olfactory bulb

Authors: *H. CHAE[1], D. KEPPLE[1,2], A. KOULAKOV[1,2], V. N. MURTHY[3], D. F. ALBEANU[1,2];
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Abstract: Characterizing the neural representation of chemical space is a formidable challenge in olfaction research. Unlike many other sensory systems, low-dimensional metrics for characterizing odors have remained elusive and it is unclear what features of chemical stimuli are represented by neurons. Here, we have endeavored to relate neural activity in the early olfactory system of mice to the physico-chemical properties of odorants. We imaged odor-evoked responses in identified tufted and mitral cells in awake mice using multiphoton microscopy. Although both mitral and tufted cells responded with diverse amplitudes and dynamics, mitral cells responses were on average sparser and less sensitive to changes in concentration of odorants compared to tufted cells. We characterized odorant features using a comprehensive set of 1,664 physico-chemical properties that has been extensively used previously. Similarity of physico-chemical features of odor pairs was a poor predictor of similarity of the corresponding neuronal representation by mitral or tufted cells. Dimension reduction revealed that ~22 dimensions could explain more than 90% of the variance in neural responses across the population, but fewer dimensions (~12) were necessary if neural activity was projected on to the space of physico-chemical properties. This suggests that factors other than the physico-chemical properties we considered, including non-sensory signals, are required to fully explain the neural responses. Responsive mitral and tufted cells were spatially dispersed, and cells within a local region were functionally heterogeneous with respect to odor identity and concentration. We used dimension reduction strategies to determine whether any odorant property is laid out in an orderly manner spatially and found only limited and variable dependence of mitral/tufted cell position on odorant characteristics. Our data indicate that novel descriptors are needed to link

chemical space to neuronal representations and that odor information leaves the bulb in a mosaic pattern, with substantial local diversity.

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Poster

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Topic: D.01. Chemical Senses

Support: Telethon GGP1116A

Title: Pharmacological rescue of altered neuronal circuits in a mouse model of X-linked intellectual disability

Authors: ***N. REDOLFI**[1], L. GALLA[1], A. MASET[1], E. SAVOIA[1], I. ZAMPARO[2], C. LODOVICHI[3];
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Abstract: Oligophrenin1 (OPHN1) encodes a Rho GTPase-activating protein, that is involved in several developmental processes such as cell migration, axon outgrowth and dendrites formation. How these cellular events can affect neuronal wiring and information processing, leading to cognitive disabilities, remains to be investigated. To address these questions we exploited OPHN1 knockout (KO) mice to analyze circuit formation and function in the olfactory system (OS), where OPHN1 is highly expressed. The OS, one of the few neurogenic niches in the adult mammalian brain, allows a window into a continuous developmental process, in which cell migration, axon outgrowth and dendritic elaboration, that may be altered by mutation in OPHN1, can be thoroughly dissected. Using a cell division marker (Bromodeoxyuridine, BrdU), we found that the generation of new cells in the subventricular zone was not hampered in OPHN1 ko mice versus controls. However, 15 days post BrdU injection the number of newborn cells was significantly decreased in the olfactory bulb. To examine the morphology of the new granule cells (GCs), neuronal precursor were labelled in the SVZ with lentivirus expressing GFP. The morphology of the new GCs was analyzed in the olfactory bulb, 30 days after the SVZ injection. By analyzing the morphology of newborn GFP-labelled GCs, we found that the length and the branching of dendrites was similar in control and experimental animals. However the proportion of filopodia -like spines was significantly increased in OPHN1 ko versus controls. To assess whether the abnormalities in the number and morphology of new GCs could depend on overactivation of the ROCK/PKA signalling, OPHN1 ko and control mice were treated with the

kinase inhibitor fasudil. The treatment successfully rescued the abnormal number and morphology of the adult generated GCs in the olfactory bulb. Our results demonstrated a clear role of OPHN1 in the neurogenesis of the OS and offered a possible new therapeutical approach.

Disclosures: **N. Redolfi:** None. **L. Galla:** None. **A. Maset:** None. **E. Savoia:** None. **I. Zamparo:** None. **C. Lodovichi:** None.

Poster

229. Olfaction: Olfactory Bulb

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 229.15/J8

Topic: D.01. Chemical Senses

Title: Abnormal olfactory behavior and neural patterning in forebrain-specific Ctgf knockout mice

Authors: *H.-C. CHANG[1], L.-J. LEE[1,2,3];
[1]Grad. Inst. of Anat. and Cell Biol., [2]Grad. Inst. of Brain and Mind Sci., [3]Neurobio. and Cognitive Sci. Ctr., Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Connective growth factor (CTGF) is a critical player in connective tissue development. However, in the nervous tissue, CTGF is expressed in some neural cells within distinct areas, such as the olfactory bulb. However, the function of CTGF in the olfactory bulb is far from fully understood. In this study, the role of CTGF in olfaction as well as the neural patterning in the olfactory bulbs was evaluated using forebrain specific Ctgf knockout (FbCtgf KO) mice. In mice, CTGF is expressed in the glomerular layers of the olfactory bulbs. In FbCtgf KO mice, the expression of Ctgf in the olfactory bulb was abolished, while the olfactory bulb was still present. The olfactory detection threshold, evaluated in buried food test, was equivalent between two genotypes. In the olfactory habituation/dishabituation test, wildtype and FbCtgf KO mice exhibited comparable sniffing time to nonsocial odors and homosexual odors, however, in FbCtgf KO mice, the sniffing time to heterosexual odors were increased in both sexes. On the other hand, the number of glomerular calretinin-positive cells was greater in FbCtgf KO mice than wildtype mice. However, the numbers of other juxtaglomerular neurons and glial cells were comparable between genotypes. Since olfactory behavior and olfactory bulb neural patterning are affected in adult forebrain-specific Ctgf knockout mice, our data suggest novel functions of CTGF in the structure and function of the olfactory system.

Disclosures: **H. Chang:** None. **L. Lee:** None.

Poster

229. Olfaction: Olfactory Bulb

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 229.16/J9

Topic: D.01. Chemical Senses

Title: Serotonergic modulation of olfactory bulb network dynamics

Authors: *M. LEWIS[1], S. T. PEACE[1], G. LI[2], T. A. CLELAND[2], C. LINSTER[1];
[1]Neurobio. and Behavior, [2]Psychology, Cornell Univ., Ithaca, NY

Abstract: The serotonergic dorsal and median raphe nuclei densely innervate the layers of the olfactory bulb, and serotonin (5-HT) has been shown to either excite or inhibit a number of olfactory bulb (OB) cell types (external tufted cells, juxtaglomerular cells, and mitral cells) through both through direct action and synaptic connections, respectively. With regard to animal behavior, 5-HT, within the OB, has been shown to be crucial in the formation of olfactory preference learning in neonatal rats. Within adult rats, we demonstrated that bilateral infusion of the 5-HT2 antagonist, cinanserin (6mM), into the OB, impaired behavior in both olfactory habituation and odor discrimination tasks (Lewis et al. in prep). While intracellular recording methods are crucial to the investigation of the effects of neuromodulators on intrinsic and synaptic properties of neurons, those methods do not allow for the observation and analysis of larger scale network activity in response to experimental manipulations. Here, utilizing a combined *in vitro* neurophysiological and biophysically-constrained modeling approach we are investigating the effects of 5-HT on OB network activity in olfactory bulb slices. To capture large-scale neurophysiological data, we recorded from 300 um thick OB slices taken from young adult (P28-P42) mice, using a 60-electrode planar microelectrode array (MEA; 200 um pitch, 30 um diameter, 30-50 kOhm impedance, 1.4 X 1.4 mm area) to record spike and local field activity under a variety of serotonergic conditions. This preparation revealed strong modulation of basal firing rates (both inhibition and excitation) and a highly robust induction of long-lasting (>1 min) gamma oscillations (OB slice: 20-55 Hz) in response to direct application of 200 uM 5-HT. These effects were blocked when OB slices were perfused with the 5-HT2R antagonist, cinanserin (20 uM) prior to 5-HT application. Current experiments are underway to both quantify these changes and to test lower doses of 5-HT which better mimic the physiological condition. Utilizing a biophysically-constrained network model developed in the NEURON simulation environment, we have begun to incorporate known effects of 5-HT from our and others' *in vitro* studies to better understand the effects of 5-HT on OB network activity.

Disclosures: M. Lewis: None. **S.T. Peace:** None. **G. Li:** None. **T.A. Cleland:** None. **C. Linster:** None.

Poster

229. Olfaction: Olfactory Bulb

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 229.17/J10

Topic: D.01. Chemical Senses

Title: Behavioral modulation of corticalbulbar feedback and olfactory bulb output in a multisensory reversal learning paradigm

Authors: *P. GARCIA DA SILVA[1,2], B. REBOUILLAT[3,1], M. DAVIS[1], D. F. ALBEANU[1]; [1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Champalimaud Neurosci. Programme, Lisbon, Portugal; [3]Ecole Normale Supérieure de Cachan, Cachan, France

Abstract: To survive, animals need to dynamically alter neuronal sensory representations such as to facilitate efficient decoding of behaviorally relevant stimuli. Depending on context, the same sensory input may require distinct actions. Rodents rely on olfaction for survival and show remarkable ability to identify and discriminate odors of interest in complex environments. Despite significant research effort, to date little is known about how the olfactory system synthesizes odor identity and value from distributed input activity patterns of glomeruli on the olfactory bulb (OB) surface. Here, we combine multiphoton microscopy with multi-dimensional analysis of system dynamics to determine how feedforward signals at the level of mitral and tufted cells (M/T), the OB output channels, are mixed with descending feedback from the anterior piriform cortex (APC) to support olfactory behaviors in a context dependent manner. Towards this end, we devised a head-fixed two modality reversal "switching" task. Mice are asked to detect a target (go) sound and a distractor (no-go) odor and report by licking, or withholding response to a water delivery port. Using a sound instead of a second odor avoids overlap in the sensory activation patterns of the two stimuli, and helps disentangling population activity that reflects the outcome of the stimulus. Once the task is learned to >70% accuracy, the reward contingency is switched. The reversal is performed in a block-like fashion (35-45 trials per block) within each session, in the absence of a cue signal for switching epochs. Mice master the task within 10-15 days (~80% accuracy, 8 mice), perform an average of 5-8 switches (~ 400 trials) per a session and take ~10 trials to switch strategies - licking to new go cue independent of modality. The learned association is robust across days, and the same animal can learn multiple odor/sound pair associations (N=3 mice, >80% accuracy). We are currently monitoring M/T cells dynamics via two photon imaging of GCaMP6f signals (TBET-Cre x Ai95 mice) and corticalbulbar axonal terminals in mice injected with AAV-GCaMP5 in the APC. Preliminary results show that, at the population, level odor and sound responses cluster differently across different task conditions (hits vs. correct rejections vs. false alarms), supportive of top-down behavioral modulation of neuronal dynamics within the OB.

Disclosures: P. Garcia Da Silva: None. **B. Rebouillat:** None. **M. Davis:** None. **D.F. Albeanu:** None.

Poster

229. Olfaction: Olfactory Bulb

Location: Hall A

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Program#/Poster#: 229.18/J11

Topic: D.01. Chemical Senses

Support: CNRS

Université Lyon 1

Ministère français de la recherche

Title: Olfactory bulb plasticity during complex perceptual learning in mice

Authors: *J. FOREST, J. SACQUET, M. RICHARD, A. DIDIER, N. MANDAIRON;
Lyon Neurosci. Res. Ctr., Lyon Cedex 07, France

Abstract: Olfaction is critical in many behaviors, such as research for food, predator avoidance or reproduction. To accomplish these behaviors successfully, an animal must be able to discriminate very close olfactory stimuli with great accuracy. Discrimination performances can be modified by perceptual learning which is defined as an increase in discrimination capabilities of two perceptually close odorants after exposure to this pair of odorants. One of the key supporting structures of this learning is the olfactory bulb (OB) (Mandairon et al. 2008). Interestingly, in the OB, granule cells, a type of inhibitory interneurons, are the target of an important adult neurogenesis originating in the subventricular zone of the lateral ventricles. Previous work showed that adult-born neurons are required for perceptual learning in mice (Moreno et al. 2009). Until now, studies have analyzed behavioral performances and neurogenic correlates during simple olfactory perceptual learning, involving only one pair of odorants. However, in real life, animals are exposed to more complex olfactory environments. Thus, in this study, we investigated how the animal adapts its perceptive abilities when exposed to more odor pairs and examined the underlying neurogenic modulations. We showed that i) increasing the complexity of perceptual learning leads to the discrimination of more odor pairs, ii) perceptual learning increased adult-born cell density independently of the complexity of enrichment (using Brdu labelling of adult-born neurons), iii) increasing the complexity of perceptual learning enhances the functional recruitment of adult-born neurons (using zif268 expression), iv) increasing the complexity of perceptual learning increases structural plasticity of adult-born neurons (labelled by GFP-expressing lentivirus), and v) structural plasticity is specific of adult-born neurons since it is not observed in neurons born during ontogenesis (using lentivirus injection at P1). All together, these results showed that increasing complexity of perceptual learning increases structural plasticity of adult-born neurons (but not of neurons born during ontogenesis) as well as their involvement in processing the learned odorants. Enhancing the complexity of the task thus intensifies adult-born neuron plasticity resulting at the behavioral level in discrimination of a higher number of perceptually similar odorants.

Disclosures: **J. Forest:** None. **J. Sacquet:** None. **M. Richard:** None. **A. Didier:** None. **N. Mandairon:** None.

Poster

229. Olfaction: Olfactory Bulb

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Topic: D.01. Chemical Senses

Support: Medical Research Council (MC_UP_1202/5)

Boerhinger Ingelheim Fonds

Title: Learning-related changes in odour representations in mitral and tufted cells of the mouse olfactory bulb

Authors: ***R. JORDAN**[1,2], I. FUKUNAGA[1], M. KOLLO[1], A. T. SCHAEFER[1,2];
[1]Neurophysiol., Francis Crick Inst. Mill Hill Lab., London, United Kingdom; [2]Neuroscience, Physiology, Pharmacol., Univ. Col. London, London, United Kingdom

Abstract: The olfactory bulb (OB) consists of feedforward and recurrent circuitry driven by sensory input. However, a large number of inputs from higher centres raises the possibility that odour representation is shaped by contextual input. While contextual changes in OB activity have been reported, the origins of these changes remain unclear. To gain mechanistic insight, we performed intracellular whole cell recordings in the OB of head-fixed mice as they learned to perform a simple olfactory go/no-go discrimination task. Concurrently, odour sampling behaviour was measured via a pressure sensor. Mice were trained to rapidly learn discrimination between two novel odour mixtures, typically reaching criterion of 80% correct responses within 40 trials (~8 mins). We recorded from 18 presumptive mitral and tufted cells (pMTCs) during successful acquisition of this task. Recordings remained stable for 19±7mins (mean±sd) with a mean access resistance of 56±37MΩ. Each odour mixture comprised 3-6 distinct monomolecular odorants known to activate dorsal glomeruli. A large proportion of recorded cells were responsive to these odours, evoking firing rate (FR) changes in 28 of 36 cell-odour pairs. During learning, 8 of 36 cell-odour pairs showed a significant change in average FR response, with either increases for the rewarded odour (CS+; +4.0±4.2Hz, n=3) or decreases for the unrewarded odour (CS-; -10.2±4.1Hz, n=5). These altered responses may arise from top-down modulation of OB processing. However, OB activity is driven by the sniff rhythm. For the cells recorded in behaving animals here, 13 of 14 pMTCs displayed significant sniff coupling of Vm. Therefore, the learning related changes could also arise from altered input patterns to the OB via changes in sniffing behaviour. To evaluate whether changes in odour response may be partially due to altered sniffing, we quantified changes in sniffing frequency during odour stimuli that occurred

across learning. Sniffing frequency changed in various ways for both CS+ (+0.22±0.73Hz) and CS- (-0.02±0.55Hz). These changes showed a significant positive correlation with changes in FR and Vm responses to odours for the CS+ (FR: R2=0.60, p=0.01, Vm: R2=0.59, p=0.002), while no such correlation was observed for the CS- (FR: R2=0.22, p=0.17, Vm: R2=0.02, p=0.61). We conclude that learning results in changes in pMTC responses to odours. However, the accompanying changes in sniff frequency suggest that odour sampling behaviour cannot be excluded as an underlying explanation for altered odour FR responses.

Disclosures: **R. Jordan:** None. **I. Fukunaga:** None. **M. Kollo:** None. **A.T. Schaefer:** None.

Poster

230. Olfaction: Higher Order Processing

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 230.01/J13

Topic: D.01. Chemical Senses

Title: fMRI study of olfaction in the olfactory bulb and higher olfactory structures of rats

Authors: ***F. ZHAO**, X. WANG, H. ZARIWALA, J. M. USLANER, A. HOUGHTON, J. EVELHOCH, C. WINKELMANN;
Merck & Co., West Point, PA

Abstract: Olfactory pathways being rich in synaptic processing would be advantageous for study of *in vivo* pharmacodynamic drug responses. Translatable method such as fMRI has been used to study odor stimulation-induced hemodynamic responses in the olfactory bulb (OB) of rodents. However, fMRI measurements of olfactory processing in other higher olfactory regions (HOR) such as anterior olfactory nucleus, olfactory tubercle and piriform cortex in rodents have not been reported. Since olfactory processing in the HOR of rats has been measured by electrophysiology recording and ultrasound, we hypothesized that fMRI would likewise detect an olfactory processing in HOR. In this study, cerebral blood volume (CBV) fMRI with superparamagnetic iron oxide nanoparticles (USPIO) as contrast agent was used to study olfactory processing in rats. fMRI data were acquired in sixteen 0.75-mm coronal slices covering the OB and HOR. For each animal, either thirty or sixty consecutive fMRI measurements were made during a 3-h experimental session, with each measurement consisting of a baseline period, an odorant-stimulation period, and a recovery period. Two different stimulation paradigms with a stimulation period of 40-s or 80-s were used to study the olfactory responses in OB and HOR, respectively. Our data shows that odorant-induced CBV increases can be robustly observed in the OB and HOR of each individual animal. The olfactory adaptation, which is characterized by the attenuation of responses to continuous or repeated stimulations, has different characteristics in OB and HOR. For the adaptation during the continuous 40-s or 80-s odor exposures, the CBV responses in the OB were stable and did not show adaptation, but the CBV responses in the HOR

were state-dependent, with no adaptation during the initial stimulations, but significant adaptation during the latter stimulations. For the adaptation to the repeated stimuli, while it was observed in both OB and HOR, the responses in HOR adapt more significant than the responses in OB. These results support previous reports that HOR plays a more significant role than OB in olfactory habituation. The technical approach with simultaneous fMRI measurements of olfaction in OB and HOR presented in this study should enable more extensive fMRI studies of olfactory processing in rats.

Disclosures: **F. Zhao:** None. **X. Wang:** None. **H. Zariwala:** None. **J.M. Uslaner:** None. **A. Houghton:** None. **J. Evelhoch:** None. **C. Winkelmann:** None.

Poster

230. Olfaction: Higher Order Processing

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: D.01. Chemical Senses

Support: Pew Scholar Program

Whitehall Fellowship

CSHL Startup Funds

Title: Top-down cortical modulation of olfactory processing

Authors: H. CHAE, *G. H. OTAZU, M. B. DAVIS, D. F. ALBEANU;
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: The olfactory bulb (OB) projects 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 bulb. To date, little is known about the dynamics and importance of these top-down feedback signals. We used multiphoton calcium imaging to monitor the activity (GCaMP5) of individual piriform cortex feedback axon boutons in the bulb of awake head-fixed mice. Responses of PC feedback boutons were sparse, odor specific and often outlasted stimulus duration by several seconds. Odor presentation either enhanced or suppressed their baseline activity. However, any given bouton responded with stereotypic polarity across multiple odors, preferring either enhancement or suppression. Pharmacological inactivation of PC increased odor responsiveness and pairwise similarity of mitral cells, but had little impact on tufted cells. We propose that PC feedback specifically acts on mitral cell representations to enable odor separation, while only mildly affecting tufted cell responses. We are currently comparing the effects of suppressing activity in the PC versus AON on the two output channels (mitral and tufted cells) of the bulb to understand the roles played by distinct

feedback signals on processing sensory input in the early olfactory circuits. To selectively suppress corticalbulbar feedback axons without disturbing the overall cortical activity, we are using optogenetic methods (JAWS) for local inactivation of the axonal terminals in the OB.

Disclosures: **H. Chae:** None. **G.H. Otazu:** None. **M.B. Davis:** None. **D.F. Albeanu:** None.

Poster

230. Olfaction: Higher Order Processing

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Topic: D.01. Chemical Senses

Support: NHMRC Project 1050832

Title: Dynamics of spontaneous activity in the mouse piriform cortex *in vitro*

Authors: ***M. L. TANTIRIGAMA**, J. M. BEKKERS;
The Australian Natl. Univ., Canberra, Australia

Abstract: The mammalian brain remains highly active even in the absence of sensory input. In cortical regions, spontaneous activity carries important information about the network's baseline state. Piriform cortex (PC) is also known to exhibit spontaneous activity, but its origin and statistical properties are unknown. Here, we simultaneously measured the activity of up to 239 neurons in the PC of anesthetized mice that are freely breathing charcoal-filtered air. We used 2-photon microscopy and functional calcium imaging, employing the calcium indicator dye Cal-520. The main input layer (layer 2) of PC contains two distinct populations of glutamatergic neurons: semilunar (SL) cells in layer 2a and superficial pyramidal (SP) cells in layer 2b. We found the properties of spontaneous calcium transients to significantly differ between SL and SP cells in their kinetics (rise time constant: SL, 63 ± 2 ms; SP, 55 ± 1 ms, $p < 0.05$; decay time constant: SL, 372 ± 16 ms; SP, 467 ± 45 ms, $p < 0.05$, mean ± SEM, t-test, n = 4 mice). The frequency of spontaneous calcium transients was higher in SL cells than in SP cells (SL, 0.20 ± 0.02 Hz; SP, 0.13 ± 0.01 Hz, $p < 0.05$). Deconvolution of the calcium signal also estimated a higher spike rate in SL cells than SP cells (SL, 1.6 ± 0.1 Hz; SP, 0.9 ± 0.1 Hz, $p < 0.05$). For both cell types, the frequency distribution deviated from a Poisson distribution in most cases (SL: 80 %; SP: 60 %, both $p < 0.05$, Chisqr test), indicating a non-random temporal structure in the spontaneous events. Topical application of the NMDA channel blocker MK801 to the PC surface completely abolished spontaneous activity (n = 2 mice), indicating a strong dependence on NMDA receptor-driven excitatory transmission. To test whether spontaneous activity is driven by nasal air flow across the olfactory epithelium, we tracheotomized mice to bypass the airway and found that spontaneous activity was unchanged (spike rate, tracheotomized vs. intact, $p = 0.68$, n = 2 mice). We next tested whether spontaneous activity is driven by intrinsic activity in

the olfactory bulb (OB). Removal of the ipsilateral OB significantly reduced the occurrence of spontaneous activity in both layers of PC (spike rate, SL, 0.06 ± 0.04 Hz; SP, 0.07 ± 0.04 Hz, n = 2 mice), and removal of OBs in both hemispheres abolished all activity in the PC. To confirm the bulbar origin of this activity, we imaged up to 112 mitral/tufted (M/T) neurons in the OB. As expected, M/T cells exhibited spontaneous calcium transients at a rate of 0.12 ± 0.01 Hz (n = 2 mice). The findings suggest that computations in PC are superimposed on cell-type specific non-random spontaneous activity that emerges from the OB.

Disclosures: M.L. Tantirigama: None. **J.M. Bekkers:** None.

Poster

230. Olfaction: Higher Order Processing

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 230.04/J16

Topic: D.01. Chemical Senses

Title: Is the innervation of piriform by the olfactory bulb random?

Authors: *P. A. RHODES;
Evolved Machines, Mountain View, CA

Abstract: Odorant representation in the vertebrate piriform as well as its homolog, the insect Mushroom body, is sparse and widely distributed, and a number of studies (Jortner et al 2006; Stettler and Axel 2009) have concluded that the cortical representation is consistent with fully random innervation. However, the plausibility of random wiring has only been assessed with simplified mathematical realizations, and the results of self-organizing wiring processes have not often been considered. In order to examine whether random wiring is consistent with the observed reliability and sparsity observed in piriform cortex *in vivo* (Stettler and Axel 2009), the wiring between an array of axons representing mitral cell output and an array of branched cortical neurons, each receiving O(103-4) synapses as seen in most insect and all vertebrate systems, was self-organized during simulated sensory input. To model the self-organization of wiring gated by local events, initially random synapses were endowed with an initial stability value which incremented or decremented upon the conjunction or disjunction of presynaptic activity with postsynaptic dendritic and neuronal firing. The sign and magnitude of these adjustments were parameters that controlled the results of the wiring process. Axonal activation patterns were modeled as abstract sets of co-active mitral axon populations as might be associated with single glomeruli, with odorants represented by conjunctions of such subsets. With random wiring, the same sensory input was provided but the wiring reorganization processes were turned off. We found, robustly across sensory environments and other parameters of this system, that the initially random wiring produced odorant encodings that were either highly overlapping, or so fragile to input variation that just a few percent jitter in spike timing

resulted in the cortical representation of repeated presentation of a given odorant varying far more than the 80% repeatability observed (Stettler and Axel 2009). In contrast, if wiring self-organized at the single neuron level so that postsynaptic firing stabilized co-active inputs then the representation that emerged was both highly stable as well as sparse, as observed in cortical systems across phylogeny. The self-organization of wiring at the single neuron level comprised of frequently coactive mitral axons was entirely consistent with the fully distributed nature of the representation of a given odorant across the cortex. The predicted neuron-level convergence of axons emanating from a single or frequently correlated glomeruli in piriform will be straightforwardly assayed by its future connectomic reconstruction.

Disclosures: P.A. Rhodes: None.

Poster

230. Olfaction: Higher Order Processing

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 230.05/J17

Topic: D.01. Chemical Senses

Title: Somatostatin interneurons in piriform cortex

Authors: *A. M. LARGE[1,2], A.-M. M. OSWALD[1,2];
[1]Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; [2]Ctr. for the Neural Basis of Cognition, Pittsburgh, PA

Abstract: Odor information is processed by the neural circuitry of the piriform cortex. Pyramidal cell responses to odor information are controlled by GABAergic inhibitory interneurons. Recent studies have characterized interneurons in piriform cortex (1) but little is known about the functions of distinct classes. Interneurons that express somatostatin (Sst) are a major class of interneurons that inhibit pyramidal cells. In this study, we analyzed the diversity and connectivity of Sst cells in APC using two transgenic mouse lines, GIN (2) and Sst-Cre (3). Previous studies have suggested that Sst interneurons can be divided into at least three classes based on their expression in distinct mouse lines: GIN, X94, and X98 (4). In contrast to many other sensory cortices, we find that APC has a dearth of GIN cells. Furthermore, using Sst-cre/lox-tdTomato mice, we find a greater proportion of fast-spiking (FS) interneurons consistent with X94 cells versus regular spiking (RS), X98-like cells. To study the targets of Sst cells, we selectively activated Sst interneurons in brain slices from Sst-cre/lox-ChR2 mice while recording inhibitory postsynaptic currents (IPSCs) in Sst(+) interneurons as well as Sst(-) interneurons and pyramidal cells in anterior piriform cortex (APC). We find that Sst cells target both types of FS cells (PV(+) and SSt(+)) as well as PCs. Moreover, the spatial profile of inhibition differs markedly between PC and interneuron cell classes. We are currently addressing the function role of Sst neurons in APC circuits and odor processing. 1) Suzuki and Bekkers (2010) Cerebral

Cortex 20:2971-84 2) Olivia et al.,(2000) J. Neurosci. 20:3354-33683 3) Taniguchi et al., (2011) Neuron 71:995-1013 4) Ma et al.,(2006) J. Neurosci 26:5069-82

Disclosures: **A.M. Large:** None. **A.M. Oswald:** None.

Poster

230. Olfaction: Higher Order Processing

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Topic: D.01. Chemical Senses

Support: DC009839

Mallinckrodt Foundation

Title: Encoding odors with ensembles of piriform cortex neurons in awake and anesthetized mice

Authors: K. A. BOLDING, *K. M. FRANKS;
Neurobio., Duke Univ., Durham, NC

Abstract: Previous studies have indicated that odors are encoded by ensembles of coordinately active neurons distributed across piriform cortex. However, the nature of these ensembles remains poorly understood. Moreover, earlier studies were performed exclusively under either awake or anesthetized conditions but it is not known how anesthesia affects cortical odor representations. We therefore recorded spontaneous and odor-evoked spiking activity from populations of piriform cortex neurons in awake, head-fixed mice. Mice were then anesthetized with ketmaine/xylazine (k/x) to generate multiple, simultaneously recorded sets of cell-odor pairs measured under both awake and anesthetized conditions. Spontaneous activity, which was higher and tonic when the mouse was awake, slowed and became strongly coupled to respiration under k/x. Despite these differences in spontaneous activity, odor responsiveness was similar in awake and anesthetized conditions: only a few cells responded to each odorant with significant increases in spiking (8% awake; 10% k/x; 1,260 cell-odor pairs), and lifetime and population sparseness were similar across conditions. Responsive cells fired in brief bursts shortly after inhalation onset, with responses being slower and slightly longer in k/x. Suppression was also observed and was more prevalent in awake compared to k/x conditions, occurred later in the sniff than activation, and typically persisted for the remainder of the sniff. Population odor responses were defined by spike counts for each cell during the first full sniff following odor onset. A linear classifier could accurately determine the odorant that evoked these responses when trained and tested on responses evoked within a condition, indicating that these ensembles do indeed encode odor identity. However, only ~1/3 of the neurons that responded to a given odor in one condition also responded to that odor in the other condition, and the classifier, while still well

above chance, performed significantly worse when trained and tested on responses from different conditions. However, cells that responded to an odor in both conditions typically responded earlier than cells that only responded in only that condition, suggesting that the temporal structure of an ensemble may also encode important information about odor identity. It also suggests that early-responding inputs may be preferentially driven by direct sensory information while inputs responding later in the sniff may be preferentially recruited by more state-dependent, intracortical activity. These studies therefore help define and provide mechanistic insight into the nature of cortical odor representations.

Disclosures: K.A. Bolding: None. **K.M. Franks:** None.

Poster

230. Olfaction: Higher Order Processing

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Topic: D.01. Chemical Senses

Support: DC 009839

Mallinckrodt Foundation

Title: Piriform outputs effect multi-stage normalization of cortical odor representations

Authors: *K. A. BOLDING, K. M. FRANKS;
Dept. of Neurobio., Duke Univ., Durham, NC

Abstract: Odor identity is thought to be encoded by ensembles of neurons in piriform cortex. Although activation of odorant receptors and olfactory bulb glomeruli scale steeply with odorant concentration, odors typically retain their perceptual identities over a large range of concentrations suggesting that olfactory information must be normalized within the bulb, within piriform cortex, or both. Previous *in vitro* electrophysiology and computational modeling studies have suggested that recurrent excitatory circuits in piriform cortex may play a major role in normalizing piriform output by recruiting strong and scaled feedback inhibition. We tested this prediction by unilaterally introducing AAVs that conditionally express tetanus toxin (TeLC) into piriform cortex of emx-1-cre mice. This strategy selectively *muted* piriform principal cells, which retained their feedforward excitatory and inhibitory inputs and could fire action potentials, but could no longer drive recurrent excitation or recruit feedback inhibition. We first recorded odor-evoked spiking to multiple odorants at different concentrations (0.03% - 1% vol./vol.) from populations of presumptive olfactory bulb mitral/tufted (M/T) cells and pirifrom cortex neurons from the uninfected hemisphere. Comparing spiking across the population at the highest and lowest concentrations, we found output increased by 75% in the bulb and 55% in piriform cortex. Some normalization of bulb output therefore occurs in piriform cortex. Analysis of

single-unit responses revealed that, while a few cells responded to higher odorant concentrations with increased firing, many other cells became increasingly suppressed. Odorants at low concentrations activated qualitatively similar ensembles in control and tetanus-infected cortices. However, increasing odorant concentration 30-fold produced a dramatic 212% increase in spiking in the muted cortex, with almost no suppression observed in single units. Principal neurons in piriform cortex therefore play a major role in normalizing their output. This normalization may occur through recruitment of cortical feedback inhibitory circuits, but TeLC will also mute piriform projections back to olfactory bulb, and these may normalize bulb output via disynaptic inhibition of M/T cells. We therefore also recorded M/T cell responses ipsilateral to the TeLC-expressing cortex and indeed found a 165% increase in spike output, indicating that some of the normalization seen in the piriform is due to suppression of bulb output. Taken together, these results demonstrate that piriform cortex neurons normalize their output at multiple stages in the olfactory pathway.

Disclosures: K.A. Bolding: None. **K.M. Franks:** None.

Poster

230. Olfaction: Higher Order Processing

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Program#/Poster#: 230.08/J20

Topic: D.01. Chemical Senses

Support: Arkansas Biosciences Institute grant

Title: State-dependent oscillatory coding and interactions between olfactory bulb and piriform cortex

Authors: *S. GAUTAM, S. CHAKRABORTY, W. L. SHEW;
Univ. of Arkansas, Fayetteville, AR

Abstract: How a neuron responds to sensory stimulation depends on the state of the network in which the neuron is embedded. The network state shifts depending on behavioral context; for example, learning, changes in attention, sleep, and anesthetic depth can result in very different ongoing network dynamics and different response to sensory input. In the olfactory system, such state-dependent coding of sensory information has been observed in olfactory bulb (OB) and, in separate studies, in piriform cortex. However, olfactory information is processed by cooperative interactions between OB and cortex and it remains unclear how changes in network state affect interactions between OB and cortex. Previous studies suggest that certain frequencies of oscillations (~10-40 Hz, in particular) mediate interactions between OB and cortex. Other studies highlight the fact that oscillations depend on network state. Indeed, changes in ongoing oscillations are often used to define changes in network state. Considered together, these

previous studies raise a basic question: how do changes in the state of the OB-cortex network impact the processing of olfactory sensory information? To address this question, we studied ongoing activity and odor-evoked activity using simultaneous dual microelectrode array recordings in OB and anterior piriform cortex (aPC) in urethane anesthetized rats. Two different odorants were administered by two different routes - either orthonasally or retronasally. As in previous studies, we observed prominent local field potential (LFP) oscillations at a few distinct frequencies during both ongoing and odor-evoked activity. Our primary finding is that the degree of oscillatory coordination between OB and aPC was dependent on both the stimulus type and the network state. For all stimuli and network states, odors evoked oscillations in both OB and aPC, but these oscillations were coordinated only when a shared frequency existed in both brain regions, typically in the 10-40 Hz range. The lower frequencies tended to be correlated with smaller phase lag than higher frequencies. Our findings demonstrate that olfactory information is relayed to cortex, no matter what the network state is - changes in network state do not gate sensory information. But, the interactions between OB and aPC vary dramatically depending on network state and stimulus type. Our results suggest that the olfactory system must cope with olfactory codes that change depending on network states.

Disclosures: S. Gautam: None. **S. Chakraborty:** None. **W.L. Shew:** None.

Poster

230. Olfaction: Higher Order Processing

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Title: The olfactory cortex areas coordinately perform inhalation-exhalation switching between superficial- and deep-layer active states

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Abstract: Olfactory perception depends on discrete respiration cycles, each consisting of an inhalation and exhalation phase. The central olfactory system is engaged in online processing of external odor information during inhalation but is isolated from the external environment during

exhalation. Although sensory processing in the olfactory cortex during inhalation has been reported previously, the functional role of olfactory cortex activity during exhalation remains unclear. To investigate the activities of the olfactory cortex during exhalation, we recorded local field potentials and unit activities in the anterior olfactory nucleus and piriform cortex in a head-fixed conscious rat using a linear multi-channel electrode. Concurrently, we monitored the respiration. Local field potentials of the olfactory cortex showed respiration-paced slow oscillations. Current source density analysis revealed fast oscillatory current sinks superimposed on a slow current sink in the superficial layer (layer I) during the inhalation phase. Surprisingly, the deep layers (layers II and III) showed oscillatory current sinks and a slow current sink during the exhalation phase. Olfactory sensory deprivation by naris occlusion or bilateral olfactory bulbectomy did not eliminate the inhalation-coupled slow current sink in the superficial layer and neither did it eliminate the exhalation-coupled slow current sink; however, whereas inhalation-coupled fast oscillatory current sinks (gamma or beta oscillations) were diminished. Both the superficial and deep layer current sinks accompanied spike discharges of olfactory cortex neurons. Importantly, the exhalation-coupled neuronal firings persisted, even after bilateral olfactory sensory deprivation, thereby suggesting intrinsic generation. These results indicate that the olfactory cortex areas simultaneously perform inhalation-exhalation switching of the operation mode. The inhalation phase is the time-window for processing olfactory sensory input in the superficial layer circuits whereas the exhalation phase is the temporal framework for the internal generation and coordination of the deep layer circuit activity across different regions of the olfactory cortex.

Disclosures: **K. Narikiyo:** None. **H. Manabe:** None. **K. Mori:** None.

Poster

230. Olfaction: Higher Order Processing

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Program#/Poster#: 230.10/J22

Topic: D.01. Chemical Senses

Title: Spatial profiles of inhibition onto semilunar and pyramidal neurons in piriform cortex

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Abstract: Inhibition regulates neural activity throughout the cortex. While numerous studies have investigated the temporal relationship between excitation and inhibition in piriform cortex, little is known about the spatial recruitment of inhibition. In this study, we investigated the spatial profiles of inhibition onto semilunar cells (SL, n=16), L2 (n=14) and L3 (n=17) pyramidal cells (PC). For each excitatory cell, we recorded IPSCs in response to optical

activation of surrounding inhibitory interneurons in sagittal slices from vGAT-ChR2 mice using two protocols- 1) *Local*: focal (~70 μm) light spots directed a grid locations separated by 150 μm, and, 2) *Global:* full field illumination. We compared the local, global, laminar, and rostral-caudal recruitment of inhibition between the three excitatory cell classes. In response to local stimulation centered on the recorded neuron, SL cells and L2PCs received comparable inhibition that was significantly weaker than inhibition received by L3PCs. However, in response to global stimulation, a subset of L2PCs received more inhibition than simultaneously recorded nearby (<50 μm) SL cells. For each cell type we analyzed the laminar profiles of inhibition as a percentage of the total inhibition. We found that SL cells received significantly greater inhibition from L1 interneurons and significantly less from L3 compared to L2 or L3 PCs. In addition, L3 pyramidal cells received significantly greater inhibition from deep L3 interneurons than SL or L2PCs. Finally, we found that SL cells as a population do not receive biased inhibition along the rostral-caudal axis. In contrast, L2 and L3 pyramidal cells received significantly caudally biased inhibition. In all cases, significance at $p<0.05$ using paired and unpaired t-tests. Taken together these results suggest that inhibition increases from L2 cells to L3 cells as well as along the rostral caudal axis in PCs. Thus, SL, L2PCs and L3PCs have both overlapping and distinct laminar sources of inhibition that may be differentially recruited by afferent or recurrent circuit activation. Ongoing studies are directed at identifying the functional role of these spatial gradients of inhibition in odor processing.

Disclosures: **N.W. Vogler:** None. **A.M. Large:** None. **A.M. Oswald:** None.

Poster

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Max Planck Society

DFG MO 432/10-1

Title: Differential odor processing in feedforward and feedback neurons in the lateral entorhinal cortex

Authors: *F. C. LEITNER[1,2], S. MELZER[3,4], H. LÜTCKE[5,6], E. C. FUCHS[3], P. H. SEEBURG[2], F. HELMCHEN[5], H. MONYER[3];
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Heidelberg, Germany; [3]Dept. of Clin. Neurobio., Med. Fac. of Heidelberg Univ. and German Cancer Res. Ctr. (DKFZ), Heidelberg, Germany; [4]Dept. of Neurobio., Howard Hughes Med. Institute, Harvard Med. Sch., Boston, MA; [5]Brain Res. Institute, Univ. of Zurich, Zurich, Switzerland; [6]ETHZ, Zurich, Switzerland

Abstract: It is established that the lateral entorhinal cortex (LEC) supports different functions, including object recognition, novelty detection and odor processing. The LEC receives direct input from the olfactory bulb (OB) and other olfactory cortical areas and conveys this information to the hippocampus. This connectivity designates the LEC as crucial relay station for odor information processing and the formation of associative memories in the hippocampus. Whereas it has been shown that single cells in LEC superficial layers respond to odors, it is not known how this activity relates to specific cell types. We characterized the two major excitatory cell types, calbindin- and Reelin-positive neurons, as well as GABAergic interneurons in layer II LEC, using anatomical approaches and *in vivo* calcium imaging combined with patch-clamp recording. Odor stimulation induced calcium transients in layer II neurons with odor responsiveness differing in a cell-type specific fashion. Reelin-expressing feedforward neurons responded with higher selectivity to specific odors compared to calbindin-expressing putative feedback neurons and GABAergic neurons. Notably, at the population level the performance in odor discrimination was comparable for Reelin- and calbindin-positive neurons, but significantly lower for GABAergic neurons. Our results highlight the functional differences among defined neuronal subpopulations in the LEC.

Disclosures: F.C. Leitner: None. **S. Melzer:** None. **H. Lütcke:** None. **E.C. Fuchs:** None. **P.H. Seeburg:** None. **F. Helmchen:** None. **H. Monyer:** None.

Poster

230. Olfaction: Higher Order Processing

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Mt. Sinai Healthcare Foundation

Title: The encoding of stimulus valence and primary reinforcers in the olfactory tubercle

Authors: *M. A. GADZIOLA, K. A. TYLICKI, D. L. CHRISTIAN, D. W. WESSON; Neurosciences, Case Western Reserve Univ., Cleveland, OH

Abstract: Sensory information acquires meaning to adaptively guide behaviors. Despite odors mediating a number of vital behaviors, the components of the olfactory system responsible for assigning meaning to odors remain unclear. The olfactory tubercle (OT), a ventral striatum structure that receives monosynaptic input from the olfactory bulb, is uniquely positioned to encode reward and transform odor information into meaningful neural codes. In recordings from mice engaged in an odor discrimination task, we report that the firing rate of OT neurons robustly and flexibly encodes the valence of conditioned odors over identity, with rewarded odors evoking greater firing rates. This coding of rewarded odors occurs prior to behavioral decisions and represents subsequent behavioral responses. To further evaluate the OT as a substrate for motivational information processing, in a second experiment we investigated whether OT neurons respond to primary reinforcers. Mice were trained to lick a spout according to a fixed-ratio (FR-18) schedule for delivery of a liquid reward. Reward type and magnitude was randomized and included tap water, 2 mM saccharine, and 1 mM quinine solutions, delivered at 4, 8 or 12 μL volumes. Firing rates of OT neurons were modulated by both the licking behavior and reward delivery, with larger volume rewards evoking greater firing rates. Taken together, these results illustrate the profound capacity for the OT to encode both stimulus valence and primary reinforcers in manners likely essential for not only basic sensory coding but also motivated and affective behaviors.

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Poster

230. Olfaction: Higher Order Processing

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Support: NIH NIDCD Grant R03DC014540

NIH NIDCD Grant DC003906

Title: The olfactory thalamus: characterizing single-unit activity of the mediodorsal thalamic nucleus in behaving rat

Authors: *E. COURTIOL[1,2], D. A. WILSON[1,2];
[1]Emotional Brain Inst., Nathan Kline Inst. For Psychiatric Res., Orangeburg, NY; [2]Dept. of Child & Adolescent Psychiatry, New York Univ. Langone Med. Ctr., New York, NY

Abstract: The thalamus is a key crossroad structure in the brain and is recognized as a major contributor to sensory perception, attention, sleep and arousal and memory. For all senses except olfaction, the information from the sensory neurons necessarily passes through a thalamic

nucleus before reaching the primary sensory cortex. However, an olfactory thalamic nucleus exists: the mediodorsal thalamic nucleus (MDT) receives direct input from different olfactory structures including the piriform cortex (PCX), and in turn has bi-directional projections with the orbitofrontal cortex (OFC). Functionally, we have shown that, in urethane-anesthetized rats, MDT units respond to a wide variety of odorants and that odor stimuli induce a conjoint emergence of beta frequency oscillations in both the MDT and the PCX. Beyond this odor responsiveness, the precise role of the MDT in olfaction remains unclear. In fact, lesion studies in both humans and animal models suggest a role for the MDT in olfactory perception, odor discrimination, learning and attention. The MDT is a higher order thalamic nucleus and is involved in various cognitive processes such as spatial and working memories, instrumental decision-making and stimulus-reward association. Despite the unusual arrangement of the olfactory pathway, how are primary sensory and higher order cognitive functions interwoven in MDT activity? To investigate precisely the role of the MDT in olfactory processing, we recorded MDT single unit activity, using a multi-tetrode drive, in 8 rats performing a two alternative odor discrimination task. We observed that a subset of MDT units are odor selective. Intermingled with this sensory function, we also observed that the MDT presents specific temporal patterns of activity during the pre-sampling period and seems to encode the direction/goal location. Our results thus reveal the involvement and the complex role of the MDT in olfactory processing. Finally, the preliminary analyses of the field potentials and MDT spike-local field potentials in both PCX and OFC will help us to better understand the role of the MDT and the relationship between MDT, its olfactory input and output.

Disclosures: E. Courtiol: None. **D.A. Wilson:** None.

Poster

230. Olfaction: Higher Order Processing

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Title: Variability and adaptation of odor representations in the zebrafish homolog of olfactory cortex

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Abstract: Odor-induced activity patterns arising from the vertebrate olfactory epithelium are initially processed in the olfactory bulb (OB), which subsequently transmits stable and decorrelated activity patterns to the olfactory cortex (OCx). We studied the dynamics of odor representations in the zebrafish homolog of olfactory cortex, area Dp, in response to repeated applications of the same odor, with the aim of analyzing the stability of odor representations. Population activity patterns were measured by 2-photon Ca2+ imaging in an ex-vivo preparation of the intact brain. Response patterns exhibited large trial-to-trial variability and marked adaptation, phenomena not observed in the OB output. To study whether synaptic plasticity mechanisms are involved in generating variable responses within OCx, we blocked NMDA receptors using APV (50 μM), which has minimal effects on OB output. In the presence of APV, odor responses were highly stable and exhibited no significant adaptation. Moreover, spontaneous activity of OCx neurons was suppressed. Two processes contributed to the variability of odor responses under control conditions. First, a small part of the variability is explained by a gradual change in the neuronal representation across trials, as correlations between pairs of response patterns decrease as a function of the separation between the trials. NMDA blockage may suppress plasticity mechanisms underlying this gradual change in representation. Second, we characterized neurons categorically as spontaneously active or silent. Spontaneously active neurons exhibited significantly higher variability in their responses, even when controlling for adaptation. This suggests that spontaneous activity in the OCx, suppressed in the presence of APV, interacts with the stable patterns arriving from the OB and contributes to response variability.

Disclosures: G.A. Jacobson: None. **R.W. Friedrich:** None.

Poster

230. Olfaction: Higher Order Processing

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Topic: D.01. Chemical Senses

Support: HHMI

Title: Electron microscopy reconstruction of Kenyon cells and their synaptic connectivity in the mushroom body of adult *Drosophila* melanogaster

Authors: *Z. ZHENG[1], J. S. LAURITZEN[1], C. B. FISHER[1], J. M. RATLIFF[1,2], B. M. HARRISON[1,2], A. E. ADESINA[1,2], C. G. ROBINSON[1], J. PRICE[3], D. MILKIE[4], O. TORRENS[4], B. KARSH[1], E. T. TRAUTMAN[1], K. KHAIRY[1], E. PERLMAN[1], M.

KAZHDAN[5], A. CARDONA[1], S. SAALFELD[1], D. D. BOCK[1];
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Abstract: The mushroom body (MB) of the *Drosophila* olfactory system is critical for olfactory learning and memory. Intrinsic neurons of the MB, Kenyon cells (KCs), receive olfactory inputs from projection neurons in the calyx, converge to form the pedunculus, and bifurcate to form the vertical and medial lobes. We used a second-generation high throughput transmission electron microscopy (EM) camera array (TEMCA2) to acquire a large-scale EM dataset of 863 serial thin (<50 nm) horizontal sections (z = ~34.5 μm, 4x4x40 nm/voxel) through the entire adult *Drosophila* brain, centered on the pedunculus of MB. After stitching and registration, the resulting image volume comprises 1.9 million 8-bit camera images consuming 12 TB of disk storage, and each section is 150,000 x 95,000 pixels (600 x 380 μm) in width and height. We used the web-based annotation tool CATMAID (Saalfeld et al. Bioinformatics 2009) to reconstruct a subset of neurons within the volume with skeleton tracing. We identified 32 KCs by their inputs from olfactory projection neurons in micro-glomeruli of the calyx (Butcher et al. J Comp Neurol 2012) and traced them to completion within the volume. Their relative locations, morphology, and trajectories in the pedunculus and γ-lobe match those of γ KCs in light microscopy data (Aso et al. eLife 2014). Our tracings reveal that γ KCs form small synapses with each other in pedunculus. Additional possible sites of synaptic interaction are observed in calyx and the γ1 lobe, often in proximity to synapses between the reconstructed KCs and a single postsynaptic MBON innervating γ1. In a parallel effort at Janelia using a FIB-SEM dataset, similar motifs have also been observed in the α-lobe of the mushroom body (Janelia Fly EM Project, unpublished data). Based on arbor structure, innervation area and γ KCs as a source of input, we identified the MBON as γ1-pedc>a/B (Aso et al. eLife 2014). A connectivity graph of 32 γ KCs that are fully reconstructed in the EM volume is revealed. After initial sampling of olfactory space by KCs in the mushroom body calyx, the synaptic interactions we observe between KCs in the pedunculus may allow channel mixing of olfactory information prior to arrival of olfactory information in the associational compartments of the MB.

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Poster

230. Olfaction: Higher Order Processing

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HHMI

Title: Plasticity-driven individualization of olfactory coding in mushroom body output neurons

Authors: ***T. HIGE**[1], Y. ASO[2], G. M. RUBIN[2], G. C. TURNER[1];
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Abstract: Although all sensory circuits ascend to higher brain areas where stimuli are represented in sparse, stimulus-specific activity patterns, relatively little is known about sensory coding on the descending side of neural circuits, as a network converges. In insects, mushroom bodies (MBs) have been an important model system for studying sparse coding in the olfactory system, where this format is important for accurate memory formation. In *Drosophila*, the 2000 Kenyon cells (KCs) of the MB converge onto a population of only 35 MB output neurons (MBONs). Here we provide the first comprehensive view of olfactory representations at the fourth layer of the circuit, where we find a clear transition in the principles of sensory coding, and demonstrate their flexibility. We show that MBON tuning curves are highly correlated with one another. This is in sharp contrast to the process of progressive decorrelation of tuning in the earlier layers of the circuit. Instead, at the population level, odor representations are reformatted so that positive and negative correlations arise between representations of different odors. At the single-cell level, we show that uniquely identifiable MBONs display profoundly different tuning across different animals. Interestingly, tuning of the same neuron across the two hemispheres of each fly was nearly identical. These results show that individual-specific coordination of tuning arises at this level of the circuit. Furthermore, we find that this individualization is an active process that requires a learning-related gene, rutabaga. Ultimately, neural circuits have to map highly stimulus-specific information in sparse layers onto a limited number of different motor outputs, in a way that is flexible. The reformatting of sensory representations we observe in the MBON layer may mark the beginning of this sensory-motor transition in the olfactory system.

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Poster

231. Auditory Processing: Neural Coding, Experiment, and Theory

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Topic: D.02. Auditory System

Support: NIH (NIDCD) 5R01DC013906-02

NSF 0924750

Title: Neural correlates of auditory scene analysis in the primate inferior colliculus

Authors: *D. S. PAGES[1,2], V. C. CARUSO[1], J. M. GROH[1,2,3];
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Abstract: Humans and monkeys can perceive multiple sounds simultaneously. However, it is still unknown how the mammalian auditory system parses free-field stimuli in order to create a 'menu' of sound streams for cortical attention processes to select from. This is a problem for the brain because in the free-field case, each sound stream generally arrives at both ears. Here, we use single-unit and multi-unit recordings in the primate inferior colliculus (IC) to investigate the encoding of scenes containing multiple sounds. A methodological challenge in interpreting neural responses to multiple stimuli is knowing which individual stimulus in the scene triggered each spike in the response; in other words, which individual stimulus is 'represented' by the neurons at each point in time. The auditory system including the IC offers a good setting to study this: spikes in the IC can phase lock to the sound that evoked them. We reasoned that it if phase locking is maintained in the presence of multiple sounds, we could use the frequency of phase locking to assign each spike in a response to the sound that evoked it: a frequency-tagging approach. . We presented auditory environments containing two simultaneous sounds to two passive rhesus monkey listeners. Sounds were always presented from separate locations and had different envelope frequencies (amplitude modulations, AM). We tested two scenarios: sounds that differed in carrier frequency (spectral content) vs. sounds that had the same carrier frequency. We found a robust relationship between the neuron's physiological frequency preferences in the single sound case and its phaselocking preference in the dual sound case. Thus, the auditory scene was parsed according to the same tonotopic gradient that was used for single sounds, creating a tonotopic 'menu' of the auditory scene. In the case when the sounds had the same spectral content, the IC used a different scheme: spatial location itself provided a means of sorting. Most neurons represented the sound contralateral to themselves, creating a spatial 'menu' of the scene. This spatial sorting cannot be explained by the spatial encoding of single-sounds, because the effect does not positively correlate with spatial tuning for single sounds, and furthermore, the dual-sound spatial tuning is significant at the population level even among the subpopulation of sites with minimal/no spatial tuning for single sounds. . Our results demonstrate that the IC is capable of parsing apart auditory scenes containing multiple sounds, and can change between different mechanisms depending on the cues available.

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Poster

231. Auditory Processing: Neural Coding, Experiment, and Theory

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Topic: D.02. Auditory System

Support: NIDCD Grant R01 DC012949

Title: The code biasing the owl's orienting behavior in situation of uncertainty

Authors: *F. CAZETTES[1], B. J. FISCHER[2], J. L. PENA[1];
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Abstract: How the brain, often a biased estimator, translates sensory information into adaptive behavioral responses is still a matter of debate. We study this question in the sound localization system of the barn owl. We have previously shown that neurons in the owl's midbrain map of auditory space are tuned to the most reliable auditory cue and that the degree to which a cue can be trusted is encoded in the shape of tuning curves. The next outstanding question is how the neural population is readout to capture cue reliability in the behavioral response. We tested the hypothesis that a population vector captures cue reliability and explains the biased orienting behavior of the owl in situation of uncertainty. We examined if a vector decoder can be computed by a population of neurons in the owl's midbrain tegmentum, which directly commands head orientation. We showed that convergence and normalization from the midbrain map leads to the emergence of a population vector in the tegmentum. We further demonstrated that manipulating the sensory input to modify specific response properties of the midbrain population can enhance or reduce the owl's behavioral bias in a manner predicted by the population vector and by the population response in the tegmentum. This is the first time a biased behavior has been explained at the encoding, decoding and behavioral levels.

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Poster

231. Auditory Processing: Neural Coding, Experiment, and Theory

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Support: NIM/NIDCD F31 DC013502

NIH/NIMH T32 MH019524

Title: Amplitude modulation rate discrimination and cortical encoding in the awake-behaving gerbil

Authors: *G. VON TRAPP, M. N. SEMPLE, D. H. SANES;
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Abstract: Fluctuations in sound amplitude (AM) are an important component of animal communication signals. Here, we explore the relationship between perceptual sensitivity and auditory cortex neuron encoding of AM rate in gerbils. Freely-moving animals were tested on an AM rate discrimination task while recording telemetrically from an electrode array implanted in core auditory cortex. Animals received a water reward on correct responses to target stimuli ("Go" trials), and no reward on trials that did not contain the target ("Nogo" trials). We measured the sensitivity of auditory cortex neurons to identical stimulus sets during two behavioral conditions: (1) Discrimination of any "Go" token greater than 4 Hz (the "Nogo") or (2) discrimination of any "Go" token slower than 32 Hz (the "Nogo"). Neural responses were also measured when animals were not engaged in the task. Psychometric analysis of AM rate discrimination thresholds showed similar sensitivity for both behavioral conditions (JND was 0.98 (n=5) and 0.91 (n=7), respectively). The sensitivity of individual auditory cortex neurons to AM rate stimuli were quantified using metrics of both discharge rate and temporal pattern, and compared to the behavioral thresholds. (Funding: F31DC013502)

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Poster

231. Auditory Processing: Neural Coding, Experiment, and Theory

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Topic: D.02. Auditory System

Support: DFG Cluster of Excellence 'Hearing4All'

Action on Hearing Loss 549:UEI:JL

Gatsby Charitable Foundation

Title: Activation of parvalbumin-positive interneurons enhances transient responses and changes tuning of offset responses in awake auditory cortex

Authors: *K. J. HILDEBRANDT[1], P. J. GANÇALVES[2], M. SAHANI[2], J. F. LINDEN[3];
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Abstract: Alterations of cortical inhibition have been proposed to play a crucial role in modulation of cortical activity. While optogenetic manipulation of different functional groups of interneurons has become an important tool for studying the roles of different cells in sensory

processing, the timing of light relative to sensory stimulation is often a confounding factor, and the pattern of supra-threshold activation of inhibitory neurons may not be physiologically accurate. Here, we circumvent these limitations by using stable step-function opsin (SSFO), which can be rendered continuously active or inactive with short pulses of light. We expressed SSFO in parvalbumin-positive (PV+) interneurons in the primary auditory cortex of mice, and recorded both local field potentials (LFP) and spiking responses to tone pips of varying frequency in awake animals. Prolonged low-level activation of PV+ cells profoundly changed the dynamics of spike responses in several and diverse ways. While spontaneous activity and sustained responses to tones were mostly suppressed during PV+ activation, onset and offset responses where either enhanced or reduced less than spontaneous responses, thus increasing signal-to-noise ratio. Frequency tuning of offset responses changed, with best frequencies shifted downwards by as much as an octave. The tuning shift was much less pronounced in sustained and onset responses. PV+ activation also resulted in narrower frequency tuning of offset responses in the majority of recorded cells. Intriguingly, tuning broadened for a small fraction of units whose spontaneous activity increased during activation. Possibly, these units were PV+ cells directly activated by SSFO. Analysis of LFP data confirmed the contrary effect of PV+ activation on transient and sustained responses. Activation of SSFO caused a decrease of the power in the high-gamma range (50-150Hz) during spontaneous and sustained tone-response phases. Both onset and offset responses were boosted compared to control, and offset responses increased more than onset responses. Generally, SSFO activation increased power in the low-frequency range of the LFP (<50Hz) and decreased power in the high-frequency range (50-150Hz, high gamma). In summary, our experiments show that prolonged low-level activation of PV+ cells with SSFO increases the signal-to-noise ratio of transient responses to sound and changes tuning of offset responses. These results indicate that alterations of inhibition have a profound impact on cortical network dynamics.

Disclosures: **K.J. Hildebrandt:** None. **P.J. Gançalves:** None. **M. Sahani:** None. **J.F. Linden:** None.

Poster

231. Auditory Processing: Neural Coding, Experiment, and Theory

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 231.05/J33

Topic: D.02. Auditory System

Support: ANR SENSEMAKER

MARIE CURIE BRAINSENSE

Title: Cortical population nonlinearities reflect asymmetric auditory perception in mice

Authors: A. KEMPF[1], T. DENEUX[1], P. EMMANUEL[2], *B. BATHELLIER[1];
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Abstract: Natural sounds display strong temporal variations of intensity, which are part of the features influencing perception and recognition of sounds. Strikingly, for example, humans perceive a tone that increases in intensity as louder than same tone with a decreasing intensity profile, although both sounds have the same energy and frequency content. The underlying neuronal mechanisms of this perceptual asymmetry are still elusive. To test if the direction of intensity variations is asymmetrically processed by the auditory system, we have measured the activity of large populations of neurons in the auditory cortex of awake mice using GCAMP6 two-photon calcium imaging. Pooling a large number of recordings together, we observed that the time integral of cortical population firing rate is much larger for sounds ramping-up than for sounds ramping-down. This asymmetry demonstrates that cortical population response is strongly non-linear. To test for perceptual consequences of this non-linearity, we performed behavioral experiments in which the saliency of a sound is measured through associative learning speed. We observed that increasing ramps are more rapidly associated to a correct behavior than decreasing ramps, showing that the asymmetry of cortical population responses reflects an asymmetry in perceived saliency. Moreover, finer analysis of cortical data indicate that ramps produce complex population activity sequences in which distinct patterns emerge depending on the direction of sound intensity variations. Based on simple population models, we show that the asymmetry of population firing rate and the complexity of activity sequences could be explained by competing populations of neurons encoding different aspects of the temporal profile (e.g. sound onset, offset). Beyond proposing a mechanism for a perceptual asymmetry that may emphasize approaching sound sources, our results suggest that non-linear interactions between temporal feature detectors could be one of the bases of sound recognition.

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Poster

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Action on Hearing Loss 549:UEI:JL

Title: Local sensory context modulates responses to complex sounds in multiple brain areas along the auditory pathway

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Abstract: Neurons in the auditory system are sensitive not only to the spectro-temporal pattern of a sound but also to the context in which that pattern occurs. Spectro-temporal sensitivities of neurons have been extensively studied using the spectro-temporal receptive field (STRF), a linear model relating a sound to the evoked response. However, little is known about the context-dependence of these sensitivities during stimulation with complex sounds. We characterized neuronal responses using the context model [1], a previously proposed form of multilinear model [2] which simultaneously characterizes both a linear "principal receptive field" (PRF) and a "contextual gain field" (CGF). The CGF describes how local gain at a particular point in the PRF is modulated by local stimulus context. We fit the context model to spiking data recorded in inferior colliculus, auditory thalamus and primary auditory cortex of rodents, as well as publicly available data sets [3] from homologous auditory areas in birds, all recorded during presentations of complex but stationary broadband sounds. In every case, the context model provided a more accurate description of neuronal responses than did the linear STRF model, yielding an increase in cross-validated predictive power of about 20%. Local facilitation in CGFs varied, but all areas shared a common pattern of reduction in input gain by preceding sound energy within about an octave range -- an input-frequency-specific, divisive form of forward suppression. The temporal structure of this suppression varied, with latencies ranging from 5-15 ms in birds and up to 80 ms in rodents. Both latency and temporal extent increased from midbrain to forebrain. Using analytical results and simulations, we demonstrate that a cascaded context model can account for the increasing extent of contextual gain effects along the ascending auditory pathway. The results suggest that these nonlinear features of sensory neurons, which are not captured by linear STRFs, are transformed along the auditory pathway in a feed-forward manner. Further exploration of these mechanisms may help to elucidate the role of nonlinear integration in generating more complex sound representations in the auditory system. References [1] Williamson, RS et al. (2011) Program No. 693.23/MM24. Neuroscience Meeting Planner. Washington, DC: SfN. Online. [2] Ahrens, MB et al. (2008) J Neurosci 28(8):1929-1942. [3] Theunissen, FE et al. (2011) CRCNS.org.

Disclosures: **A.F. Meyer:** None. **J.F. Linden:** None. **M. Sahani:** None.

Poster

231. Auditory Processing: Neural Coding, Experiment, and Theory

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Topic: D.02. Auditory System

Support: NIDCD DC-011580

Title: Swarm intelligence meets the brain: estimating cellular parameters related to auditory processing in young and aged rats using particle swarm optimization

Authors: *B. S. COVENTRY[1], A. PARTHASARATHY[1], E. BARTLETT[1,2];
[1]Weldon Sch. of Biomed. Engin., [2]Dept. of Biol. Sci., Purdue Univ., West Lafayette, IN

Abstract: Age-related hearing loss affects 29% of male and 23% of females over the age of 60 (Yamasoba et al, 2013) and is marked by deficits in processing speed (Wingfield et al, 1985) and difficulty with speech in complex listening environments (Frisina and Frisina, 1997). The inferior colliculus (IC) is a major auditory integrative center, receiving inputs from cochlear nuclei, the superior olivary complex and nuclei of the lateral lemniscus (Kelly & Caspary, 2005). How these inputs converge to generate complex frequency and temporal processes is not well understood. Moreover, while the synaptic and membrane properties that shape this processing have largely been examined in isolation, their interactions are poorly understood. In addition, age-related changes in GABAergic markers upset the delicate balance of excitation and inhibition in the IC (Caspary et al, 1999; Palombi and Caspary, 1996). Here we utilize a modified version of our previously published IC computational model (Rabang et al., 2012) to elucidate synaptic input parameters that fit individual recorded single unit responses in IC, using the NEURON programming environment under control of Python (Hines et al, 2009; Carnevale and Hines, 2005). To fit responses of the model to responses from single unit recordings of the rat IC, the model was modified to incorporate particle swarm optimization (PSO), an optimization method which models social networks of flocking birds or schooling fish to solve mathematical problems (Kennedy and Eberhart, 1999). We have recently developed a new PSO social network that mimics visual cortex winner-take-all coding (WTAPSO). WTAPSO has shown excellent performance in higher dimensional problem solving (Coventry & Bartlett, in prep) compared to other PSO algorithms. With computational models linked with WTAPSO, we will test whether age-related changes in frequency tuning can be explained by changes in synaptic parameters, such as GABAergic inhibition, consistent with what has been seen *in vivo*. Additionally, we will explore other synaptic mechanisms, such as those involved in onset and sustained responses and how they contribute to age-related processing deficits. WTAPSO can be applied widely to optimization problems such as those encountered in neuronal modeling.

Disclosures: B.S. Coventry: None. **A. Parthasarathy:** None. **E. Bartlett:** None.

Poster

231. Auditory Processing: Neural Coding, Experiment, and Theory

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Support: American Federation for Aging Research

NIDCD DC-011580

Title: Representations of voice onset timing cues in the inferior colliculus of young and aged rats

Authors: *C. S. SOVERNS[1], A. PARTHASARATHY[2], E. L. BARTLETT[1,2];
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Abstract: Specific timing cues are important in addition to firing rate for speech recognition in the auditory pathway. Of particular interest are neural representations of complex sounds and sound attributes, such as the consonant-vowel transition, and how such patterns change with age. Here we have investigated specific firing patterns in response to a vocalization as voice onset time (VOT) was varied along the ba-pa spectrum. Extracellular recordings were made in the inferior colliculus (IC) of anesthetized young adult and aged male rats in response to the acoustic stimuli. Standard and novel analysis techniques were employed to extract specific features of the responses. Such features identify representation strategies likely to be used by IC neurons to communicate VOT-related information leading to perception of the respective consonant-vowel combination. The Victor-Purpura spike distance metric was utilized to contrast firing patterns across stimuli and units and to investigate potential degradation of repeatability with age. A recent fusion of wavelet decomposition and information theory was utilized for identification of time scales and temporal locations of characteristic features. With knowledge of such features we sought to construct distinct firing pattern templates for the stimuli, identify patterns of age-related degradation, and inform future efforts to restore perception in degraded cases. Rather than a global agreement or disagreement of response patterns across the population, we have found evidence for local clustering of neurons with distinct regions sharing similar response properties.

Disclosures: C.S. Soverns: None. **A. Parthasarathy:** None. **E.L. Bartlett:** None.

Poster

231. Auditory Processing: Neural Coding, Experiment, and Theory

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Topic: D.02. Auditory System

Support: BFNT 01GQ0840

Title: Chronic calcium imaging of neuronal ensembles in the mouse auditory cortex

Authors: *D. F. ASCHAUER[1], J.-B. EPPLER[2], M. KASCHUBE[2], S. RUMPEL[1];
[1]FTN - Rumpel Lab., Johannes Gutenberg-University Mainz, Mainz, Germany; [2]FIAS, Johann Wolfgang Goethe Univ., Frankfurt, Germany

Abstract: We recently found that the activity of local neuronal ensembles of the auditory cortex encode broad sound categories into non-linear population response modes (Bathellier et al., Neuron 2012; 76, 435-449). The discrete responses of these ensembles generate a basis set of perceptual categories that can be used to drive behavioral decisions. Additionally, chronic *in vivo* imaging approaches have demonstrated that the structural correlate of the postsynaptic component of excitatory synapses, dendritic spines, are highly dynamic throughout the life time of an animal (Loewenstein et al., J. Neurosci. 2011; 31(26):9481-9488). The interplay between these two very distinct but inherently interconnected types of dynamics has been challenging to address. In particular, how do functional neuronal ensembles emerge in the context of dynamic connectivity? How is information (e.g. sensory perceptions) maintained in such constantly rewiring neural circuits? Classical neurophysiological approaches, like extracellular recordings of action potentials, have largely not been able to provide chronic observations of activity dynamics due to technical limitations. Towards this goal, we established a procedure to monitor the activity of local neuronal ensembles of the auditory cortex in response to sounds over prolonged periods of time in the awake animal. Using AAV-mediated neuronal expression of the genetically encoded calcium indicator GCaMP6m enabled measurements of a few hundred individual cells simultaneously. Alignment of neuronal populations within a recording session to compensate for movements of the animal and alignment across imaging sessions at a two day interval is controlled by the co-expression of the stable nuclear marker H2B:mCherry. We developed automated image analysis routines that allow compensation for movement and detection of frames that cannot be reliably aligned. We are currently analyzing this dataset to measure the stability of functional neuronal ensembles forming auditory representations over the period of several days to more than a week. This question is of fundamental interest given the volatility in structural connections between neurons of the neocortex.

Disclosures: **D.F. Aschauer:** None. **J. Eppler:** None. **M. Kaschube:** None. **S. Rumpel:** None.

Poster

231. Auditory Processing: Neural Coding, Experiment, and Theory

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Program#/Poster#: 231.10/J38

Topic: D.02. Auditory System

Support: BFNT 01GQ0840

Title: Modeling discrete cortical representations and their stability in the presence of synaptic turnover

Authors: ***J.-B. EPPLER**[1,2], D. ASCHAUER[3], S. RUMPEL[3], M. KASCHUBE[1,2];
[1]FIAS, Frankfurt, Germany; [2]Johann Wolfgang Goethe Univ., Frankfurt, Germany; [3]FTN - Rumpel lab, Johannes Gutenberg Univ., Mainz, Germany

Abstract: Synapses in the auditory cortex display prominent changes in size over a period of several days, even in the absences of any explicit learning paradigm (Loewenstein et al., J. Neurosci. 2011; 31(26):9481-9488). It is currently unclear to what extent this volatility in structural connections impacts the functional properties of cortical circuits. Here we address this issue by studying the robustness of responses to brief complex sounds in a circuit model of mouse auditory cortex. Population responses in this system were observed to typically cluster into one out of a small number of observed states (Bathellier et al., Neuron 2012; 76, 435-449), a property we aim to exploit here to reveal the stability of such near discrete representations under synaptic turnover. First, we propose a model class consisting of networks of firing-rate neurons, which robustly displays several key properties of population activity in the mouse auditory cortex, including a skewed distribution of activity across neurons and a clustering of broad sound categories into non-linear population responses. In a broad parameter regime where recurrent connections are sufficiently heterogeneous and the network is dominated by inhibition, different sounds modeled as temporally varying random inputs are grouped spontaneously into a small number of different activity clusters. The degree of clustering is largely independent of network size (after rescaling synaptic strengths appropriately). Second, we use this model to study systematically the impact of synaptic turnover on collective response properties. We change the strength in a subset of connections and monitor the amount of drifts this induces in the activity vectors themselves and in the cluster means. We also assess to what extent stimuli get re-mapped to different activity clusters under synaptic turnover. Predictions of our model are tested in parallel experiments employing chronic two-photon imaging of population activity in mouse auditory cortex.

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Poster

231. Auditory Processing: Neural Coding, Experiment, and Theory

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Topic: D.02. Auditory System

Support: BFNT #01GQ0811

Title: Prediction of neural population responses to complex acoustic stimuli

Authors: *D. LYZWA;
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Abstract: Reliable prediction of neural responses to complex sounds in the midbrain is desirable and would have important medical applications. In this work, a generalized linear model for

responses from single neurons and groups of a few neurons is presented. It models responses from the midbrain of guinea pigs and covers the main auditory frequency range, and furthermore includes several values for amplitude modulation preferences. The model is derived from neural responses to a broad range of acoustic stimuli, including artificial sounds such as white noise, pure tones and clicks, as well as complex natural sounds like calls and songs. This generalized linear model is compared to previous findings which predict temporal spiking responses to acoustic stimuli based on a) specific frequency tuning of the neurons, b) biophysically detailed modeling, or c) based on first and second order spectrotemporal receptive fields. This model reliably predicts a range of complex natural sounds.

Disclosures: D. Lyzwa: None.

Poster

231. Auditory Processing: Neural Coding, Experiment, and Theory

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Title: Change in brain state leads to stimulus information degradation through the thalamocortical circuit

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Abstract: Brain state is known to influence evoked activity in the cortex and is ultimately involved in sensory perception. For example, wakefulness is associated with a high degree of perception, whereas little sensory information is perceived during sleep. Quite how and where information degrades through cortical circuits remains unknown. Here we look at evoked spiking activity at the first stage of cortical processing using recordings from the auditory thalamus (MGB) and across layers in primary auditory cortex (A1) in rats. 5ms white noise was presented over a range of intensities in two distinct brain states: the inactivated state, characterised by

strong collective activity across units; and the activated state, characterised by desynchronised firing activity. The inactivated state is natural under urethane anaesthesia, whilst the activated state was induced through electrical stimulation of the nucleus basalis, a region known to be involved in neuromodulation of cortical dynamics. We find that multiunit spiking activity differs depending on recording location and brain state, and that A1 evoked activity consists of separate initial and secondary components. Initial A1 activity correlates strongly with MGB activity, and secondary A1 activity correlates strongly with initial A1 activity, demonstrating propagation of activity through the circuit. Using mutual information to quantify stimulus information in spike counts at the single unit level, we find that during the initial stage of activity the most significantly modulated units are found in MGB, thalamorecipient layer and upper infragranular layers. These locations also contain the most informative units and display significantly more stimulus information during the activated state. During secondary activity, we find that much fewer units are significantly modulated by stimulus than in the initial activity. These units are mostly found in upper infrangranular layers, where the most informative units are found. Again, stimulus information is significantly greater during the activated state. The secondary stage is less informative than the initial stage, but relative decrease in information between states is larger, suggesting an increased propensity for local information loss in the inactivated state in cortical circuits. In summary, our results suggest that information flows through this first stage of cortical sensory processing similarly between brain states, but that the inactivated state is associated with less informative input from the thalamus, worse stimulus intensity representation in A1, and increased degradation of information as activity propagates through cortical circuits.

Disclosures: **J. Bamber:** None. **S. Sakata:** None. **J. Herrmann:** None.

Poster

231. Auditory Processing: Neural Coding, Experiment, and Theory

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Title: Quantifying temporal structure of auditory cortex multineuronal patterns

Authors: ***J. DECHERY**[1,2], J. N. MACLEAN[3,1];
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Abstract: The complexity of natural auditory stimuli is derived from the temporal features of one dimensional pressure fluctuations in atmospheric pressure. Multineuronal activity patterns in auditory neocortex, whether evoked by sound or arising spontaneously, can be similarly characterized by their temporal features. A fundamental step toward understanding the neural

representations of sound is a quantitative description of the dynamics of these multineuronal patterns. This is especially true in neocortex, where recurrent connectivity dominates and neurons must work in concert to encode behaviorally relevant stimulus features. Precision of spike timing is of particular interest, due to its potential for reliable information representation and its elusive mechanistic underpinnings. Here, we analyze temporal aspects of naturalistic network dynamics in A1 acute slices at the single cell, pairwise, and network level. Spiking activity is measured in populations as large as 1000 neurons with two photon calcium imaging. By comparing these recordings to simulated data that preserves firing rates but destroys pairwise temporal information, we statistically determine the temporal characteristics not driven solely by spike rate. We identified subsets of neurons that have very reliable and temporally structured activation patterns relative to both intrinsic and extrinsic reference points. At the pairwise level, highly correlated neurons acted as building blocks that together formed reliable sequences of propagating network activity. Finally, we explored the dynamics underlying an observation that spike-time variability increases throughout a multineuronal pattern. This analysis acts to both describe the dynamics of neocortical neurons solely driven by local connectivity, as well as to contribute to the conceptualization of networks as more than the sum of their components. Our results imply that activity in A1 can be described as sequences of reliable and structured multineuronal activity patterns. Characterizing the variance and stereotypy of activity propagating through A1 networks will be key to understanding how the underlying synaptic inputs drive spiking and how the networks themselves encode and transform auditory input.

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Poster

231. Auditory Processing: Neural Coding, Experiment, and Theory

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Title: Cortical representations of stimulus intensity of cochlear implant stimulation in awake marmosets

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Abstract: Electrical stimulation of the cochlear nerve via a cochlear implant (CI) is successful in restoring auditory sensation to individuals with profound hearing loss. However, many questions

remain unanswered regarding how the central auditory system processes the electrical stimulation. Understanding how the brain processes CI stimulation should help guide improvement of CI technology, but techniques for studying human cortical processing have low spatial resolution, and the extent to which non-primate or anesthetized animal models represent the human case is unclear. We therefore developed an alert non-human primate CI model in the common marmoset (Callithrix jacchus) by implanting a multi-channel electrode array in one cochlea while leaving the other cochlea acoustically intact. This preparation allows us to directly compare a cortical neuron's responses to acoustic and CI stimulation in the awake condition. We found that acute, episodic CI stimulation was less effective in activating primary auditory cortex (A1) neurons compared to acoustic stimulation. This may be explained by broader cochlear excitation areas caused by electric stimulation compared to acoustic stimuli, because many cortical neurons exhibit narrow frequency tuning and sideband inhibition. For neurons driven by both CI and acoustic stimuli, we characterized responses as a function of current level and sound intensity. A majority of these neurons showed monotonic responses to CI stimuli; less than 20% had non-monotonic responses. This compares to acoustic responses which had 40% non-monotonic responses. We observed that ~40% of non-monotonic CI-driven neurons showed monotonic responses to acoustic stimuli, while ~25% of monotonic CI-driven neurons showed non-monotonic responses to acoustic stimuli. This change of response pattern in the same neuron suggested that CI and acoustic stimuli evoked different neural circuits. Consistent with clinical psychophysical data from CI users, dynamic ranges of A1 cortical neuron responses to CI stimuli were much smaller than those to acoustic stimuli (3.4 dB vs 32 dB in our experiments). For a given A1 neuron, thresholds of CI and acoustic stimuli were positively correlated, but their dynamic ranges were not significantly correlated. Response latencies were also positively correlated between the two stimulation types. In addition, acoustic stimuli usually evoked greater firing rates than CI stimuli. These findings suggest that the coding mechanism for stimulus intensity differs between the stimulation modes, and that CI stimulation is less efficient in driving A1 neurons.

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Poster

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Action on Hearing Loss (UK-US Fulbright Commission scholarship; M.S.)

Title: Spectro-temporal dynamics of excitatory, suppressive, and inhibitory influences in the auditory nerve and ventral cochlear nucleus following sensorineural hearing loss

Authors: *M. G. HEINZ[1], K. S. HENRY[3], M. SAYLES[2,4];
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Abstract: Nonlinear cochlear signal transduction, reflecting outer-hair-cell function, manifests as suppressive spectral interactions; e.g., decreased auditory-nerve-fiber (ANF) firing rate to one tone when a second, simultaneous tone is presented. For broadband sounds, there are multiple interactions between spectral components. These frequency-dependent nonlinearities are important for neural coding of speech. Outer-hair-cell damage in hearing-impaired mammals is associated with loss of nonlinearity, which auditory prostheses attempt to restore, e.g., with "multi-channel compression" algorithms. At the first stage of brain-stem processing, the ventral cochlear nucleus, many neurons receive frequency-tuned inhibitory input in addition to ANF excitatory input. Emerging evidence from evoked-potential studies suggests "central gain" changes following acoustic trauma; which may indicate altered inhibitory-excitatory balance in synaptic input to brain-stem nuclei, or changes in cochlear nonlinearity reflected in their ANF input. We used spike-triggered neural-characterization techniques to probe altered spectro-temporal dynamics of excitatory and suppressive/inhibitory influences on ANF and VCN-unit firing probability in anesthetized chinchillas following noise-induced hearing loss.Hearing-impaired (HI) animals had elevated single-unit excitatory thresholds (by ~20-40 dB), broadened frequency tuning, and reduced-magnitude distortion-product otoacoustic emissions; consistent with mixed inner- and outer-hair-cell pathology, and loss of nonlinearity. We characterized suppression (ANFs) and suppression/inhibition (VCN units) using second-order Wiener-kernel (h_2s) analyses of responses to broadband noise (ANFs: 92 NH, 148 HI; VCN units: 122 NH, 95 HI). Using singular-value decomposition, we factored h_2s into excitatory and suppressive/inhibitory sub-kernels, to quantify the relative strength and spectro-temporal characteristics of suppression/inhibition. Suppression strength was decreased in HI ANFs, which correlated with broadened frequency tuning. In the VCN, we recorded from all major unit types in NH and HI animals, with no difference in their relative distribution (χ^2, $p>0.05$). We found evidence for altered balance between suppressive/inhibitory and excitatory influences on VCN units following noise-induced hearing loss, consistent with recent evidence for central-gain change following acoustic trauma. These data help guide novel amplification strategies, particularly for complex listening situations (e.g., speech in noise), in which current hearing aids struggle to restore intelligibility.

Disclosures: **M.G. Heinz:** None. **K.S. Henry:** None. **M. Sayles:** None.

Poster

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Topic: D.02. Auditory System

Title: Single trial decoding of task-relevant variables in auditory and frontal cortex during sensorimotor decision-making

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Abstract: Single-unit activity recorded from behaving animals can often have heterogeneous response profiles. A fraction of recorded cells typically exhibit trial-averaged responses with obvious task-related features, such as pure tone frequency tuning in the auditory cortex, or ramping activity in secondary motor areas. However, a substantial number of cells do not appear to fire in a task-related manner and require novel analytical methods. We analyzed single-units recorded from rats during a frequency recognition task in order to identify to what extent task variables are reflected in individual spike trains of every recorded neuron - independently or as part of small ensembles of cells. Adult rats were trained on a go/no-go frequency recognition task that required them to nosepoke to a single target tone for food reward and withhold from responding to multiple nontarget tones. Using multielectrode arrays we recorded from 59 single-units in the auditory cortex and 57 single-units in the frontal cortex (FR2). Spike trains were complex and highly variable from trial to trial. However, these single-trial responses are the only signals available to downstream neurons. Accordingly, we devised a novel unbiased spike-timing based algorithm for trial-by-trial decoding. Surprisingly, we found that frontal cortex is more informative about stimulus category than auditory cortex, and cells in auditory cortex can be informative about the upcoming behavioral choice. Furthermore, decoding improves when using ensembles of cells demonstrating that the approach is scalable. Our method reveals that responses from nominally non-responsive cells, which are often neglected, can contain a significant amount of information about task relevant variables.

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Program#/Poster#: 231.17/J45

Topic: D.02. Auditory System

Support: NIH Grant DC011843

Title: Encoding and decoding of amplitude modulated signals amongst populations in auditory core of awake squirrel monkeys

Authors: *M. J. RUNFELDT[1], B. J. MALONE[2];
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Abstract: Neurons in primary auditory cortex simultaneously encode information about multiple stimulus dimensions, including the envelope and fine structure of modulated signals. Individual neurons can exhibit carrier-bandwidth, carrier-frequency, and carrier level-specific modulation tuning, which can be expressed in the neuron's firing rate and/or the timing of spikes relative to the phase of the modulating waveform. It is unclear how the response properties of individual neurons shape the information available to downstream 'read-out' neurons that receive multiple simultaneous inputs. Using simultaneous multichannel recordings from awake squirrel monkey cortex, we analyzed the responses of local neural populations to sinusoidal amplitude modulation (4 to 512 Hz) applied to pure tone and noise carriers. We focus our analyses on how network- and stimulus-driven spiking correlations, temporal coherence, and diversity within single neuron tuning properties impact population decoding of signal modulation frequency. In a simple convergent model based on spike summation, pooling the activity of multiple neurons can either increase or decrease decoding performance depending on the interplay between spiking patterns among neurons comprising the encoding population. For example, pooling across neurons that fire at different phases with respect to the modulation envelope will limit the synchrony of the pooled response, and decrease information about the envelope carried by spike timing. Analyzing multi-unit activity, we found that increasing the number of simultaneously-recorded channels that contribute to a summed population response is detrimental to the decoding of modulation frequency on average. When intertrial correlations were removed by shuffling responses across trials, however, adding more channels almost always improved performance. We explore whether this reflects loss of information due to signal averaging and/or redundancy in local populations. Additionally, using single-unit activity from spike-sorted data, we identify optimal encoding populations and characterize the features that contribute to their performance.

Disclosures: **M.J. Runfeldt:** None. **B.J. Malone:** None.

Poster

231. Auditory Processing: Neural Coding, Experiment, and Theory

Location: Hall A

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Program#/Poster#: 231.18/J46

Topic: D.02. Auditory System

Title: Differential effects of multiple anaesthetic regimes on spontaneous, sensory and multisensory recordings from guinea pig cortex

Authors: *O. WOOLNOUGH, J. I. BERGER, B. COOMBER, M. N. WALLACE, A. R. PALMER, C. J. SUMNER;
MRC Inst. of Hearing Res., Nottingham, United Kingdom

Abstract: Anaesthesia is widely used in *in vivo* studies of sensory neural processing. Previous studies of the effects of systemic general anaesthesia on neurons in the auditory cortex have shown significant changes to frequency tuning and responses to basic features within the stimulus such as onsets and offsets. Studies of this nature have typically used a single anaesthetic agent, comparing awake and anaesthetised states, and it remains unclear to what extent the choice of anaesthetic agent will affect basic response properties of neurons to sensory stimulation. EEG is a widely used technique in humans and as it is a relatively simple protocol to implement in animals could provide an important methodological bridge between species. However, the effects of anaesthesia on EEG are relatively poorly characterised in animals. Electrophysiological recordings were made with chronically implanted, extradural electrodes, positioned over auditory and visual cortices. Recordings were made both while awake and under a range of anaesthetic regimes including opiates, NMDA antagonists and GABA potentiators. Recordings of spontaneous oscillations replicate the results of previous human studies, showing a rapid increase in power of low-frequency (<10Hz) oscillations at loss of consciousness, most prominently in the visual regions, and suppression of high frequency activity - effects which appear mostly independent of anaesthetic regime. Responses to a range of basic sensory stimuli such as auditory clicks, visual flashes and sequences of adapting stimuli were also recorded. Unlike spontaneous activity, we observed substantive differences in processing of even basic sensory stimuli between anaesthetic regimes. For example, the cortical auditory evoked potential in response to a tone shows an early (~25ms) and late (~50ms) deflection. Under ketamine anaesthesia both potentials were suppressed, diazepam supressed the early potential but enhanced the later one and fentanyl and urethane both increased the size of the early potential but abolished the later one. We have also observed significant changes between anaesthetics in the time constants governing the onset and recovery from adaptation and, under all anaesthetic regimes tested, auditory responses in visual cortex were absent. In conclusion, we have demonstrated that anaesthesia has significant effects on systems level sensory processing and that the choice of anaesthetic used for recording can have grossly different effects on the response to even simple sensory stimuli.

Disclosures: O. Woolnough: None. **J.I. Berger:** None. **B. Coomber:** None. **M.N. Wallace:** None. **A.R. Palmer:** None. **C.J. Sumner:** None.

Poster

231. Auditory Processing: Neural Coding, Experiment, and Theory

Location: Hall A

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Program#/Poster#: 231.19/J47

Topic: D.02. Auditory System

Support: NIH Grant R01 DC009215

Title: Neural dynamics for encoding stationary sound stimuli in the primary auditory cortex of marmoset monkeys

Authors: *W. SUN, D. L. BARBOUR;
Dept. of Biomed. Engin., Washington Univ. in St. Louis, Saint Louis, MO

Abstract: Neural systems usually respond to external stimuli with rich dynamics, even when the stimulus itself is stationary. In primary auditory cortex, responses to stationary sounds contain two dynamic components. The onset component occurs immediately following stimulus onset, which spreads across a wide range of neurons at high population activity level. As the stimulus proceeds, responses of many neurons fade away, leaving only a small subset of neurons firing sustainably throughout the stimulus duration. This sustained response is the second component. Previous studies showed higher response selectivity for sustained responses and proposed distinct functions for the two response components, where onset responses encode the occurrence of a sound onset and sustained responses convey stimulus-specific information. To quantitatively investigate sound encoding properties of the two components, we recorded neural spiking activities from the primary auditory cortex of awake marmoset monkeys in response to pure tones. Through population decoding methods, we found that both response components contained stimulus-specific information. Moreover, to achieve a particular decoding accuracy, the required window length for onset responses was shorter. Along the entire stimulus interval, analysis windows with the same number of spikes showed the same decoding accuracy. Thus, the higher-rate onset responses can perform encoding at a faster speed. Finally, we showed that the observed relationship between activity level and decoding speed can be explained by the inherent properties of Poisson neurons, in which higher rates result in more reliable rate estimation and thus a shorter encoding interval. In contrast to onset responses, sustained responses were more energy efficient with their lower spiking rates. Instead of uniformly lowering the activity level of each neuron, however, a large number of neuron responses faded away while only a small subset remained active. This sparseness potentially helps maintain the statistical power of the system in the sustained low activity level regime, and improves code orthogonality, thereby preserving encoding capacity for new stimuli. The trade off, however, is a slower encoding speed. This unified theory accounts for the existence of primary-like neuronal responses that can rapidly encode sensory changes in the environment when they occur while also efficiently maintain that representation in face of no additional stimulus changes. These complementary strategies arise out of constraints from behavior, neuronal biophysics and metabolic factors.

Disclosures: **W. Sun:** None. **D.L. Barbour:** None.

Poster

231. Auditory Processing: Neural Coding, Experiment, and Theory

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Topic: D.02. Auditory System

Title: Rapid estimation of neuronal frequency response area using Gaussian process regression

Authors: *X. D. SONG, W. SUN, D. L. BARBOUR;
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Abstract: Frequency response area (FRA), an auditory neuron's firing rate to tonal stimuli as a function of tone frequency and intensity, is a basic characterization that provides useful information about the neuron under investigation, such as the distribution of excitatory and inhibitory inputs. Traditionally, FRA is obtained by sampling the frequency/intensity plane in a grid fashion, then interpolating the measurements for a smoothed estimate. Due to the many frequency/intensity combinations, acquiring data for FRA is often time-consuming and risks loss of contact with the neuron or animal status change. We derived a procedure for rapid FRA estimation using Gaussian process (GP) regression, a Bayesian machine learning technique, to estimate the entire FRA function from a relatively small set of responses. Furthermore, these responses can be determined by a Bayesian active learning strategy to successively select stimuli informative to the FRA estimate, thereby speeding data acquisition. Neurons in primary auditory cortex of marmoset monkeys were tested with tones in a frequency/intensity grid. A GP with a two-dimensional squared exponential kernel was first conditioned on a random subset of these responses. The GP posterior was then used to predict firing rates and to quantify confidence of the prediction. Next, for the active sampling strategy, the frequency/intensity combination with the lowest prediction confidence was added to the conditioning subset. Derived FRA accuracy was assessed by comparison to 1) FRA estimation with all samples smoothed by a traditional method, 2) FRA estimation using GP regression with all samples, and 3) observed firing rates averaged over multiple trials. Efficacy of the active sampling method was evaluated by comparison to GP estimates derived from randomly selecting the same number of samples. FRA estimates produced by the GP were comparable to the smoothed and trial-averaged firing rates, and provided principled estimates of neural responses outside the range of collected data. As the number of data points used to condition the GP increased, the accuracy of the estimate also improved. Compared to the traditional grid search strategy, the Bayesian active learning sampling strategy demonstrated a more rapid increase in estimation accuracy as a function of size of the sample set, achieving the same level of accuracy with considerably fewer samples. These results demonstrate that GP regression can be used to build principled estimates of FRAs, and along with Bayesian active learning, can produce accurate FRA estimates with limited data, thus expediting the experimental process.

Disclosures: X.D. Song: None. **W. Sun:** None. **D.L. Barbour:** None.

Poster

231. Auditory Processing: Neural Coding, Experiment, and Theory

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Title: Neural signatures of predictive coding in cortical pitch processing

Authors: *W. SEDLEY[1], P. E. GANDER[2], S. KUMAR[1], H. OYA[2], H. KAWASAKI[2], M. A. HOWARD, III[2], T. D. GRIFFITHS[1];
[1]Newcastle Univ., Newcastle Upon Tyne, United Kingdom; [2]Univ. of Iowa, Iowa City, IA

Abstract: Accumulating evidence suggests that perception is not simply the consequence of serially processed input from sensory organs, but results from a weighted combination of this information with prior expectations based on a generative model of the environment. In predictive coding (1,2), prior expectations take the form of predictions, represented by their mean and precision (inverse variance), and sensory information in violation of these is called prediction error, which is related to surprise (the negative log probability of a sensory event occurring). Theoretical models based on cortical microarchitecture (3) propose that predictions are represented by local field potential oscillations in the beta band (~20 Hz), and prediction errors in the gamma band (> 40 Hz), though empirical evidence is limited and mainly relates to gamma band activity. In the present study of cortical pitch processing, we varied the fundamental frequency of an otherwise constant pitch stimulus in order to explicitly manipulate and dissociate prediction error, surprise, prediction mean and precision. Recordings were made from primary and non-primary auditory cortex (in Heschl's gyrus and superior temporal gyrus) from awake patients undergoing invasive monitoring for epilepsy localisation. We found that the amplitude of gamma correlated with surprise (rather than prediction error), beta with changes to prediction mean, and theta (~6 Hz) with precision of predictions. The findings relating to beta and gamma lend strong support to existing hypotheses, and for the first time demonstrate quantitative relationships between the relevant neuronal and computational processes. Theta oscillations are of fundamental importance to a wide range of cognitive and perceptual processes, and have been shown to have an organising and modulatory effect on higher-frequency oscillations. The present results suggest that one such role may be to encode the precision of predictions passed from higher centres, which could be achieved through dynamic modulation of higher frequency prediction and error signals. - 1.Rao, R. P. & Ballard, D. H. Predictive coding in the visual cortex: a functional interpretation of some extra-classical receptive-field effects. Nat. Neurosci.

2, 79-87 (1999). 2.Friston, K. A theory of cortical responses. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 360, 815-36 (2005). 3.Bastos, A. M. et al. Canonical microcircuits for predictive coding. Neuron 76, 695-711 (2012).

Disclosures: **W. Sedley:** None. **P.E. Gander:** None. **S. Kumar:** None. **H. Oya:** None. **H. Kawasaki:** None. **M.A. Howard:** None. **T.D. Griffiths:** None.

Poster

231. Auditory Processing: Neural Coding, Experiment, and Theory

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Topic: D.02. Auditory System

Support: NIH DC009215

Title: Single and population neural discrimination of vocalizations in noise

Authors: ***R. NI**[1], D. A. BENDER[2], J. R. GAMBLE[1], D. L. BARBOUR[1];
[1]Dept. of Biomed. Engin., [2]Dept. of Biol., Washington Univ. in St. Louis, Saint Louis, MO

Abstract: Robust sensory perception is critical for prompt behavior responses. Vocal communication in particular requires reliable auditory processing for both human and animals to interact with conspecifics. The nature of robust neural representation of vocalizations is incompletely understood. Recent research has revealed the existence of background-invariant neurons in higher auditory areas of songbirds, as well as site-dependence of the effect of masker type upon high-level neural representation. Auditory cortex contributes to the processing of complex natural sounds in primates, but relatively little is known about the impact of different noises on cortical encoding of vocalizations. Here we investigate the influence of two kinds of noises on the single and population neural discrimination of vocalizations in a highly vocal primate, the common marmoset monkey (Callithrix jacchus). Single-unit activities in auditory cortex were recorded from alert adult marmoset monkeys while animals passively listened to the playback of natural and modified conspecific vocalizations. White Gaussian noise (WGN) and 4-vocalization babble noise were mixed with five natural marmoset vocalizations at eight different signal-to-noise ratios. To quantify the influence of noise on individual neuron responses, we implemented spike-train distance-based discrimination analysis and further derived a robustness index for each neuron. An intensity invariant index corresponding to each vocalization was also developed in a similar way. We further trained diverse classifiers on pooled population response to assess the robustness of population encoding. Over the 224 primary auditory cortex neurons studied, the robustness indices of individual neurons against two noises are poorly correlated, revealing only a weak positive relationship. No significant relationship was shown between single units' intensity invariance and noise robustness under both noise cases. With regard to

population coding, WGN leads to a lower detection threshold for the presence of target vocalizations than babble. Additionally, optimized subpopulation neurons outperformed the whole population for each of vocalization-noise pairs. Our results are consistent with our previous finding based upon correlation-based analysis and indicate individual neuron responses across different sound background are context-dependent in primate primary auditory cortex. The general influence of noise on cortical representation is revealed successfully by population coding and suggests that a variety of individual neuron responses is a prerequisite for robust coding across different conditions.

Disclosures: **R. Ni:** None. **D.A. Bender:** None. **J.R. Gamble:** None. **D.L. Barbour:** None.

Poster

231. Auditory Processing: Neural Coding, Experiment, and Theory

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Topic: D.02. Auditory System

Support: NIH Grant U01NS090569

Title: Avalanche dynamics of population activity in the auditory cortex

Authors: D. WINKOWSKI[1], S. SESHADRI[2], D. PLENZ[2], *P. O. KANOLD[1];
[1]Biol., Univ. of Maryland, College Park, MD; [2]NIMH, Bethesda, MD

Abstract: The layered organization of cortex fundamentally organizes how information is processed in the brain. Sensory input enters layer 4 (L4) from which activity quickly spreads to superficial layers 2/3 (L2/3), deep layers 5/6 (L5/6) as well as to other cortical areas. In primary auditory cortex (A1), our recent *in vivo* 2-photon experiments revealed a homogeneous frequency (tonotopic) organization in L4, which is more heterogeneous in the next layer, L2/3. The organization of stimulus preference in L2/3 is currently not well understood, yet, numerous studies have shown that L2/3 incorporates ongoing cortical activity, usually in the form of reverberating activity from within or distant cortical regions, that reflect the state and behavioral context of the animal. A promising candidate dynamics are neuronal avalanches which emerge in L2/3 and provide a scale-invariant organization of activity patterns that correlate distant cortical sites in a highly selective manner, a prerequisite to form perceptual categories. Towards understanding the spatiotemporal properties of population activity in A1 and the functional relationship of neurons participating in neuronal avalanches we investigate the presence of neuronal avalanches in A1. We use *in vivo* 2-photon imaging of spontaneous and sound evoked activity of A1 in awake mice using GCaMP6. We deconvolve the signal from ROIs to derive the instantaneous neuronal firing rates. Non-zero firing rates from local clusters were calculated and the cluster size distributions were examined for power law behavior to identify avalanche

dynamics. We then compare avalanche dynamics of ongoing and evoked activity in different lamina in the anesthetized and awake state. Together our experiments characterize the spatiotemporal population activity in the auditory cortex.

Disclosures: **D. Winkowski:** None. **S. Seshadri:** None. **D. Plenz:** None. **P.O. Kanold:** 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.; NIH U01NS090569.

Poster

231. Auditory Processing: Neural Coding, Experiment, and Theory

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Topic: D.02. Auditory System

Support: NIH Grant R01DC014279

The Pew Charitable Trusts

Title: Determining the joint neural encoding of phonetic and speaker features with EEG

Authors: B. KHALIGHINEJAD, M. YANG, J. O'SULLIVAN, *N. MESGARANI; Columbia Univ., New York, NY

Abstract: Humans possess the ability to simultaneously and continually perceive speech and speaker information. This perceptual ability requires the extraction of linguistic features such as consonants and vowels from a complex acoustic signal, and the linking of these features to the speaker that uttered them. Recent invasive and non-invasive studies have shown an acoustic phonetic feature representation of speech in human auditory cortex. However, how speaker specific variability affects the representation of phonetic features remains unknown. Here, we addressed this question by analyzing scalp electroencephalography (EEG) data recorded from subjects who listened to continuous stories with alternating sentences read by a male and female speaker. To ensure the attentional engagement of the participants, they were required to answer questions regarding the content of the stories. The stories were segmented into time-aligned sequences of phonemes, and the neural responses to all instances of each phoneme were obtained. We then grouped these responses into phonetic feature categories and found that the neural responses to different phonetic categories (e.g., consonant-vowel, manner of articulation) were discriminable. In order to determine the dependence of such a phonetic representation on speaker specific characteristics, we compared the average responses to each individual phoneme spoken by each speaker. We used the pairwise Euclidian distance between phoneme pairs as a measure of dissimilarity. We found a varying degree of overlap between the phonetic feature representations of the two speakers. Furthermore, by performing similar analysis on the average

spectrograms of each phoneme, we found that the joint acoustic similarity of speaker and phonetic features determined the organization of the neural responses. These results suggest a joint encoding of speaker information and phonetic features, which is a fundamental step towards determining the neural basis of the ability to attend to a single speaker in multi-talker and noisy environments.

Disclosures: **B. Khalighinejad:** None. **M. Yang:** None. **J. O'Sullivan:** None. **N. Mesgarani:** None.

Poster

231. Auditory Processing: Neural Coding, Experiment, and Theory

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Topic: D.02. Auditory System

Support: NIH NIDCD

Title: Characterizing the dynamic representation of acoustic spectral regularity in humans

Authors: ***A. M. GIFFORD**, M. KAHANA, Y. COHEN;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Auditory perception depends on the auditory system's ability to detect and segregate or group the spectrotemporal regularities in the acoustic environment. In particular, the pattern of spectral progression over time is widely thought to be a signal that the auditory system can use to group and segregate acoustic events into distinct sounds. However, it is currently unclear how the auditory system tracks and reflects changes in spectral regularity over time in an ongoing, dynamic stimulus. Moreover, much of the previous work on the brain's representation of spectral regularity focused either explicitly on the contributions of the core auditory fields or was conducted with techniques that have poor spatial localization (such as electroencephalography). As a result, little is known about the extent to which aspects of spectral-regularity representation are specific to either the core or higher-order auditory fields, or general to the auditory system as a whole. The goals of this study were (1) to test how the human auditory system reflects local spectral regularities on short time scales in ongoing acoustic-tone sequences with pseudo-random structure and (2) localize these representations in cortex. Because neural oscillatory activity influences the timing of neural spiking and correlates with deviations in spectrotemporal regularities, we examined the contribution of oscillatory activity to dynamic spectral regularity representation by measuring electrocorticographic activity in human epileptic patients. Subjects listened passively to sequences of alternating tone bursts of two frequencies presented in a pseudorandom order. We analyzed oscillatory activity as a function of temporally local configurations of tones within the overall sequences (e.g., subsets of tone triplets). We found

generally that, whereas analytic phase concentration was positively correlated with the degree of spectral regularity on local timescales, analytic amplitude was not. Additionally, we found that these phase-concentration effects were widespread across cortex, present not only in regions along the classical auditory pathways, but also other more multisensory regions. These findings support a role for oscillatory activity in dynamically tracking local spectral regularities and organizing the auditory scene.

Disclosures: A.M. Gifford: None. **M. Kahana:** None. **Y. Cohen:** None.

Poster

231. Auditory Processing: Neural Coding, Experiment, and Theory

Location: Hall A

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Program#/Poster#: 231.26/K6

Topic: D.02. Auditory System

Title: The encoding mechanism throughout the human auditory pathway

Authors: *Q. ZHANG[1], X. HU[2];
[1]Computer Sci. and Technol., [2]Tsinghua Univ., Beijing, China

Abstract: It is unclear that whether there is a consistent encoding mechanism throughout the human auditory pathway. In this paper, we used an unsupervised deep neural network, named sparse-HMAX, which is inspired by the V1 simple cell and complex cell to modulate the speech data. In the first layer of the network, the neurons decompose the speech in frequency domain, which is just like the cochlea neurons. In the next two layers, the neurons fired as IC neurons. They have similar receptive fields with corresponded acoustic features, such as harmonic stacks, onsets and terminations, and also more exotic structures as checkerboard patterns and spectral motion patterns. Then in the fifth layer, the neurons tend to represent the phonetic features such as plosive and nasal, which is very similar to the human auditory neurons. Finally, in the last layer of our network, the neurons can represent phonemes with population coding methods. Our results suggest that the sparse coding mechanism and the simple/complex cell pattern are widely used throughout the human auditory pathway.

Disclosures: Q. Zhang: None. **X. Hu:** None.

Poster

232. Striate Cortex: Receptive Field Organization

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Topic: D.04. Vision

Support: CRSNG

FCI

Réseau de la Recherche en Santé de la Vision FRQS

Title: Geometric diversity of axons in afferent and efferent projections of the mouse visual cortex

Authors: P. RÉGNIER, I. MASSÉ, R. TREMBLAY-LALIBERTÉ, *D. BOIRE;
Anatomie, UQTR, Trois-Rivieres, QC, Canada

Abstract: The branching structure of axons shows the spatio-temporal distribution of information in the brain. In addition, the structure of the arborisation is relevant to computational properties that can take place at the axonal level. Diameter inhomogeneities such as bifurcations and varicosities can alter spatio-temporal propagation of action potentials along the branching tree. Specifically, abrupt changes in the diameter of axons as found at varicosities and differences in the diameter ratio of the mother and daughter branches at an axonal bifurcation could introduce delays in the transmission of action potentials (Goldstein & Rall, 1974). It has been suggested that the structural diversity of axon projections between cortical areas form a complex network of lines of communication with different geometrical and time computing properties (Innocenti, Vercelli, & Caminiti, 2014). This study compares the geometric structure of cortico-cortical axons to understand their underlying principles. This analysis helps evaluate if functional differences of intracortical connections are reflected in axon topology. MATERIALS AND METHODS: Iontophoretic micro-injections of high molecular weight biotinylated dextrans (10kDa) were made in the primary visual cortex (V1), somatosensory barrel field (S1BF) and auditory cortex (Au) of C57BL/6 mice. Three-dimensional reconstructions were performed to obtain complete arbors of axons for projections from V1 to the extrastriate visual areas LM and AL; for the intermodal projections from V1 to S1BF and S1BF to V1 and from Au to the lateral portion of V2 (V2L) and to V1. Analysis of axon diameter at branching points, segment lengths and density of varicosities were made with Neurolucida (Microbrightfield Bioscience). Geometric ratios of mother and daughter branches were calculated for every branching point. RESULTS: Reconstructed axons showed a significant diversity of branching structure, total length, density of varicosity, average diameter at origin point and values of geometric ratios. The diameter of mother branches showed little variation along the axon arbor. There was no relation between the position of branching points along the axon and the values of their geometric ratio. There seems to be a relation between mother branch diameters and the maximum possible value of their geometric ratios. This tendency seems to be common among all axons observed. CONCLUSION: There were significant differences in axonal morphology between the studied projections and also a significant diversity of geometric and possible computational properties of axonal arbors within each of these projections.

Disclosures: **P. Régnier:** None. **I. Massé:** None. **R. Tremblay-Laliberté:** None. **D. Boire:** None.

Poster

232. Striate Cortex: Receptive Field Organization

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Topic: D.04. Vision

Support: NIH R01 EY020765-04

NSF GRFP DGE-1321851

Title: The spatiotemporal distribution of synaptic inputs to simple cells of V1: challenging a classical model of receptive field structure

Authors: ***M. M. TAYLOR**, M. SEDIGH-SARVESTANI, L. E. VIGELAND, L. A. PALMER, D. CONTRERAS;
Neurosci., Perelman Sch. of Med., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Simple cells in layer 4 (L4) of primary visual cortex (V1) have receptive fields (RFs) composed of at least one elongated sub-region sensitive to either bright or dark contrast (Hubel & Wiesel, 1962). Adjacent sub-regions are sensitive to opposite contrasts, making these cells responsive to edges in the visual field. Extracellular observations of these cells' visual responses led to a push-pull model of RF organization: within a sub-region, one stimulus contrast increases firing rate (push), while the opposite contrast decreases firing rate (pull) (Palmer & Davis, 1981; Jones & Palmer, 1987). The mechanisms of push and pull were later interpreted as pure excitatory and inhibitory postsynaptic processes, respectively (Ferster 1988; Hirsch et al., 1998; Anderson et al., 2000). A synaptic push-pull theory in simple cells would entail opponent excitation and inhibition in response to opposite contrasts within a sub-region, leading to spatially segregated excitation and inhibition across sub-regions. Here, using intracellular recordings in cats *in vivo* from simple, regular spiking cells in V1 (Area 17) L4, we estimate the underlying synaptic structure of simple cell RFs. We use single or pairs of optimally oriented bars flashed in different positions within the RF. We record membrane potential (Vm) with various levels of current injection, and we calculate synaptic conductance and Vrev, from which we estimate excitatory and inhibitory conductances. Our results depart from key predictions of push-pull in at least two ways. First, we find that a stimulus that evokes an excitatory conductance also evokes a comparable inhibitory conductance. We find that this inhibition from excitatory stimuli is delayed relative to excitation. Second, while excitation is spatially segregated according to RF sub-region, we observe broad inhibition across the RF. We further show that delayed inhibition plays a functional role in reducing the "window of opportunity"

(Pinto et al., 2000) for synaptic inputs to trigger spikes, in effect increasing the precision of the cells' output, as we have proposed previously (Cardin et al., 2010), and as we and others have demonstrated in other sensory systems (Wilent & Contreras, 2005; Higley & Contreras, 2006; Wehr & Zador, 2003). Our results suggest not only that synaptic inhibition suppresses responses to non-preferred stimuli and thereby shapes the cell's RF; additionally, in response to excitatory drive from preferred stimuli, inhibition dynamically shapes the timing of a V1 simple cell's spiking output.

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Poster

232. Striate Cortex: Receptive Field Organization

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Program#/Poster#: 232.03/K9

Topic: D.04. Vision

Title: The effect of callosal projections on stimulus responses of layer 2/3 neuronal populations in rodent visual cortex

Authors: *V. RAMACHANDRA, V. PAWLAK, J. N. D. KERR;
Behavior and Brain Organization, Res. Ctr. Caesar, Bonn, Germany

Abstract: In rats, left and right primary visual cortex (V1) are linked by strong, reciprocal and anatomically specific callosal projections which are most prominent in the binocular region. However, these interhemispheric projections' effect on visual responses remain poorly understood. We recorded spontaneous and stimulus-evoked population activity in layer 2/3 of anesthetized rat V1 while reversibly inactivating the contralateral region projecting to the imaged area. We first anatomically labeled the contralateral region projecting callosally to V1 with cholera-toxin based retrograde tracers, and functionally located it within the binocular region using intrinsic optical imaging. To reversibly inactivate this contralateral region, we expressed the light-activated proton pump archerhodopsin in neurons using adeno-associated virus. Using two-photon imaging of the calcium indicator Oregon green BAPTA-1, we measured population responses to drifting gratings (8 orientations in both directions) with and without inactivation of contralateral V1. Inactivating contralateral V1 reduced visual responses in a subpopulation of neurons, leading to a significant overall decrease in responsiveness, but did not change neurons' preferred orientations or reduce spontaneous activity. Responsiveness to stimulation was reduced for either eye, but was stronger for the ipsilateral eye. Thus callosal projections contribute significantly to stimulus responses of neurons in rat V1 while maintaining their targets' preferred stimuli.

Disclosures: **V. Ramachandra:** None. **V. Pawlak:** None. **J.N.D. Kerr:** None.

Poster

232. Striate Cortex: Receptive Field Organization

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Support: HHMI

Gatsby Foundation

Title: Neural circuits for the cortical control of the optokinetic reflex

Authors: ***B. LIU**[1], A. HUBERMAN[1], M. SCANZIANI[1,2];
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Abstract: The cerebral cortex of mammals has the ability to control and modulate innate, reflexive behaviors mediated by subcortical structures. By adjusting these behaviors to the prevailing conditions or according to past experiences the cortex greatly expands the behavioral repertoire of mammals. One example of such cortical modulation is the impact of visual cortex on the optokinetic reflex (OKR), a compensatory eye movement that stabilizes retinal images during self-motion. The initial stages of the OKR are mediated by phylogenetically old subcortical brainstem nuclei of the accessory optic system (AOS). Here we study the cortical control of the OKR as a model system to understand the mechanisms enabling cortex to modulate innate behaviors. We optogenetically silenced the visual cortex of mice to evaluate the cortical contribution to the OKR and to the activity of AOS nuclei. We observed that cortical silencing moderately but significantly reduced the OKR gain by 10-30%, depending on visual stimulus parameters. Furthermore the reduction in OKR gain upon cortical silencing could be largely accounted for by the concomitant decrease in AOS activity without affecting the sensorimotor transformation function. We further demonstrate that layer V neurons in the visual cortex project to and form functional excitatory synapses onto AOS neurons, implying that the cortex can modulate AOS activity through this direct projection. Finally we discover that surgical disruption of the vestibulo-ocular reflex, another gaze stabilization mechanism, leads to a compensatory enhancement of OKR gain and that this enhancement is achieved, primarily if not exclusively, by the visual cortex increasing its contribution to AOS activity. Our results thus indicate that visual cortex, via its direct corticofugal projection, amplifies the activity in the AOS to modulate OKR behavior. This circuit enables visual cortex to compensate for disruptions in reflexive gaze stabilization mechanisms.

Disclosures: **B. Liu:** None. **A. Huberman:** None. **M. Scanziani:** None.

Poster

232. Striate Cortex: Receptive Field Organization

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Title: Effect of running on surround suppression in inhibitory neurons of mouse visual cortex

Authors: *M. DIPOPPA[1], A. RANSON[2,1], M. KRUMIN[1], M. CARANDINI[1], K. D. HARRIS[1]; [1]Univ. Col. London, London, United Kingdom; [2]Cardiff Univ., Cardiff, United Kingdom

Abstract: The dynamics of cortical networks are shaped by functional connectivity between different classes of inhibitory interneurons. In the superficial layers of the mouse primary visual cortex (V1), interneurons expressing somatostatin (Sst) have been implicated in surround suppression of pyramidal neurons, in a model where they increase their activity with visual stimulus size (Adesnik et al., 2012). Interneurons expressing vasoactive intestinal peptide (Vip) are thought to disinhibit pyramidal cells during running, by inhibiting Sst interneurons (Fu et al., 2014). Also, running decreases surround suppression in pyramidal neurons, suggesting an interaction between the two effects (Ayaz et al., 2013). We asked how this interaction emerges from the local cortical circuit, and what is the role of the different interneuron classes. We used two-photon calcium imaging in V1 of multiple transgenic mouse lines to study how locomotion modulates neural activity and size tuning in Vip, Sst, parvalbumin-expressing (Pvalb), and unidentified (putatively mainly pyramidal) cells. Mice were head-fixed and free to run on an air-suspended ball while visual stimuli of different sizes were presented. To identify interneuron classes we generally expressed GCaMP6m in all neurons and tdTomato in a specific subclass. In control experiments we expressed GCaMP6m in only Sst neurons, to study their responses in the absence of out-of-focus fluorescence. In all cell classes visual responses increased during running. For Sst cells, this result is at odds with the disinhibitory model of modulation by running. Consistent with prior reports, Sst neurons did not typically show surround suppression during running - however, in the stationary condition, all cell types showed surround suppression. Finally, while pyramidal and Vip neurons increased their spontaneous activity during running, spontaneous activity of Pvalb and Sst neurons could show either a decrease or an increase. This last result may in part explain the discrepancy of different studies on effects of running in the Sst population (Polack et al., 2013; Fu et al., 2014). Using a mean field model of the recurrent cortical network, we show how the effects we observed can be produced by a running-induced rebalancing from recurrent to feedforward excitation – a canonical effect of

neuromodulators such as acetylcholine. We conclude that the reduction of surround suppression during running cannot be explained by the disinhibitory model. Instead, we suggest that it arises from more complex network dynamics, in which excitatory recurrent connectivity is controlled by neuromodulation.

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Poster

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Klingenstein Foundation

Title: Functional properties of deep layer projection neurons in the primary visual cortex

Authors: ***G. LUR**, L. TANG, J. A. CARDIN, M. J. HIGLEY;
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Abstract: In the mammalian neocortex, pyramidal neurons (PNs) in layer 5 provide the principal source of efferent information to subcortical structures. Within layer 5, distinct pools of PNs can be identified based on their morphology, physiological properties, local connectivity, and subcortical projection patterns. However, most data regarding the properties of L5 PNs has been obtained from *in vitro* preparations. Whether these disparate characteristics translate into distinct functional roles (e.g., the ability to encode sensory information) *in vivo* is unclear. Here, we group L5 PNs in the mouse primary visual cortex based on their projections to either the superior colliculus, the dorsal striatum or the contralateral cortex. First, we show that these groups, identified by retrograde tracers, comprise largely non-overlapping populations. We further demonstrate that all three groups receive monosynaptic thalamic input from the lateral geniculate nucleus, suggesting they are well-suited to encode basic features of visual inputs. We then use *in vivo* video-rate calcium imaging of GCaMP6 in targeted subsets of L5 PNs to monitor their activity in response to visual stimulation. We find that visual response properties are highly heterogeneous across the different populations. Tuning for orientation and spatial frequency is broadest in cells projecting to the superior colliculus. Furthermore, responsiveness of superior colliculus-projecting neurons saturated at moderate contrast levels while other L5 PNs exhibited significantly broader dynamic range. Finally, we have characterized the synaptic inputs to each class of cells *in vivo*. We find that inputs to the apical tuft of single L5 PNs exhibit narrow orientation and spatial frequency tuning, consistent with receiving direct sensory-related

information. We also find evidence for both local and dendrite-wide spiking that suggests dendritic nonlinearities contribute to visual processing. In summary, our findings highlight substantial functional differences between distinct populations of L5 PNs in the mouse visual cortex. We suggest that superior colliculus-projecting cells are best described as “detectors”, while other populations may be more suited for discrimination. These results indicate that L5 consists of multiple parallel processing streams for routing specific features of sensory information to their target structures.

Disclosures: **G. Lur:** None. **L. Tang:** None. **J.A. Cardin:** None. **M.J. Higley:** None.

Poster

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Title: Binocular organization of cortical maps for ON/OFF spatial phase and orientation in primary visual cortex

Authors: *J. KREMKOW[1,2], J. JIN[2], Y. WANG[2], J.-M. ALONSO[2];
[1]Inst. for Theoretical Biol., Humboldt Univ. of Berlin, Berlin, Germany; [2]State Univ. of New York, Col. of Optometry, New York, NY

Abstract: A hallmark of the primary visual cortex (V1) is the systematic mapping of different stimulus attributes such as eye input, orientation preference and ON/OFF spatial phase (Wang et al. 2015). We have recently showed that cortical maps for ON/OFF spatial phase are closely related to the geometry of cortical orientation maps in cat V1 (Kremkow et al. 2014). However, it is not fully understood in what extent the relation between these maps depend on eye input. To address this question we performed horizontal penetrations through the primary visual cortex in cat with 32-channel multielectrode arrays (inter-electrode separation: 100 microns) and studied the binocular organization of ON/OFF spatial phase and orientation/direction preference. To characterize the orientation map, we presented moving bars and calculated the orientation/direction preference as the angle that generated the maximum response. To characterize the ON/OFF spatial phase map, we measured the ON/OFF receptive fields (RF) with light and dark sparse noise. ON/OFF retinotopy was then characterized by the center-of-

mass of the ON/OFF receptive subfields and spatial phase by fitting a Gabor function to the ON-OFF receptive field. Our analysis shows that the cortical maps for orientation and direction are precisely matched across eyes: the mean difference in orientation preference was only 13 deg (n=1417 with orientation selectivity > 0.3) and the mean difference in direction preference was 17 deg (n= 550 with direction selectivity > 0.3). In contrast, the cortical map for ON/OFF spatial phase was more variable across eyes. In accordance with previous studies (DeAngelis et al. 1991), we found cortical sites that were precisely matched for spatial phase and cortical sites that were mismatched (median phase difference = 43 deg, n = 324). The diversity of binocular spatial phase was also reflected in the organization of binocular ON/OFF retinotopy. While on average ON/OFF retinotopy was well matched across eyes (mean distance between same sign receptive subfields = 0.27 RF diameters, n=648), we found also cortical sites with retinotopic distances larger than 0.5 RF diameters (n=94/648). Interestingly, our data also show that OFF retinotopy is less scattered than ON retinotopy across eyes (ON-ON distance / OFF-OFF distance = 1.5, n=324, $p<0.001$, Wilcoxon test), and moreover, that the ON-ON distance is more strongly correlated to spatial phase differences ($c=0.54$, $p<0.00001$) than the OFF-OFF distance ($c=0.3$, $p<0.001$). Our results suggest that ON/OFF spatial phase maps are, on average, well matched across eyes and that phase mismatches are more likely caused by binocular differences in ON retinotopy.

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Poster

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Title: Direction selectivity of thalamic excitation onto single neurons in mouse primary visual cortex

Authors: ***A. D. LIEN**[1], M. SCANZIANI[2];
[1]GIND, Gladstone Inst. of Neurolog. Dis., San Francisco, CA; [2]HHMI/University of California San Diego, San Diego, CA

Abstract: Primary visual cortex (V1) contains neurons that are selective for the direction of movement of visual stimuli, a feature thought to play a key role in representing the visual world for perception and behavior. Our understanding of the underlying neuronal circuit mechanism is incomplete. Sensory information reaches V1 neurons via excitatory synaptic input from the thalamus. Whether the thalamic excitation exhibits direction selectivity and how such selectivity

might arise remain open questions. We address these issues by isolating and recording thalamic excitation onto mouse L4 V1 neurons in response to visual stimuli consisting of drifting or static gratings using *in vivo* whole-cell recording and optogenetic silencing of intracortical excitation. In response to drifting gratings thalamic excitation fluctuates in amplitude at the temporal frequency of the grating (F1 modulation). We find that while the total excitatory charge (or average excitation) across the stimulus duration was similar regardless of stimulus direction the amplitude of the F1 modulation was selective for direction. That is, large amplitude fluctuations (strong F1 modulation) occurred in the preferred direction and small ones (weak F1 modulation) in the opposite. How can the temporal dynamics of thalamic excitation be so different in response to the same spatial pattern moving in opposite directions? We hypothesize that at certain spatial phases the drifting grating triggers thalamic excitation with a prolonged time course while at other phases the same grating triggers transient excitation. Thus, if the movement of a drifting grating occurs such that phases evoking prolonged excitation precede phases evoking transient excitation it will produce optimal temporal summation and strong F1 modulation. Movement in the opposite direction, i.e. transient phases before prolonged ones, will result in poor temporal summation and weak F1 modulation. Consistent with this hypothesis, in neurons with direction selective thalamic excitation, the time course of thalamic excitation in response to static gratings varies as a function of the spatial phase of the grating with certain phases producing a prolonged excitation and others producing a transient one. The preferred direction of thalamic excitation was well predicted by the relative spatial phase of the prolonged and transient responses. In conclusion, we find that direction selectivity is present in the thalamic excitation onto V1 neurons and that such direction selectivity is likely generated by an asymmetric distribution of the time course of thalamic excitation across the receptive field.

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Poster

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Title: Laminar processing of global visual contours in V1 and V2

Authors: R. CHEN[1], F. WANG[1], H. LIANG[2], *W. LI[1];
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Abstract: Linking line segments into global contours is important for coherent visual perception. Increasing evidence indicates important roles of both feedforward and feedback connections in

contour integration, but the underlying intra- and inter-cortical circuitry is unclear. Using a pair of laminar electrodes (U-probes, Plexon Inc), each with 24 recoding contacts spaced 100 μm, we simultaneously recorded multiunit activities from different layers in monkey V1 and V2 at retinotopically matched locations. The monkeys were trained to detect visual contours formed by different number of collinear bars embedded in a background of randomly oriented bars. Referring to the known anatomical structures and using current source density analysis, we separated each cortical area into three laminae approximate to the supra-granular (SG), granular (G) and infra-granular (IG) layers. Cells showing responses correlated with contour length were defined as the contour cells. The distribution of contour cells across cortical layers were different. V1 contour cells were largely confined in the SG and IG layers, scarcely seen in the G layer, while in V2 the contour cells were mainly located in the SG, G and upper IG layers. The time course and the strength of contour-related signals also varied across layers. In V1 contour signals did not emerge until ~100 ms after stimulus onset for almost all contour cells, and the contour signals became progressively earlier and stronger towards the upper SG (~cortical layer I/II). In V2 a small proportion (~10%) of contour cells in the SG and G layers started to signal the global contour since their visual response onset (~50 ms after stimulus onset); the contour signals in the remaining cells were much delayed, similar to V1 contour cells, but the shortest latencies and maximal strengths of contour signals were seen in the lower SG layer of V2. The overall contour signals in V2 were much stronger than V1. The global contour induced a Mexican-hat response profile in both V1 and V2, facilitating neurons with receptive fields on the contour and suppressing those on the background. The facilitation preceded the suppression, and this difference systematically varied across layers. Conditional Granger causality analyses showed interdependence of contour signals across different layers in V1 and V2. Our results suggest that different cortical layers and areas play different roles in contour integration, and that the inter-laminar and inter-areal processes work synergistically to amplify the contour signals and suppress interfering image components.

Disclosures: **R. Chen:** None. **F. Wang:** None. **H. Liang:** None. **W. Li:** None.

Poster

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Topic: D.04. Vision

Title: Two-photon imaging of neuronal populations in the primary visual cortex representation of the overhead visual field

Authors: ***S. RULLA**[1], B. NG[1], J. MACKE[2], D. WALLACE[1], J. SAWINSKI[1], J. KERR[1]; [1]Dept. Behavior and Brain Organization, [2]Res. Ctr. Caesar: A Max Planck Inst., Bonn, Germany

Abstract: Rodents have a large binocular field of view that extends from the snout to over the animals head. Recent experiments have shown that rodents have a strong, innate, evasive behavior evoked exclusively by stimuli presented above them. However, little is known about the functional properties of cortical neurons that represent the overhead visual field. Here we describe a method for allowing direct optical recording from populations of neurons representing the overhead visual field. Firstly, the conventional microscope objective has been replaced with a periscope coupled to a miniature objective to facilitate placement of a stimulus monitor above the rat's head. Secondly, we developed a method for presentation of visual stimuli on the OLED display of a tablet running the Android OS, and a camera-based method for calibrating the position of the stimulus display in relation to the animals head. Using this setup, we recorded in rats the activity of neurons in the representation of the overhead visual field of the primary visual cortex in response to a range of stimuli. Neurons were labeled with the calcium indicator OGB-1 with counterstaining of astrocytes using sulforhodamine 101. Stimuli were either an expanding or contracting looming dot, or a moving dot that moved at constant speed along multiple trajectories to cover all positions within the display. In both stimulus types, differing sets of foreground/background luminance were used. Preliminary results show that 19% of the neurons responded with clear and reproducible transients to the looming dot stimulus, and 30% were responsive to moving dot stimuli. The response profiles of neurons to different stimulus types and parameters were further analyzed in detail and compared between cortical areas and receptive field properties established for this cortical region.

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Poster

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Title: Cortical spread of correlations between orientation selectivity and orientation preference in primary visual cortex

Authors: ***J. JIN**[1], J. KREMKOW[1,2], Y. WANG[1], R. LASHGARI[1,3], J. ALONSO[1]; [1]SUNY-Optometry, New York, NY; [2]Inst. for Theoretical Biol., Humboldt Univ. of Berlin, Berlin, Germany; [3]Neurosci. and Neural Engin. Res. Lab., Iran Univ. of Sci. and Technol., Tehran, Iran, Islamic Republic of

Abstract: The primary visual cortex of carnivores and primates contains a systematic map of stimulus orientation. Within this map, the highest orientation selectivity is found at regions with the slowest orientation gradient and the lowest selectivity at regions with the fastest gradient. Although this relationship between orientation selectivity and orientation gradient appeared weak or inexistent in early studies, recent work demonstrates that it is robust (Nauhaus et al. 2008) and could play a potential role in cortical map development. To investigate how the relationship between orientation selectivity and orientation gradient varies with cortical distance and eye input, we performed horizontal penetrations through the primary visual cortex with 32-channel multielectrode arrays (inter-electrode separation: 100 microns). We selected recording sites that responded robustly to moving bars either when stimulated through the contralateral eye (n=3077) or the ipsilateral eye (n=1912) and then fitted a von Misses function to calculate orientation preference and orientation selectivity. The orientation preference was calculated as the angle that generated the maximum response and the orientation selectivity as the ratio between the responses to the preferred orientation and orthogonal orientation. For each possible pair of recordings, we calculated the average orientation selectivity and orientation gradient (difference between orientation preferences) and then measured the correlation between both as a function of cortical distance (from 100 to 2500 microns). This analysis demonstrate that orientation selectivity is negatively correlated with orientation gradient at distances of 100 microns ($r=-0.38$, $p<0.0001$ for contralateral eye and $r=-0.39$, $p<0.0001$ for ipsilateral eye), the correlation fully decays within 500 microns (e.g. $r=0.02$, $p=0.2$ for contralateral eye) and then becomes slightly positive ($r = 0.1$, $p < 0.0001$ at 600 microns) before returning to baseline. The functions relating cortical distance with the selectivity/gradient correlation were remarkably similar between eyes ($R^2 = 0.99$, slope = 1.03) and could be accurately described with a difference of Gaussian functions with standard deviations of 322.13/391.52 microns for contralateral eye ($R^2=0.97$) and 309.69/379.96 for the ipsilateral eye ($R^2=0.94$). These results demonstrate that the correlation between orientation selectivity and orientation gradient is a robust feature of cortical orientation maps, it is remarkably similar across the two eyes and has a cortical spread comparable to the size of a column for spatial phase in visual cortex (Kremkow et al., 2014; Wang et al., 2015).

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Poster

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EY013312

Title: Cortical topography and cross-orientation interactions in visual cortex

Authors: *E. KOCH, J. JIN, M. JANSEN, C. PONS, J. ALONSO, Q. ZAIDI;
SUNY Col. Optometry, New York, NY

Abstract: The primary visual cortex of carnivores and primates is organized into functional maps for orientation preference that contain regions where the orientation is similar among neighboring neurons (iso-orientation domains) and regions where it is very different (pinwheel centers/ fractures). Within this cortical map, cross-orientation interactions are thought to be important for making a more efficient population code for orientation processing, but it is currently unknown if cross-orientation suppression varies across the different regions of the map. To address this question, we performed horizontal penetrations with multi-electrode arrays in the primary visual cortex of anesthetized cats, and sampled populations of neurons in different orientation domains (32-channel Neuronexus probe, 100 microns inter-electrode distance). Neurons were stimulated with a sequence of flashed (100 msec) sinusoidal gratings of 8 orientations, 8 contrasts, and 4 phases, and pairs of these gratings (plaids). We measured the semi-saturation constant (C50), orientation tuning bandwidth measured as half width at half height (HWHH), and local homogeneity index (LHI) at each site. The LHI gives a measure of the orientation gradient: closer to 0 in pinwheel centers/ fractures and closer to 1 in iso-orientation domains. A suppression index (SI) was calculated by subtracting the plaid response from the summed responses to the individual gratings, and normalizing by the maximum response. Recent studies indicate that cross-orientation suppression originates from contrast saturation in thalamic inputs. Consistent with these studies, we found a negative correlation between C50 and SI ($r=-0.58$, $p< 0.0001$, $n=150$). In addition, we found a positive correlation between LHI and SI ($r=0.39$, $p<0.0001$), implying that there is more suppression in iso-orientation domains than pinwheel centers. HWHH was correlated with LHI ($r=-0.33$, $p<.0001$) but not with C50 ($r=0.008$, $p=0.93$). LHI was also correlated with C50 ($r= -0.4$, $p<0.0001$) indicating that neurons located in iso-orientation domains generate responses that saturate more with luminance contrast than those located around pinwheel centers. A step-wise regression demonstrated that LHI had a significant effect on SI beyond that of C50 (combined $r=0.66$; LHI coefficient: 0.31, $p<0.001$). Existing models of cross-orientation suppression can predict the relation of C50 with SI and LHI but not the relation between SI and LHI. Therefore, we conclude that cortical topography for orientation preference significantly affects cross-orientation interactions in visual processing, a finding that may require rethinking existing models.

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Title: Specificity in the spatial organization of receptive fields supporting multiple functional maps in tree shrew visual cortex

Authors: *K.-S. LEE[1,2], X. HUANG[1], D. FITZPATRICK[1];
[1]Max Planck Florida Inst., Jupiter, FL; [2]Integrative Biol. and Neurosci. Grad. Program, Florida Atlantic Univ., Boca Raton, FL

Abstract: *In vivo* 2-photon imaging of calcium sensors in visual cortex has provided a host of new insights into the fine scale columnar mapping of response properties like orientation, direction, and visual space. However, it remains unclear how the inputs supplied by the LGN are spatially arranged to generate these functional maps. Extracellular recording in carnivores have shown that nearby geniculate afferents in layer 4 exhibit spatially offset ON and OFF centers, which could provide the basis for the generation of orientation selectivity. In the tree shrew, the ON and OFF pathways remain segregated in layer 4, but converge for the first time in layer 2/3, where selectivity for orientation emerges. In this study we used 2-photon imaging of GCamP6s calcium fluorescent signals to map the receptive fields of neurons in layer 2/3 of tree shrew visual cortex with reverse correlation using sparse noise. We found a diverse array of receptive field properties in layer 2/3 including neurons with classic simple, complex and single sign receptive fields (either ON or OFF). The ON and OFF subfields in layer 2/3 were found to exhibit topologically distinct relationships with the maps of visual space and orientation preference. In most cases, the centers of OFF subfields for neurons in a given region of cortex were confined to a compact region of visual space and displayed a smooth retinotopic progression, while the centers of the ON subfields were distributed over a wider region of visual space and displayed less retinotopic precision. Consistent with the arrangement of ON and OFF subfields of simple cells in other species, the angle of displacement in visual space of the ON and OFF subfields for individual neurons could be used to predict the organization of the orientation map. Taken together, these results suggest that the differential arrangement of ON and OFF subfield centers by cortical circuits meets the conjoint constraints of mapping both visuotopy and orientation in a single population of neurons and in a fashion that preserves continuity for both stimulus features.

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Poster

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SPCCR

University of Fribourg (CH)

Title: Phase and structure selectivity depend on grating class in primary (V1) and secondary (V2) visual cortex

Authors: *P. DE LUNA, J. POIROT, R. KRETZ, G. RAINER;
Univ. of Fribourg, Switzerland, Fribourg, Switzerland

Abstract: Many studies have shown that neurons in early visual cortex are tuned for the structure, i.e. orientation and spatial frequency, of Cartesian gratings, and often exhibit activity modulations dependent on spatial phase. Here we systematically explored how neural responses in V1 and V2 of the tree shrew (species Tupaia belangeri) depend on structure and phase of gratings taken from the Cartesian, as well as hyperbolic and polar grating classes. We analyzed single neuron activity from 86 cells, as well as visual evoked potentials (VEPs). As expected, structure selectivity was greatest for Cartesian gratings for both neural signals, but unexpectedly, phase sensitivity was best revealed using hyperbolic gratings (3-way ANOVA, $p<0.01$). We observed a number of differences between V1 and V2: V2 neurons exhibited greater structure selectivity than V1 neurons for Cartesian gratings, whereas the reverse was true for the VEP (unpaired t-tests: $p<0.05$). At the same time, the VEP was more sensitive to phase in V1 than in V2. By presenting gratings at 1x, 2x and 4x the size of the minimum response field, we also investigated how contextual modulation affected neural responses. We observed that visual stimulation of the surround often resulted in an enhancement of the responses for single neurons and VEPs, with suppression occuring only rarely. Surround enhancements were more pronounced for Cartesian than other grating classes. Our findings support recent evidence about a functional organization of neurons according to phase, i.e. phase columns, in V1 but not V2. At the same time, phase sensitivity based on Cartesian grating responses may underestimate the actual sensitivity of visual cortical neurons to phase, which can be revealed by hyperbolic grating stimulation. We discuss our findings in regard to the extent to which they may reflect general principles of visual cortical function or species-specific adaptations that are characteristic of the tree shrew.

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Title: Orientation- and direction- selective responses of GABAergic neurons in ferret visual cortex

Authors: *D. E. WILSON[1,2], G. B. SMITH[1], A. JACOB[1], T. WALKER[1], J. DIMIDSCHSTEIN[3], G. J. FISHELL[3], D. FITZPATRICK[1];
[1]Functional Architecture & Develop. of Cerebral Cortex, Max Planck Florida Inst. For Neurosci., Jupiter, FL; [2]Integrative Biol. and Neurosci. Grad. Program, Florida Atlantic Univ., Boca Raton, FL; [3]NYU Neurosci. Inst., New York Univ. Langone Med. Ctr., New York, NY

Abstract: The response properties of inhibitory neurons critically shape the function of the circuits in which they participate. Genetic tools in the rodent visual cortex have revealed that GABAergic inhibitory neurons show weak response selectivity across all subtypes, but it remains unclear whether these results generalize to other mammalian lineages. We used an adeno-associated viral construct to express GCaMP6f in GABAergic neurons in the ferret visual cortex. The orientation and direction selectivity of these neurons was assessed through *in vivo* two photon calcium imaging and inhibitory subtypes were identified using post hoc immunostaining. We find that the virus effectively labels inhibitory neurons with high specificity across multiple subtypes, including both somatostatin-positive and parvalbumin-positive interneurons. In contrast to the mouse visual cortex, we find that multiple subtypes of inhibitory neurons exhibit strongly selective responses for orientation and direction of motion in the ferret visual cortex. Neighboring inhibitory neurons share similar orientation and direction preferences and are organized into columnar maps, that are consistent with maps of population activity derived through intrinsic signal imaging. The degree of orientation and direction selectivity is similar to excitatory neurons and appears to vary based on location within the orientation map. These findings suggest that feature-selective inhibition plays a prominent role in shaping the response properties of cortical circuits that manifest columnar architecture.

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Title: End-stopped cells help detect natural contours

Authors: *G. C. MEL, P.-O. MARTIN, B. MEL;
USC, Los Angeles, CA

Abstract: Our abstract is forthcoming

Disclosures: G.C. Mel: None. **P. Martin:** None. **B. Mel:** None.

Poster

232. Striate Cortex: Receptive Field Organization

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 232.17/K23

Topic: D.04. Vision

Title: Self-organization of receptive fields and orientation maps in layer 2/3 of the primary visual cortex: Comparisons between cats and rodents

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Abstract: Spatial representation of preferred orientations is revealed to be salt-and-pepper-like in the rodent visual cortex, whereas that in the cat visual cortex exhibits an orderly map structure. Recently, our mathematical model of the activity-dependent self-organization of geniculocortical inputs has demonstrated that the connection probability of lateral excitations (p) determined which type of orientation representation is formed in layer 4; the salt-and-pepper-like representation emerges at lower probability p, whereas orderly maps appear at higher p. Irrespective of p values, almost all model layer 4 neurons were oriented and characterized by simple-cell response properties. In the present study, we extended the model by taking into account fast and slow decay time constants of postsynaptic currents of neurons in layer 2/3. In the framework of the cascade model, we performed computer simulations of self-organizing feedforward connections from layer 4 to layer 2/3 as well as from the lateral geniculate nucleus to layer 4 at different values of p. Simulations showed that average values of orientation selectivity indices of neurons in model layer 2/3 were higher than those in model layer 4. As in layer 4, orientation representation changed from orderly maps to salt-and-pepper-like structures when p became smaller than about 0.1. To classify model neurons into simple and complex cell

types, we calculated the ratio of F(2) to F(0) harmonics in responses to sinusoidal gratings drifting in the preferred direction for each neuron in model layer 2/3. The number of neurons classified into complex cells evidently decreased when orientation representation became salt-and-pepper-like. In the cascade model, the phase invariance of complex cells in layer 2/3 is achieved by the integration of feedforward inputs from layer 4 simple cells with similar preferred orientations over various spatial phases. In the cortex exhibiting salt-and-pepper-like orientation representation, it may be difficult to find sufficient phase variation of neurons with similar preferred orientations within a short distance to eliminate phase dependence. Thus, it is suggested that in layer 2/3 of the visual cortex, simple cells are abundant in rodents, whereas complex cells are rich in cats.

Disclosures: **M. Miyashita:** None. **N. Wakabayashi:** None. **S. Tanaka:** None.

Poster

232. Striate Cortex: Receptive Field Organization

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MOST 103-2321-B-002-028

Title: Receptive fields mapped with natural and artificial stimuli in macaque monkey V1

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Abstract: Our visual system has evolved to process natural stimuli that are very complex in both spatial and temporal aspects. However, properties of visual neurons in the primary visual cortex V1, the first cortical stage of visual processing, are primarily studied with artificial stimuli that have simple and well characterized properties (Felsen and Dan 2005; Rust and Movshon 2005). Few studies had used both artificial and natural stimuli to map V1 receptive fields (Ringach et al 2002; Sharpee et al 2006), but it remains unclear whether natural and artificial maps are comparable or not. We addressed this question by using both stimulus types to measure the receptive field of neurons in different layers of macaque monkey V1. The artificial stimulus is a binary white noise (m-sequence, 16x16 pixels, Reid et al 1997), and the natural stimulus is a movie (48x48 pixels) recorded from a camera attached to the head of the cat walking in

grassland and forest (Kayser et al 2003). In comparison with the m-sequence, the movie has lower spatial and temporal frequencies, and has more low-luminance pixels (lower than the mean luminance). The frequency power of the movie is higher along the vertical than the horizontal axis, so we also rotate the movie by 90 degrees to measured receptive fields of the same neurons. We used a multi-electrode matrix (8x8 array, 200 um spacing, Neuronexus) to simultaneously record from many neurons in different layers of V1. Receptive fields were calculated by reverse correlation. Preliminary results show that the receptive field size and the separation of different subareas tend to be larger for natural maps than for artificial maps. The change in the frequency power of the natural movies [from vertical dominated to horizontal dominated] also has an effect on receptive field properties. Overall, receptive fields of V1 neurons are not fixed and can vary with different stimulus ensembles.

Disclosures: **H. Wu:** None. **Y. Hsu:** None. **C. Yeh:** None.

Poster

233. Extrastriate Cortex: Motion Processing

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Topic: D.04. Vision

Support: NHMRC of Australia Grant 10005427

Title: Impact of speed and contrast on response latency and spatial representations in primate area MT

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Abstract: The response latency of neurons in visual pathway depends on features of the visual stimulus, including its speed and contrast. Stimulus dependence of response latency may influence motion computations, which depend on the fine temporal structure of visual response, and thus contribute to speed-dependent biases in the perceived position of a moving object. To assess this we measured spiking activity in area MT of sufentanil-anaesthetised marmoset monkeys (n=4) using 10x10 planar electrode arrays. Response latency depended on the speed and contrast of a large field of moving white dots, in agreement with previous work. Latency decreased from 73 ms for speeds of 1 degree/s to 58 ms at 80 degrees/s; for dot fields moving at 20 degree/s, latency decreased by 20 ms when Weber contrast was increased from 22 to 100%. Regression analysis shows that response latency depends on speed, contrast and response

amplitude, but not motion direction. To determine if changes in response latency influence the representation of spatial position we analysed responses to a single white disk (contrast 22 or 100%), moving steadily at speeds ranging between 12.5 and 100 degrees/s. The spatial profile of receptive fields obtained at each speed and contrast could be brought into register by assuming a single fixed response latency. A Bayesian decoder trained on population response at the lowest speed was capable of predicting the position of the disk at higher speeds without bias. Similarly, a decoder trained on population response to a 100% contrast disk predicted the position of a 22% contrast disk without bias. We conclude that while speed and contrast can influence the latency of population response in area MT, they have little influence on its representation of the position of a single moving object.

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Poster

233. Extrastriate Cortex: Motion Processing

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Program#/Poster#: 233.02/K26

Topic: D.04. Vision

Title: Response properties of neurons specialized in motion detection in extrastriate area 21a of the cat cortex

Authors: *H. ASLANYAN[1], A. KHACHATRYAN[2], D. KHACHVANKIAN[1], B. HARUTIUNIAN-KOZAK[1], A. GHAZARIAN[1];
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Abstract: The neurophysiological mechanisms underlying central information processing in the cortical visually sensitive areas, including extrastriate area 21a, is still a fundamental problem in neuroscience. Since the pioneering studies of Hartline and Hubel and Wiesel the “receptive field” (RF) concept of a single visually sensitive neuron has become the most important substrate for the interpretation of mechanisms governing the perception of stationary and moving visual images. A concept was introduced, according to which the receptive field stationary structure, as a rule, predetermines the transformation and central processing of incoming visual information concerning the moving visual stimuli. Numerous subsequent studies have confirmed this concept, however earlier experiments from our group showed that certain discrepancies in this approach are evident. In our investigations of the neuronal mechanisms of visual information processing of moving visual images, we observed only a weak correlation, and in some cases, an almost complete absence of correlation between the RF stationary structure and the neuron response patterns to moving stimuli. In the present study we present further observations concerning a small group of neurons in extrastriate area 21a which did not react to the stationary

flashing light spot positioned in its RF, determined by the hand held stimuli, but responded strongly to the moving visual stimuli. We propose that there is a minority of neurons (5.2%) that lack responses to stationary visual stimuli yet reveal a strong ability to detect moving images by discriminating contrasts, sizes, directions and orientations of the motion of visual images. It is likely that these neurons participate in the central processing of visual information by acting as highly specialized motion-detectors. Our results allow us to suggest that a small group of neurons in central visual pathways while being unresponsive to stationary visual stimuli, with great probability are strictly specialized in motion detection, a result of modulatory influences of the activated adjacent neurons, having RFs overlaping with the RF of the neuron under investigation. It is likely that such interactions may be temporary events taking place during the time of moving image motion. Further investigations are needed to elucidate the nature and neurophysiological mechanisms underlying such specialized temporary influences in the central processing of visual information.

Disclosures: H. Aslanyan: None. **A. Khachatryan:** None. **D. Khachvankian:** None. **B. Harutiunian-Kozak:** None. **A. Ghazarian:** None.

Poster

233. Extrastriate Cortex: Motion Processing

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Support: BMBF Grant 01GQ1005C

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Title: Motion-direction tuning in the post-saccadic remapped response in macaque MT

Authors: *T. YAO[1,2], S. TREUE[1,3,2], S. KRISHNA[1];
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Abstract: In the neural phenomenon of perisaccadic remapping, neurons in several visual and attentional/oculomotor brain areas respond to stimuli that appear in their post-saccadic receptive fields (RFs) before the saccade occurs. A robust debate exists regarding its properties and potential function with one view emphasizing its role in keeping track of relevant locations across saccades (the “attentional pointer” hypothesis) while others additionally emphasize its role in transsaccadic feature integration and the perception of visual stability across saccades. A critical difference between these two views lies in whether the remapped response contains only information about stimulus location, but no information about features like orientation and motion direction (as the attentional pointer hypothesis predicts). We previously showed that

neurons in macaque MT show a remapped post-saccadic response that was greater for task-relevant stimuli, but was not tuned for stimulus motion direction. However, the neurons in our study, like those in all other prior studies of remapping, mostly had RF centers well away from the fovea. Visual experience in humans and macaques is predominantly influenced by foveal processing, and saccadic eye movements serve to bring interesting locations in the visual scene onto the fovea. Further, it is known that attention shifts towards the saccade target (ie. the future foveal stimulus) just prior to saccade execution. We therefore trained two monkeys to fixate and attend to a moving random dot pattern (RDP) in order to respond to a brief direction change with a manual response. In addition, during this period, the fixation point could disappear, serving as the cue for the monkey to make a saccade towards the RDP. The monkey had to continue to monitor the RDP and respond to the direction change when it occurred. While the monkeys performed this task, we recorded from neurons with foveal or parafoveal RFs in area MT. The RDP moved in one of four directions and was located well outside the neurons' RF before the saccade, until the saccade brought the RDP into the neurons' RF. Critically, on about half the trials, the RDP disappeared just before the saccade, so that the RDP never appeared within the neurons' RF. On these trials, our preliminary results indicate that the post-saccadic response of MT neurons differs depending on the motion direction of the RDP that occupied the spatial location of the post-saccadic RF (but before the saccade). This suggests that the role of remapping can extend beyond merely serving to remap the retinotopic position of attended locations across saccades to possibly playing a role in transsaccadic motion integration.

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Poster

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Support: Alfred P. Sloan Foundation (JDG)

H. Dean and Susan Regis Gibson Research Award (DB)

Title: Topographic maps of depth in human visual cortex

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Abstract: Depth is a frequently overlooked aspect in vision research, especially in humans, despite the fact that recognizing and perceiving depth cues are essential when it comes to appropriately interacting with our surroundings. Behavioral and physiological studies have provided a solid framework for understanding depth perception, but we have yet to establish the

precise neural organization of depth representation in human visual cortex. In order to advance our understanding of this question we mapped depth representations via fMRI using a phase-encoded stimulus that travels along the z-axis (depth), analogous to the standard 2D retinotopic mapping paradigm (wedges and rings; Engel et. al., 1994; Sereno et. al., 1995). Our stimulus was a large 2D patch that consisted of rapidly moving black and white dots. The stimulus traversed along 13 discrete depth planes either forwards or backwards (in alternating runs), completing a full cycle every 28 seconds. Subjects wore red/green anaglyph glasses while in the scanner so that they could perceive depth. Using a standard phase-encoded cross-correlation analysis, we found voxels selective for different depth planes in several intermediate and later visual areas. Depth representations appear to be organized into a large-scale map-like representation across visual areas V3d, V3A, V3B, and V7. Furthermore, this result was seen to have high within-subject reliability across multiple scanning sessions. Our findings provide critical insights regarding the neural correlates in which depth is represented, and we detail for the first time depth maps in human visual cortex.

Disclosures: D. Berman: None. **N.J. Finlayson:** None. **J.D. Golomb:** None.

Poster

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Emory SIRE Program

Title: Evidence for neurochemical alterations in area MT following damage to V1 in monkeys

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Abstract: Primates with damage to primary visual cortex (V1) show some recovery or sparing of visual function, in particular an ability to detect and discriminate moving stimuli. This ability is likely subserved by spared neural activity in motion-related cortical areas MT and MST. In humans, the residual vision is typically not conscious and has thus been referred to as 'blindsight'. However, the remaining visual activity in MT/MST is weaker than in intact animals, and the preserved motion-processing abilities are correspondingly abnormal. Here, we hypothesized that alterations in calbindin expression might be associated with these changes in MT function. Calbindin D-28K (Cal) is a calcium-binding protein that is both preferentially associated with inhibitory cell populations in cortex and with buffering of calcium transients that

impact cellular excitability throughout the brain. Moreover, earlier studies in cats (Huxlin & Pasternak, 2001, *J. Comp Neurol*.), showed that lesions of areas 17/18 produce decrements in expression of Cal in the supragranular layers of area PMLS, a proposed homolog of MT. To address the possibility of similar alterations in primates, we quantified Cal neuron populations in the supragranular layers of MT/MST in four *Macaca rhesus* with unilateral (left) lesions of central V1 in either adulthood (2 animals) or infancy (5-6 wks of age, 2 animals). After a survival period of 4-7 yrs, Cal immunoreactivity was revealed in 50 µ brain slices using the ABC method, DAB as the chromogen, and Giemsa counterstaining. Cal-immunoreactive cells were counted in six areas of 5-8 sections per hemisphere using the optical disector method and a counting frame of 120 by 80 µ. Using each lesioned case as its own control, we found significantly reduced densities of Cal neurons of layer III specifically in the foveal portion of MT (but not peripheral MT or MST) of the damaged hemisphere in two of the cases and a marginally significant difference in this same comparison in a third. Age at lesion was not a factor. The hemispheres did not differ in calbindin density in any region sampled in two intact control brains. However, comparison of blood vessel density between the hemispheres of the lesion cases indicated that alterations in vascularization may accompany the changes in Cal populations. It remains to be seen whether the changes in Cal density found reflect changes in expression or loss of specific Cal populations. The results contribute to our understanding of the suite of neurochemical, structural and connectional changes that accompany damage to V1 in a monkey model of 'blindsight'.

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Poster

233. Extrastriate Cortex: Motion Processing

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Title: Dynamics of heading and choice-related signals in areas PIVC, VIP and MSTd

Authors: *J. FERNANDEZ-LEON[1,2], A. ROSENBERG[1], A. CHEN[3], A. ZAIDEL[4], G. C. DEANGELIS[5], D. E. ANGELAKI[1];
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China Normal Univ., Shanghai, China; [4]Bar-Ilan Univ., Tel Aviv, Israel; [5]Brain and Cognitive Sci., Ctr. for Visual Science, Univ. of Rochester, New York, NY

Abstract: Recent studies reveal that multiple cortical areas in non-human primates represent heading. However, little is known about the dynamics by which heading and choice are represented in neural activity. To disassociate the time-varying contributions of these signals to neural population activity, we trained macaque monkeys to report the direction of self-motion relative to straight ahead in either visual or vestibular heading discrimination tasks. Using electrophysiological techniques, we quantified the contributions of heading and choice to neural responses in two areas that exhibit multimodal (visual-vestibular) heading signals (dorsal medial superior temporal, MSTd; ventral intraparietal, VIP) and in an area (parieto-insular vestibular cortex, PIVC) with only vestibular activity. To separate heading- and choice-related components of each cell's response dynamics, we computed partial correlations between spike counts, heading, and choice, and then squared to calculate the accounted variance between neural activity and heading/choice. By averaging the squared partial correlation coefficients across all cells within an area, we could estimate population dynamics explained by heading and choice. Vestibular heading signals were similar in PIVC and MSTd, and slightly weaker in VIP. Choice-related activity was strongest in VIP, with MSTd showing the weakest choice activity. Similar observations were found for visual heading signals in VIP and MSTd. Targeted dimensionality reduction techniques were used to further examine how much variance in the population responses could be explained by the velocity and acceleration components, as well as choice. For vestibular signals, the time course of the targeted dimensions revealed that cell activities followed the dynamics of the velocity and acceleration components of the heading signals. The largest contribution of velocity signals was in MSTd, with weaker contributions in PIVC and VIP. Acceleration signals were strongest in PIVC and MSTd, and weakest in VIP. Corroborating the partial correlation analysis, choice-related activity was strongest in VIP and weakest in MSTd. Similar results for the visual condition were found except that the acceleration component was not observed in MSTd and VIP (PIVC does not show visual responses). We conclude that visual-vestibular signals are differentially represented across areas, with the strongest vestibular acceleration in PIVC, visual-vestibular components of velocity in MSTd and choice in VIP. Our dimensionality reduction analyses support this observation because the population activities of these areas occupy different regions of the state space.

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Poster

233. Extrastriate Cortex: Motion Processing

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Title: Contributions of visual and extra-retinal mechanisms to rotation-tolerant heading selectivity in macaque area MSTd

Authors: *A. D. DANZ[1,2], D. E. ANGELAKI[3], G. C. DEANGELIS[2,1];
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Abstract: Optic flow provides a reliable cue for heading estimation when the eyes do not rotate relative to the scene. When the eyes rotate relative to the world, a global component of motion is added to the retinal flow field, thus confounding the relationship between optic flow and heading. To overcome this confound, the visual system could use extra-retinal (e.g., efference copy) signals to compensate for the effects of eye rotation on responses of heading-selective neurons. Alternatively, the visual system may have mechanisms to decompose the flow field into translational and rotational components based on the differential depth dependencies of these flow components. Previous neurophysiological studies have shown partial compensation for eye rotations in heading tuning curves of neurons in area MSTd. However, the relative contributions of visual and extra-retinal signals to this compensation remain unclear because these previous studies either used an improper visual control for eye rotation or did not include a visual control. In addition, it remains unclear whether visual mechanisms of compensation require rich depth structure in the scene. We measured the heading tuning of MSTd neurons while macaque monkeys actively pursued a moving target or maintained fixation while eye rotations were simulated visually. Depth cues were manipulated by presenting either a 3D cloud or a fronto-parallel (2D) plane of random dots on a visual display subtending 90x90° of visual angle. Heading was varied within the horizontal plane, spanning the full range of 360° in 45° intervals. Measuring full tuning curves allowed us to distinguish between tuning shifts, response gain changes, and bandwidth changes resulting from real or simulated eye rotation. Compensation for rotation was quantified using a novel analytical method (Sunkara et al. 2015, eLife) that extracts tuning shifts due to rotation while controlling for changes in response gain and expected changes in tuning bandwidth. Results to date show stronger rotation compensation of heading tuning with the 3D virtual environment than with the 2D scene structure, for both real and simulated eye rotations. In the 2D environment, real eye rotation results in greater compensation than simulated rotation. In all conditions, the extent of rotation compensation is greater than what is expected from rotation-intolerant neurons. These data suggest that both visual and non-visual mechanisms contribute to generating a neural representation of heading in area MSTd that is partially tolerant to eye rotations. We will compare our findings to recent results from the ventral intraparietal area (Sunkara et al. 2015).

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Poster

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Support: NIH DC007620

NEI EY016178

Title: Contributions of visual and vestibular cues to rotation-tolerant heading perception

Authors: *A. SUNKARA[1], B. LI[1], G. C. DEANGELIS[2,3], D. E. ANGELAKI[1];
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Abstract: Navigating through the world requires estimating one's heading, i.e. egocentric translation direction (T), but optic flow cues to heading are confounded by eye or head rotations (R). Several sources provide information about T and R, including visual and vestibular cues, as well as efference copy (EC) signals. Most human psychophysical studies have examined the roles of EC and visual cues (optic flow) in estimating heading in the presence of R. Since optic flow patterns are a sum of T and R components, it is hypothesized that EC is used to estimate R, which is then subtracted from the optic flow (Royden et al., 1992; Royden, 1994). Theoretically, heading in the presence of R can also be calculated based on purely visual cues (Longuet-Higgins & Prazdny, 1980) or through multi-sensory integration of visual and vestibular cues. Importantly, T can be estimated without the need for a separate estimate of R by using motion parallax cues from optic flow or otolith signals from the vestibular system. Since previous studies did not use vestibular T signals, it is unclear how T is extracted from optic flow and how vestibular cues contribute to heading perception in the presence of R. To address these questions, we used a 2-alternative forced choice task in which subjects (n = 8) reported their perceived heading relative to straight ahead. First, we evaluated the role of visual cues by using 3D optic flow stimuli that simulated T and R. All subjects had heading biases that were significantly smaller than the bias expected (20°) for no rotation compensation. These results show that visual cues can be used to partially compensate for R (consistent with neuronal data; Sunkara et al., 2015). Next, we evaluated the role of vestibular cues in heading perception by adding vestibular T to optic flow using a motion platform. This significantly reduced heading biases compared to the visual-only condition, showing that the integration of visual and vestibular translation signals plays a key role in estimating heading. In contrast, when vestibular R was added to the visual stimuli using a yaw motor to passively rotate subjects, heading biases increased substantially. Furthermore, when both vestibular T and R were added, biases were significantly greater than when only vestibular T was added. These results contradict the prevailing hypothesis that an estimate of R is subtracted from optic flow, which would predict smaller biases in heading perception when more information about R is present (in the form of vestibular R cues). Hence,

our results support an alternative hypothesis that heading is derived directly through multisensory combination of visual and vestibular T signals, without first estimating R.

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Poster

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Title: Multisensory representation of self-motion in macaque area 7a

Authors: ***K. LAKSHMINARASIMHAN**[1], E. AVILA[1], G. C. DEANGELIS[2], D. E. ANGELAKI[1,3];
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Abstract: Area 7a of macaque posterior parietal cortex, which projects to the hippocampal formation, is believed to be critical for spatial perception (Andersen, 1989) and has previously been shown to contain neurons sensitive to the pattern of optic flow (Motter and Mountcastle, 1981; Steinmetz et al., 1987; Siegel and Read, 1997; Merchant et al., 2001). Whether the same neurons are also tuned to non-visual self-motion signals remains unknown. To test whether area 7a, like other parietal areas, is multisensory, we used visual, vestibular and multisensory heading stimuli to characterize the tuning of 7a neurons. We recorded from seventy-five single units in area 7a of a macaque monkey trained to passively view different patterns of optic flow while fixating at the center of the screen (visual condition). Different optic flow patterns depicted the movement of the animal along 8 different directions spanning 360° on the azimuth plane of a three-dimensional environment. A subset of sixty neurons was additionally recorded while the monkey was passively translated along the same directions by a motion platform (vestibular condition), as well as simultaneously subjected to both visual and vestibular stimulation (combined condition). Roughly half of the neurons (n=37/75) exhibited significant tuning ($p < 0.05$; ANOVA) to egocentric heading direction in the visual condition. The median strength of their tuning, as quantified using a standard direction discrimination index (DDI), was found to be 0.67±.2. A vast majority of the neurons also exhibited significant direction tuning ($p < 0.05$) under the vestibular (67%, n=40/60) and combined (70%, n=42/60) conditions and their DDIs were comparable to the visual condition (vestibular: DDI=0.6±.2; combined: DDI=0.65±.2). To our knowledge, these findings constitute the first evidence for a multisensory representation of

self-motion in area 7a. A comparison of the DDIs suggests that the precision of heading representation in area 7a is comparable across both modalities. This complements several anatomical studies (Andersen et al., 1990; Lewis and Van Essen, 2000) and supports the idea that heading representation in area 7a may be acquired from convergent inputs from other parietal areas.

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Poster

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EY017866

JSPS

Title: Perception of object motion during self-motion: multisensory contributions to flexible reference frame transformations

Authors: *R. SASAKI[1], D. E. ANGELAKI[3], G. C. DEANGELIS[2];
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Abstract: When a moving observer views a moving object, the retinal image velocity of the object is the vector sum of two components: object motion in the world and self-motion. To estimate object motion in the world, one has to compensate for the effects of self-motion on the retinal image. Other times, we may need to judge how an object moves relative to our head/body, which does not require compensating for self-motion. The brain must therefore perform computations of object motion that flexibly incorporate self-motion signals based on task demands, and we hypothesize that vestibular signals regarding self-translation play an important role. Our goal was to develop a behavioral paradigm for macaques that can be used to study how neurons mediate this flexible computation of object motion. We trained two monkeys to report whether an object moves upward/rightward or upward/leftward by making a saccade to one of two choice targets. In the world-coordinate task, the monkey is required to judge whether the object moved to the left or right of vertical in the world; in the head-coordinate task, the animal reports left or right relative to the head. The two tasks are randomly interleaved, as cued by fixation point shape. In the absence of self-motion (Object only condition), the two task

conditions are equivalent. However, in the presence of lateral self-motion, the animal must compensate for self-motion to perform the world-centered task. Self-motion information was provided by background optic flow (Object+Visual condition) or by a combination of optic flow and translation of a motion platform (Object+Combined condition). Perceptual biases were measured by constructing psychometric functions that plot the proportion of 'rightward' choices as a function of object direction in world coordinates. If the animal compensates fully for self-motion in the world-coordinate task, psychometric functions for rightward and leftward self-motion should overlap. Monkeys successfully switched between performing the world- and head-coordinate tasks. In the world-coordinate task, perceptual biases are substantially greater in the Object+Visual condition than the Object+Combined condition, indicating that vestibular self-motion signals contribute to perception of object motion in the world. Performance was largely accurate in the head-coordinate task, indicating that self-motion signals can also be gated out of the computation of object motion. Our findings demonstrate that perception of object motion incorporates self-motion signals as dictated by task demands, and they set the stage for electrophysiology experiments to explore flexible neural representations of object motion.

Disclosures: R. Sasaki: None. **D.E. Angelaki:** None. **G.C. DeAngelis:** None.

Poster

233. Extrastriate Cortex: Motion Processing

Location: Hall A

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Program#/Poster#: 233.11/K35

Topic: D.04. Vision

Support: ARC Grant FT120100816

NHMRC Grant APP1005427

Title: Decoding feature spaces in primate MT: An evaluation of multivariate pattern analysis methods

Authors: *T. A. CARLSON[1], E. GODDARD[1], H. HOGENDOORN[2], S. S. SOLOMON[3], S. G. SOLOMON[4];
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Abstract: In recent years there have been substantial developments in the application of multivariate pattern analysis methods to neuroscience data, e.g. fMRI, EEG/MEG, and single unit recordings. Many of these developments have come from studies examining the brain's coding of visual objects, a model system in which the underlying coding principles are poorly understood. In contrast, the coding of visual motion in the brain is relatively well known. We

know the fundamental features of motion, i.e. direction and speed, and understand how these features of visual motion are coded in MT, the brain area most often associated with representing motion. In the present study, we leveraged our knowledge of visual motion to study the effectiveness of inferential and exploratory methods for analysing multivariate brain recordings. We analysed single unit activity in multielectrode recordings from area MT of sufentanil-anaesthetised marmoset monkeys. The visual stimuli were moving dot patterns varying in speed and direction (7 speeds x 12 directions), or moving grating patterns varying in spatial frequency (SF), temporal frequency (TF), and direction, (3 SF x 3 TF x 12 directions). For each dataset, we used a linear classifier to compute decoding performance from the recordings for all possible pairwise combinations of stimuli. We first applied representational similarity analysis (RSA; Kriegeskorte, 2008), an inferential model testing approach, and confirmed that speed, direction, SF and TF were represented in population response. We then explored the utility of multidimensional scaling (MDS), an exploratory method of "discovering" important feature dimensions in the neural code. MDS was capable of revealing speed and direction feature dimensions for moving dot patterns; for grating patterns, however, MDS conflated SF and TF, and gave no indication that motion direction was relevant. We then applied hierarchical cluster analysis, another exploratory method that assumes categorical structure and aims to recover the categorical divisions. Cluster analysis conflated the feature dimensions in both dot and grating datasets, and provided limited interpretative value. Our findings show that in a well characterised system (visual motion analysis in area MT), where critical stimulus features have been explicitly manipulated, that exploratory analyses have limited value in "discovering" the underlying coding principles used by the brain. Theory and explicit model testing provide better means to uncover the brain's neural code.

Disclosures: **T.A. Carlson:** None. **E. Goddard:** None. **H. Hogendoorn:** None. **S.S. Solomon:** None. **S.G. Solomon:** None.

Poster

233. Extrastriate Cortex: Motion Processing

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Title: Direction tuning of response variability in populations of MT neurons is different in awake versus anesthetized recordings

Authors: *J. LOMBARDO, M. MACELLAIO, B. LIU, L. C. OSBORNE, S. E. PALMER; Univ. of Chicago, Chicago, IL

Abstract: Populations of sensory neurons exhibit variable firing in response to repeated stimulus presentations. This trial-to-trial stochasticity arises from variability in transmitter release, differences in underlying network state, and uncontrolled sensory inputs. The structure of this variability directly affects the information coding capacity of the population as quantified by the Fisher information. Many models of neural responses assume a Poisson or gamma-Poisson distribution of spike counts. These models fit data well, with spike count variance being proportional to the underlying firing rate, and exhibit a Fano factor (FF) of 1 or greater. While we find our recordings from MT neurons in anesthetized macaques to be consistent with a gamma-Poisson model with a moderate amount of "extra" variance, we find lower levels of variability in recordings from alert macaques, (FF significantly less than 1), arising from shorter time scales of response autocorrelation in the alert state. The difference in FF in the alert and anesthetized states,, and the difference in spike count correlations over time, suggest that consciousness alters the reliability of cortical responses at the level of single neurons. We observed a significant tuning of variability with respect to the preferred motion direction in MT neurons in alert recordings, with FF decreasing for preferred stimuli. We observed the opposite tuning in anesthetized macaques, with FF increasing for preferred stimuli as firing rate increases. New models of stimulus-dependent variability in MT responses are needed to account for these data, and for testing theories of population coding capacity in MT.

Disclosures: J. Lombardo: None. **M. Macellaio:** None. **B. Liu:** None. **L.C. Osborne:** None. **S.E. Palmer:** None.

Poster

233. Extrastriate Cortex: Motion Processing

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Luckhardt Endowment

Title: Synergistic coding of motion direction and speed in MT neurons in monkeys

Authors: *M. V. MACELLAIO, B. LIU, L. C. OSBORNE;
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Abstract: Neurons often encode multiple features of sensory stimuli. For example, in the middle temporal cortical area (MT), many neurons are selective for visual motion and are tuned for both direction and speed. Using one neural population to represent multiple stimulus features presents a challenge to downstream circuits that need to recover information about multiple features, e.g. direction and speed. For example, smooth pursuit eye movements translate visual estimates of target direction and speed arising from a population of MT neurons to rotate the eye in order to cancel retinal image motion. We ask how the joint encoding of direction and speed in MT neurons affects the precision of internal motion estimates and how that in turn affects pursuit. While we cannot directly observe an internal estimate of direction or speed, we can use the eye movement as a loyal representation of internal sensory estimates because <10% of the variation in pursuit arises from motor processing. We use a combination of extracellular recording in area MT of alert monkeys and analysis of eye movement behavior to analyze information about motion direction and speed over time, both at the level of single neurons and at the behavioral output. We find that MT neurons encode motion parameters synergistically, i.e. they encode more information about direction and speed together than the sum of the information about each singly. Synergistic coding arises from nonlinearities in the cortical input-output functions.

Disclosures: M.V. Macellaio: None. **B. Liu:** None. **L.C. Osborne:** None.

Poster

233. Extrastriate Cortex: Motion Processing

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Title: Navigation and decision in mouse parietal cortex

Authors: *M. KRUMIN, K. D. HARRIS, M. CARANDINI;
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Abstract: Posterior parietal cortex (PPC) in primates is believed to be involved in cognitive operations such as coordinate transformations and decision making. In rat PPC, some

experiments revealed decision signals, but others revealed signals related to navigation. Does mouse PPC carry signals related to decision or to navigation, or a mixture of these and other signals? To investigate the role of PPC in mice, we developed a two-alternative forced choice task based on a virtual T-Maze. The initial corridor contains a grating, whose contrast and position (left wall or right wall) are varied randomly across trials. The mouse indicates the position of the grating by turning to that side at the end of the corridor, and receives a water reward for correct choices. Wrong choices are marked by a brief sound. Mice learned the task after 2-3 weeks of training. During the behavior we recorded neural activity in PPC and other areas of the visual cortex, using two-photon imaging of the calcium indicator GCaMP6f through a chronic window. A typical imaging session yielded the activity of several hundreds of neurons (predominantly from layer 2/3) within ~0.5x0.5 mm. The firing of PPC neurons was strongly modulated by the virtual position and heading of the mouse. We expressed these responses as a function of two variables: virtual position of the mouse along the main corridor (z, between 0 and 100 cm), and virtual head direction (θ, between -30 and 30 degrees). Many of the cells had localized 'place-heading fields' in these coordinates. Predicting calcium signals from just these two variables (and not from variables such as speed, stimulus position, or behavioral choice) gave a reasonable prediction of each neuron's activity. These place-heading fields could occur at any location in z-θ space, and indeed the neurons recorded in a single session typically tiled the T-maze . In some cells, the strength of the place-heading field was additionally modulated by the contrast and position of the visual grating (on left or right wall). However, the final choice made by the animal did not seem to affect the responses of PPC cells beyond what could be simply predicted from their place-heading field. These results indicate that mouse PPC is largely concerned with the use of visual information in spatial behavior, including navigation. Some PPC cells showed an interaction of visual information with spatial position, which resembles the interaction of visual stimulation with eye position observed in monkey PPC (Andersen et al, Science 1985). Perhaps PPC plays an analogous role in monkeys and mice, by converting visual information from one coordinate system to another.

Disclosures: **M. Krumin:** None. **K.D. Harris:** None. **M. Carandini:** None.

Poster

234. Eye Movements: Saccades

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 234.01/K39

Topic: D.06. Eye Movements

Title: Bimodal-distributed saccadic reaction time of predictive and reactive saccades

Authors: *K. NI[1], Z. HAN[2], M. ZHANG[2];
[1]Inst. Of Neurosci., Chinese Acad. of Sci., Shanghai, China; [2]Beijing Normal Universisty, Beijing, China

Abstract: To boost the chances of survival in competition, animals especially primates have evolved with not only reactive responses, but also predictive responses to the environment. We find that saccadic reaction time (SRT) is bimodal-distributed when monkeys perform the memory-guided saccade task. The second modal of the SRT distribution should be from reactive saccades triggered by fixation point off, and the first modal is hypothesized to be from predictive saccades triggered by internal timing. To causally prove the relationship of the first modal and temporal prediction, we first lengthen the delay period to prove the sufficiency of temporal prediction to the first modal, and then randomize the delay period to prove the necessity. By comparing the differences between predictive and reactive saccades, this behavior paradigm can be used to study the neuronal mechanism of temporal prediction.

Disclosures: K. Ni: None. **Z. Han:** None. **M. Zhang:** None.

Poster

234. Eye Movements: Saccades

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Topic: D.06. Eye Movements

Support: NIH Grant EY014885

Title: Saccade trajectory curvature induced by planning of sequential saccades

Authors: *R. AZADI, R. M. MCPEEK;
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Abstract: Saccadic trajectory curvature can be modulated by different factors; for example, when a target is presented along with a distractor, saccades to the target show greater curvature in their trajectories than when the target is presented without a distractor. Here, we investigated the effect on saccade trajectory of the planning of a subsequent saccade. In this experiment, subjects were instructed to perform a sequence of two saccades, as quickly as possible, when a central fixation point disappeared. The first saccade was a vertically-directed movement to a visual target presented below the fixation point and the second saccade was either a leftward or rightward movement to one of two additional targets, flanking the first target. The direction of the second saccade was visually cued by a small oriented bar presented at the fixation point during the whole fixation period (1000±250ms). We also included a control condition, in which subjects were cued to only make a single vertical saccade but no second saccade. The three trial types (leftward, rightward and no second saccade) were randomly interleaved in the experiment.

Thus, in each trial, the peripheral visual stimuli were identical and the initial saccade was directed to the same location; only the direction of the second saccade differed. Across these three conditions, we did not find any significant differences in landing position or peak velocity of the initial saccade. But in agreement with earlier studies, when two sequential saccades were made, the latency of the initial saccade was slightly longer than when only a single movement was made. More importantly, the trajectory curvature of the initial saccade differed significantly among the three conditions: on average across all subjects, the vertical saccades in the control condition showed less curvature than when the same movement was followed by a second saccade. Moreover, when two sequential saccades were made, the initial saccade tended to curve away from the goal of the second saccade. These results indicate that planning of a subsequent saccade induces systematic saccade curvature even when the visual stimulus and initial saccade are kept constant. This is consistent with the idea that planning of a saccade sequence involves activation of a future saccade goal at a relatively low level in the oculomotor system (e.g., superior colliculus), where it can influence the execution of the current movement.

Disclosures: **R. Azadi:** None. **R.M. McPeek:** None.

Poster

234. Eye Movements: Saccades

Location: Hall A

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Topic: D.06. Eye Movements

Support: NIH Grant EY019258

Title: The effects of visual target goal on the adaptation of saccades

Authors: *R. SOETEDJO;
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Abstract: The ability of saccades to recover following nerve palsy and extraocular muscle injuries suggests that the saccadic system has access to adaptation mechanisms that gradually correct persistent motor errors. In the laboratory, a more practical intrasaccadic-step paradigm is used to simulate saccade dysmetria and produce adaptation. Our understanding of the neuronal mechanisms underlying adaptation of saccades is based almost solely on data obtained from targeting (visually guided) saccades. Several studies show that adaptation of targeting saccades involves the cerebellar oculomotor vermis (OMV). The OMV receives mossy fiber projection from the nucleus reticularis tegmenti pontis that appears to relay desired saccade vector signal from the superior colliculus (SC). Recent studies of the SC indicate that SC activity may also encode target goal signal. How these two different signals from the SC affect saccade adaptation is unknown. Targeting saccade, a task used in most saccade adaptation studies, cannot

distinguish the effects of the two signals because its target goal and desired saccade vectors are congruent. Therefore, we employed double-step saccade task (DST) to dissociate the two vectors. In this task, two sequential saccades are made in the dark following two flashes of a small target spot in different locations on a tangent screen. The task allows us to vary the eccentricity of target goal while keeping the desired saccade vector of the second saccade constant, and vice versa. We tested whether saccade adaptation relies on the context of the desired saccade vector or target goal by measuring the transfer of adaptation of targeting saccades to the second saccades of DST, and vice versa. If adaptation of saccades modifies desired saccade vector signal, the two types of adaptation (targeting and second saccade of DST) would transfer to each other as long as their desired saccade vectors are identical. On the other hand, if adaptation modifies target goal, then the amount of transfer would be determined solely by the target goal vector irrespective of the desired saccade vectors. We show that transfer of adaptation of targeting saccades to the second saccades of DST is a function of how close the target goal of the second saccades to the adapted saccade target vector is. If the target goal of the second saccades and the adapted saccade target vector were dissociated, very limited or no transfer was observed even though the two saccades had identical desired saccade vectors. Our results suggest that adaptation of targeting saccades modifies the target goal, but not the desired saccade vector signal.

Disclosures: R. Soetedjo: None.

Poster

234. Eye Movements: Saccades

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Topic: D.06. Eye Movements

Support: NIH R01 NS078311

Title: Modulation of vigor during a temporal discounting task

Authors: *T. REPPERT[1], K. M. LEMPERT[2], P. W. GLIMCHER[2], R. SHADMEHR[1];
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Abstract: Value-based decision making is a complex process depending on factors such as socio-economic status, age, and impulsivity. Previous studies of value-based decision making have focused on explicit choices as the primary measure of preference. Given an individual's choices in a well-designed binary choice task pitting small, immediate rewards against large, delayed rewards, we can derive an accurate, precise measure of subjective valuation of rewards. Here, we present findings that suggest that movement vigor serves as an implicit measure of choice preference. Specifically, vigor of saccades made before the time of decision reflects the

subjective valuation of the rewards. Sixty subjects participated in a standard temporal discounting task with a series of choices between small, immediate monetary rewards and large, delayed rewards (e.g., "$10 today or $20 in 30 days"). The delay was held constant at 30 days, and we presented each subject with the same group of 60 distinct reward pairings, displayed in random order. Each reward pairing was presented twice, for a total of 120 trials, each lasting 16.5 sec. As the subjects made their decisions, we monitored their eye movements. We found that, before a decision was made, saccade vigor was higher to the reward which was eventually chosen. Moreover, vigor of saccades was modulated by the difference in subjective values of the reward offerings. In this manner, vigor served as an implicit measure of choice preference. We also found that vigor of saccades dropped significantly after a decision was made, corroborated by the fact that vigor decremented most steeply on trials in which subjects made relatively quick decisions. Thus vigor also encoded timing of decision, in a manner consistent with previously established behavioral measures of decision making, such as pupillometry. We present vigor of saccades as a novel tool to be leveraged to assess value-based decision making.

Disclosures: **T. Reppert:** None. **K.M. Lempert:** None. **P.W. Glimcher:** None. **R. Shadmehr:** None.

Poster

234. Eye Movements: Saccades

Location: Hall A

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Program#/Poster#: 234.05/L1

Topic: D.06. Eye Movements

Title: On the role of intrinsic microsaccadic biases in influencing behavior in Posner cueing

Authors: ***X. TIAN**, Z. M. HAFED;
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Abstract: Microsaccades are modulated in systematic manners during Posner cueing. Immediately after spatial-cue onset, microsaccades are biased in the cue's direction, and they then flip to being opposite the cue. Microsaccade frequency is also modulated. We recently suggested that both direction and frequency modulations reflect a resetting of microsaccadic oscillatory rhythms by cue onset (Hafed & Ignashchenkova, J. Neurosci., 2013). Moreover, we also found that subsequent behavioral performance modulations observed in Posner cueing (e.g. increases or decreases in reaction time, RT, to the post-cue target) can be accounted for by the instantaneous phase at which post-cue targets appear (Tian & Hafed, Vision Sciences Society, 2014, 2015). That is, microsaccades occur in a repetitive manner, and are anti-correlated in direction relative to the previously executed microsaccade. If a target appears spatially-congruent with the current microsaccadic plan, performance is facilitated; if it appears incongruent, performance is impaired (as in inhibition-of-return). However, it remains unclear why on a given

trial, a cue may or may not influence microsaccades. According to the microsaccadic rhythmicity hypothesis, if a monkey has an intrinsic microsaccade direction bias (e.g. largely purely downward), then cue onset (e.g. either purely above or purely below fixation) would be more or less likely to occur congruent with the current microsaccadic plan. This would alter how a cue might influence microsaccades, and could potentially help explain asymmetries in final attentional performance (e.g. RT) as a function of visual field location. We ran two monkeys in a Posner cueing paradigm. In each trial, a cue/target (1 deg diameter white circle) appeared at 5 deg eccentricity, either horizontally or vertically. Cue-to-target onset asynchrony (CTOA) was random (between 32 ms and 1532 ms), and the post-cue target could appear either in the "same" or "opposite" cued location. We collected >4000 trials per monkey and measured RT to the target. In monkey P, intrinsic microsaccade bias was purely downward. In monkey N, the bias was more rightward. In both monkeys, cue onset opposite the microsaccade bias was more effective in driving early microsaccades towards its location. Subsequent microsaccadic direction oscillations reflected the intrinsic bias. These results suggest that the efficacy with which cues influence both microsaccades and final performance can depend on individual intrinsic biases in subliminal, fixational oculomotor activity. This has implications on our understanding of the neuronal bases for visual field asymmetries in attentional performance.

Disclosures: X. Tian: None. **Z.M. Hafed:** None.

Poster

234. Eye Movements: Saccades

Location: Hall A

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Topic: D.06. Eye Movements

Support: NIH Grant EY021286

Title: Foveal attention amplifies a position-correcting mechanism during ocular pursuit

Authors: *S. J. HEINEN[1], E. POTAPCHUK[1], S. WATAMANIUK[2];
[1]Smith-Kettlewell Eye Res. Inst., San Francisco, CA; [2]Wright State Univ., Dayton, OH

Abstract: Previously we showed that more catch-up saccades occur during pursuit when the stimulus has a central target than when it does not, and that even more catch-up saccades occur when the central target is attended (Heinen et al., SfN 2013; 2014). Here we investigate the mechanism underlying these results. Since catch-up saccades are thought to be generated by a combination of retinal position and velocity errors (deBrouwer et al., 2002), we asked how the presence of a central pursuit target and attention interact with these factors. We first investigated the influence of a central target. Stimuli were either a single spot, four peripheral dots which formed a virtual diamond, or a 5-dot composite of these stimuli. Critically, all these stimuli are

symmetrical, and create identical magnitude position and velocity errors on the retina when they move. Therefore, they should generate identical numbers of catch-up saccades during pursuit. However, the four dot stimulus produced fewer saccades than the two stimuli with central elements for the same combinations of position and velocity error, indicating that these errors are not the only factors contributing to catch-up saccade generation, and that the presence of a foveal target is also a factor. Since attention shifts precede saccades (Peterson et al., 2004), and saccades correct position error, we wondered whether attention to the foveal target influenced the position mechanism during pursuit. We directed attention to and away from the fovea using a detection task in which either the central target or one of the peripheral ones briefly dimmed. Drawing attention to the central target increased saccade frequency, while drawing it away reduced it. Importantly, the relative contribution of position error to catch-up saccades was greater when the stimulus had a central target, and increased further when that target was attended. Conversely, removing the central target, or taking attention away from it, produced saccades driven mostly by velocity error. The results suggest that attention to a foveal target amplifies position error signals during pursuit, leading to more catch-up saccades.

Disclosures: **S.J. Heinen:** None. **E. Potapchuk:** None. **S. Watamaniuk:** A. Employment/Salary (full or part-time):; Smith-Kettlewell Eye Research Institute.

Poster

234. Eye Movements: Saccades

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Topic: D.06. Eye Movements

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NIH R21-EY019713

Title: Inter-trial correlations in sequences of saccade endpoints: further reflections on fractal structure in motor control

Authors: *M. J. SHELHAMER[1], A. WONG[2], P. FEDERIGHI[3];
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[3]Univ. of Firenze, Florence, Italy

Abstract: This work continues our investigation into inter-trial correlations between consecutive saccades, made in response to periodically paced visual targets. There is significant variation in the performance of such saccades from one trial to the next, even if paced at a constant rate with two targets at fixed locations. Previously, we demonstrated that variations in the amplitudes of sequences of these saccades exhibit a fractal structure: the power spectrum of the sequence of amplitudes has a power-law form, reflecting a gradual power-law decay of the autocorrelation.

This in turn implies that performance information is stored from one trial to the next, enabling the programming of subsequent movements in a predictive (anticipatory) manner. We found that a larger magnitude of spectral slope is associated with faster saccade adaptation in a subsequent double-step task, suggesting that subjects who adapt faster are better able to utilize prior experience to modulate future behavior. Why the inter-trial correlations are power-law and not exponential remains a mystery. Here, we investigate similar properties in inter-trial correlations in the endpoints of sequences of predictive saccades. Subjects made sequences of predictive saccades, paced periodically between two fixed targets, along a horizontal or vertical axis. Variations in endpoints were examined for inter-trial correlations and fractal structure, along the axis of the targets and orthogonal to that direction. In the primary movement direction (aligned with the targets), there were little or no inter-trial correlations, as reflected in an approximately flat power spectrum (similar to white noise): slopes of the spectra in log-log coordinates were not significantly different from zero. In contrast, in the direction orthogonal to target motion, the endpoints did exhibit inter-trial correlations: slopes were 0.40±0.19 (H) and 0.33±0.21 (V). These results are different from those with saccade amplitudes, where scaling was significant in the direction of target motion. While saccade amplitudes are programmed neurally, the endpoints reflect movement error. Hence, uncorrelated errors along the direction of target motion suggest that the maximum amount of information has been extracted from previous movements for the programming of the next saccade: the sequence of residuals is white (uncorrelated). In the direction orthogonal to target motion there are remaining inter-trial correlations, suggesting that more information could have been extracted from this sequence. Thus, subjects control movement accuracy by utilizing previous performance information only along task-relevant directions.

Disclosures: **M.J. Shelhamer:** None. **A. Wong:** None. **P. Federighi:** None.

Poster

234. Eye Movements: Saccades

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UAB School of Optometry

Title: Short-term saccadic adaptation to intra-saccadic conjugate and disconjugate background shifts

Authors: *C. BUSETTINI[1], K. P. SCHULTZ[2];
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Abstract: The double-step paradigm elicits short-term adaptive changes in saccadic gain. The subject is asked to respond to a first target shift with a (primary) saccade. During its execution, the target is shifted a second time, introducing an artificial landing error. In a previous report on macaques, using small targets in a dichoptic viewing, we studied the adaptive process elicited by introducing the same error in both eyes (conjugate error) and equal and opposite in both eyes (symmetric disparity error) after a conjugate primary saccade. At the start of the session the animal responded to conjugate errors with a corrective (secondary) saccade and to disparity errors with a smooth vergence response. Repeating this process gradually caused the saccadic system to alter the primary saccade to include the landing error. The evidence suggested that the mechanism involved in compensating the disparity error, by making the saccade asymmetric, has different properties than the mechanism compensating the conjugate error. These results indicated that saccadic adaptation is a binocular process and not a monocular mechanism where each eye recalibrates its gain independently from the other. To further explore this aspect, again in macaques, we modified the paradigm by adding to the target a large 40x40° b/w random-dots background. The dots were 2° in size with an approximate 50% density. The target (0.5°) is now used only to drive the primary saccade and it is extinguished at the same time that the background is shifted. Thus, the landing error is on the background only. There is no primary shift of the pattern. The primary steps were conjugate 10° rightward or leftward in a randomized sequence starting from center and the initial position of the background was randomized to eliminate local cues. The errors were +2° or -2° conjugate or 2° symmetric crossed or uncrossed disparity. Conjugate shifts of the background did not elicit saccadic adaptation, but only a robust, unspecific decrease in the gain of the primary saccade, suggesting that the disappearance of the small target prior to the end of the primary saccade put the saccadic system in a post-saccadic visual open-loop state which, as default, decreased the gain of the saccade. On the contrary, disparity errors elicited powerful disconjugate adaptation, with time constant similar or even faster than the one we observed with no background. We also tested anti-correlated backgrounds (one image the negative of the other). Our studies on short-latency vergence with anti-correlated patterns observed transient vergence responses in the opposite direction but also not giving depth perception. No disconjugate adaptation was observed.

Disclosures: C. Busettini: None. **K.P. Schultz:** None.

Poster

234. Eye Movements: Saccades

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 234.09/L5

Topic: D.06. Eye Movements

Title: Camera-based calibration system for obtaining gaze vectors from freely moving rats in a screened arena

Authors: *M. E. SILVA PRIETO, K.-M. VOIT, D. S. GREENBERG, G. NOTARO, J. SAWINSKI, D. J. WALLACE, J. N. D. KERR;
Behavior and Brain Organization, Res. Ctr. Caesar: A Max Planck Inst., Bonn, Germany

Abstract: Detection and escape from predatory threats is an innate behavior in rats, even those bred in captivity. Escape responses that are potentially similar to those evoked by predators are observed when rats are exposed to specific overhead visual stimuli. We used this behavioral readout to measure what part of the overhead visual field rats respond to, prior to their escape response. With the recent advances in camera-based tracking techniques, we were able to record the eye movements during these escape responses. We achieved this, by implanting miniaturized head-mount cameras on freely moving rats, placed in an arena consisting of a base surrounded by side screens, and a screen located overhead. Simultaneously, we tracked the animal's position and head orientation with four overhead cameras. With this data, we could translate the gazing vectors into the static coordinate frame of the overhead camera setup. To relate these gazing vectors to the displayed stimuli, we used the same camera system that is also employed to track the animal's head position. However, some of the screens were out of view of the cameras - especially the overhead screen - and none of them emitted IR light, so were invisible to the tracking cameras. To address these problems, we equipped each screen with four IR LEDs, and used a calibration mirror, which also contained IR LEDS, so that all screens could be viewed by the tracking cameras. This allowed us to accurately relate the LED positions to pixel coordinates of the stimuli. We next extended the beam-path model of our software framework for camera tracking to take into account the mirror, including refraction in the glass layer above the reflective coating. We verified the accuracy of our mirror tracking framework by comparing the reconstructed 3D model of the screen setup with physical measurements of the real-world setup, both regarding distances between LEDs on a single screen and distances between two different screens. With the setup presented, we are able to determine the gazing vectors with respect to the stimuli positions on the screens for freely moving rats.

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Poster

234. Eye Movements: Saccades

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 234.10/L6

Topic: D.06. Eye Movements

Title: Rat eye movements diminish visual field blind spots during free movement

Authors: *J. N. KERR, D. S. GREENBERG, J. SAWINSKI, Jr, D. J. WALLACE;
Behavior and Brain Organization, Res. Ctr. Caesar: A Max Planck Inst., Bonn, Germany

Abstract: Rats have a large visual field that extends vertically from below their snout, over the top of the head to almost 60 degrees beyond vertical behind their head. This large coverage is due, in part, to the eye optics, which have a large angle of acceptance (>180 degrees), and in part to the semi-lateral position of the two eyes optical axis, (~58 degrees from frontal plane and ~36 degrees above horizontal). When freely moving, rats exhibit large disconjugate eye movements that are strongly correlated with head rotations. As a consequence of the position of the eyes on the head and the large disconjugate eye movements observed, rats forgo constant binocular fusion. We suggested that one purpose of these eye movements is to reduce 'blind spots' which can involve either or both eyes thereby increasing the ability to detect overhead threats. Here, we examined the effects of eye movements on the coverage of visual space above the animals head during free movement to test whether blind-spots were reduced by eye-movements. We computed a probability map for coverage of visual space around the animal (n = 3 animals, 6 recording sessions, 92519 time points, range of head pitch 120º , range of roll 60º). Altogether, the area covered included 76.9% of all space above the horizon or 15868.6 square degrees. When this map was computed with the eyes fixed to their mean positions the total covered area above the horizon was reduced to 67.9% or 14011.8 square degrees, introducing additional blind spots of 1856.8 square degrees; this was especially obvious in the overhead region. Thus, when including all regions of visual space and all orientations of the head without distinction, the overall effect of eye movements is a reduction of blind spots above the horizon. We next examined whether there were particular head positions that eye movements had greatest effect in reduction of blind spots. By dividing head- and eye-tracking data into five equally sized segments based on head pitch (-90º to -63º , -63º to -36º , -36º to -º 9, -9º to 18º , and 18º to 45º) we computed the probability of visual space coverage as a function of elevation and azimuth, both including observed eye movements and with the eyes fixed to their mean positions. The greatest differences in coverage were seen in the two groups with most extreme head pitch. During periods of downward pitch of the head, eye movements always increased the average coverage of visual space above the horizon These measurements show that although the eye movements of the rat are disconjugate, one purpose they serve is to maintain a stable visual coverage above the horizon and to reduce blind spots in the visual field, especially overhead.

Disclosures: J.N. Kerr: None. **D.S. Greenberg:** None. **J. Sawinski:** None. **D.J. Wallace:** None.

Poster

234. Eye Movements: Saccades

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 234.11/L7

Topic: D.06. Eye Movements

Title: Oculo-videography of eye movements in freely moving ferrets and tree shrews

Authors: *D. J. WALLACE[1], D. S. GREENBERG[1], J. SAWINSKI[1], R. CORLEW[2], D. FITZPATRICK[2], J. N. D. KERR[1];
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Abstract: Most cortical and subcortical regions processing visual information are organized in a retinotopic manner. Both the movements of the two eyes and how the movements of left and right eye are coordinated critically determine the visual information that the brain has to process. In humans and primates, the coordination of eye movements is such that both foveas are continuously directed towards the same visual target, and both fixation and ocular-alignment are tightly controlled. We recently showed that the coordination of eye movements in the rat is dominated by the vestibulo-ocular reflex (Wallace, Greenberg, Sawinski, Rulla, Notaro, and Kerr (2013) Nature. 498: 65-9). The vestibular-driven movements combined with the animals' laterally-pointing optical axis has the consequence that ocular-alignment is not continuously maintained. The continuously changing ocular-alignment has significant implication for binocular processing in the cortex. To investigate the extent to which the coordination of eye movements is dominated by the vestibulo-ocular reflex in other animal species we recorded eye movements in freely moving ferrets and tree shrews. Simultaneous recording of the movements of both left and right eyes were acquired using custom-built, miniature oculo-videography systems while the animals were exploring or pursuing objects in a large arena (2.6x1.3m for ferrets and 1.3x1.3m for tree shrews). Simultaneous tracking of the animals position and head orientation was conducted using a custom built overhead tracking system, consisting of 6 high-speed digital cameras. In freely moving ferrets, like in freely moving rats, there was a strong relationship between horizontal eye position (along the nasal-temporal axis) and the degree of head pitch. Strong nose-down head pitch results in marked temporal movement of both eyes, while nose-up pitch results in nasally directed movement of both eyes. During the temporal movements there was a strong misalignment of the optical axes, similar to that seen in rats. However, unlike rats, we did not observe a strong relationship between roll of the head and vertical eye position. Eye movements observed in freely moving tree shrews consisted mainly of very rapid, saccade-like conjugate movements, and neither correlations between head pitch and horizontal eye position nor head roll and vertical eye position were observed. Preliminary analysis indicates that alignment of the optical axes was continuously maintained. Each of the three species (ferret, tree shrew and rat) show distinct and different coordination of eye movement, with the implications for processing of binocular information being distinct for each species.

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Poster

234. Eye Movements: Saccades

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 234.12/L8

Topic: D.06. Eye Movements

Title: Fixation eye movements of monkeys studied with bright and dark background

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Abstract: Fixation eye movements may contribute to visual perception. However, the evidence supporting this assertion relates mostly to photopic vision. In photopic vision, saccades and fixation direct the fovea to interesting locations in the scene. In scotopic vision, the fovea is largely insensitive. Directing the fovea to interesting locations seems pointless; it is not clear what exactly the functions of saccades and fixation are in scotopic conditions. Interestingly, even if the (small) target is bright and only the background is dark, monkeys direct their eyes so that the fovea is above the target. This background-luminosity-contingent 'upshift' of gaze was described in several studies; we recently showed that the upshift continues for the entire duration of 2-s fixations. The aim of the present study is to test whether the upshift is accompanied by changes in fixation eye movements; that is, if fixation eye movements depend on background luminosity, and how this putative dependence relates to the upshift. The upshift is a gradual phenomenon; dim luminosities were previously shown to yield small upshifts, with upshift being larger the darker the background is. Here we test whether fixation movements change with background luminosity, similarly to the upshift. We test two possibilities: either the change is in individual movement parameters (such as microsaccade amplitude) or it reflects combinations of states, a bright-background state and a dark-background state. In the present study we have collected data from 3 monkeys. We used two tasks. In both tasks, the monkey fixates target dots appearing in 24 locations, arranged 3 concentric circles. Both tasks test fixations with and without a fixation spot. In both tasks there were blocks of trials with background being dark (0 cd/m2) or bright (6 cd/m2). One task explored intermediate background luminosities, which might mimic mesopic-like conditions. This task contained also blocks with background being one of three levels (0.1, 0.3, and 0.7 cd/m2). The second task tested the effect of dark adaptation. During dark-adapted testing, targets were dim, barely above threshold. In all other testing, targets were bright (44.6 cd/m2). Dark adaptation was obtained by waiting in the dark for 40 min. Altogether we now have more than 20000 fixation trials. At the time of writing this abstract, the data is being analyzed. Our hope is that the findings on fixation eye movements in different background luminosities will reflect on the visuomotor processes that occur in photopic, scotopic, and mesopic vision.

Disclosures: O. Spivak: None. **P. Thier:** None. **S. Barash:** None.

Poster

234. Eye Movements: Saccades

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Title: Characteristics of spontaneous square-wave jerks in the healthy macaque monkey during visual fixation

Authors: F. M. COSTELA[1,2], J. OTERO-MILLAN[1,3], M. B. MCCAMY[1], S. L. MACKNIK[4,1], L. L. DI STASI[1,5], H. RIEIRO[1,6], J. R. LEIGH[7], X. G. TRONCOSO[1,8], A. NAJAFIAN JAZI[1], *S. MARTINEZ-CONDE[4,1];
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Abstract: Saccadic intrusions (SIs), predominantly horizontal saccades that interrupt accurate fixation, include square-wave jerks (SWJs; the most common type of SI), which consist of an initial saccade away from the fixation target followed, after a short delay, by a return saccade that brings the eye back onto target. SWJs are present in most human subjects, but are prominent by their increased frequency and size in certain parkinsonian disorders and in recessive, hereditary spinocerebellar ataxias. SWJs have been also documented in monkeys with tectal and cerebellar etiologies, but no studies to date have investigated the occurrence of SWJs in healthy nonhuman primates. Here we set out to determine the characteristics of SWJs in healthy rhesus macaques (Macaca mulatta) during attempted fixation of a small visual target. Our results indicate that SWJs are common in healthy nonhuman primates. We moreover found primate SWJs to share several characteristics with human SWJs, including the relationship between the size of a saccade and its likelihood to be part of a SWJ. One main discrepancy between monkey and human SWJs was that monkey SWJs tended to be more vertical than horizontal, whereas

human SWJs have a strong horizontal preference. Yet, our combined data indicate that primate and human SWJs play a similar role in fixation correction, suggesting that they share a comparable coupling mechanism at the oculomotor generation level. These findings constrain the potential brain areas and mechanisms underlying the generation of fixational saccades in human and nonhuman primates.

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Poster

235. Vestibular Hair Cells, End Organs, and Nerve

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 235.01/L10

Topic: D.07. Vestibular System

Support: NIH Grant DC02058

Title: Line of polarization reversal (LPR) located lateral to the striola in the chinchilla utricular macula

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Abstract: Traditionally, the striolar region in the vestibular otolith endorgans in vertebrates has been defined by a lower hair cell density, the presence of complex calyces, smaller otoliths, and hair cells and calyces with a greater diameter (Lindeman 1969; Fernandez et al., 1988, 1990, 1995). Our laboratory used the marker, calretinin, to define the striolar region (Desai et al., 2005a,b) based upon the finding that calretinin labels pure calyx afferents (Desmadryl and Dechesne, 1992), a class of vestibular afferents with distinct physiological traits (Baird et al., 1988; Goldberg et al., 1990; Lysakowski et al., 1995). The Desai definition matched the earlier areal delineation, but was not based upon a clear definition of the reversal line, which was depicted running down the middle of the striolar region. Recent studies have determined that the line of polarity reversal (LPR) in the utricular macula is located lateral to this striolar region in mice (Li et al., 2008) and rats (Schweizer et al., 2009). Individual organs were dissected and their hair bundles were removed by gentle sonication. Hair-bundle polarization was determined by the kinocilium location, indicated by a hole in the cuticular plate, which was otherwise labeled by spectrin. We now have evidence for a finding similar to that in mice and rats in a third rodent species, Chinchilla laniger, from a different Rodentia suborder, Caviomorpha, albeit also lateral-eyed. The large majority (95%) of calyx afferents, as in previous studies, were located

medial to the LPR. The implications for sensory processing are profound. The results are of interest because utricular afferents originate to one side or the other of the LPR, separating the utricular macula into a medial and lateral extrastriola. According to Maklad et al. (2010), the medial extrastriola projects to the vestibular nuclei, while the lateral extrastriola projects to the cerebellum. If this is true, then the entire population of calyx afferents projects to the vestibular nuclei and none to the cerebellum.

Disclosures: A. Lysakowski: None. **S.D. Price:** None. **J.M. Goldberg:** None.

Poster

235. Vestibular Hair Cells, End Organs, and Nerve

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 235.02/L11

Topic: D.07. Vestibular System

Support: NIDCD R21 DC013358

Title: Whole-cell recordings from type II vestibular hair cells with basolateral processes in the mature mouse utricular macula

Authors: *A. GONZALEZ GARRIDO[1], J. S. STONE[2], R. A. EATOCK[1];
[1]Neurobio., Univ. of Chicago, Chicago, IL; [2]The Virginial Merrill Bloedel Hearing Res. Ctr. and the Dept. of Otolaryngology-Head and, Univ. of Washington, Seattle, WA

Abstract: The vestibular epithelia of amniotes have two hair cell types, type I and type II, contacted, respectively, by large calyceal afferent or more compact bouton terminals. Recently it was discovered that type II hair cells in mature mammalian vestibular epithelia can have extensive basal (sub-nuclear) cytoplasmic projections that contact supporting cells, neurons and even other hair cells (Golub et al., J Neurosci 32:15093, 2012; Pujol et al., J Comp Neurol 522:3141, 2014). To evaluate whether hair cells with basolateral processes are a distinct type II population, we are measuring their electrophysiological properties in semi-intact utricular epithelia from mature mice. Here we report whole-cell recordings from the lateral extrastriolar zone of epithelia from mice between 3 and 7 weeks of age (34 ± 2.9 postnatal days, n=13). The epithelia were bathed in L-15 culture medium at room temperature and recording pipettes contained conventional KCl-based solutions and fluorescent dye, either sulforhodamine 101 (n=11) or Lucifer Yellow (n=2). In 10 successful fills, 8 had either simple or branched processes similar to those described in Golub et al. (2012) and 2 had no cytoplasmic processes. In no case did we observe dye transfer from a recorded hair cell to any of its neighbors, consistent with the lack of evidence for gap junctions (Pujol et al. 2014). The electrophysiological properties in our sample resembled those provided in previous reports for younger mouse utricular type II hair cells. Input resistance was 1.0 ± 0.3 GΩ (mean ± SEM, n=13 cells), capacitance was 4.4 ± 0.5 pF

and resting potential was −64 ± 2 mV (junction potential corrected). In all cells, hyperpolarizing voltage steps evoked inward HCN currents and depolarizing steps evoked outward currents that activated positive to resting potential and showed some inactivation during 200 ms. For the outward currents, Boltzmann fits of the tail current activation curve yielded a midpoint of −30 ± 1.4 mV (n=13), a slope factor of 9 ± 1 mV and a maximum steady-state conductance of 21 ± 5 nS. The activation curve parameters are in good agreement with previous values reported for outward K+ currents in type II hair cells and clearly different from values for currents in type I hair cells, which activate more negatively. Voltage responses to current steps in current clamp experiments were also consistent with previous reports for type II cells: positive steps evoked a sharp initial peak and a strongly rectified response, and negative steps evoked a "nose" attributable to HCN current. These preliminary results suggest that hair cells with basolateral processes are typical type II hair cells rather than a distinctive sub-population.

Disclosures: A. Gonzalez Garrido: None. **J.S. Stone:** None. **R.A. Eatock:** None.

Poster

235. Vestibular Hair Cells, End Organs, and Nerve

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Support: VIEP-BUAP SAL/G/2015

PIFI-SEP 2014

VIEP-BUAP 00072/2015

Title: Modulatory actions of metabotropic glutamate receptors on spontaneous electrical activity of the vestibular afferents: an ontogenic study

Authors: R. VARELA[1], E. MONJARAZ[1], *F. GALINDO[1], J. CEBADA[2], A. FLORES[1]; [1]Inst. de Fisiología, Benemerita Univ. Autonoma De Puebla, Puebla, Mexico; [2]Facultad de Medicina, Benemérita Univ. Autónoma de Puebla, Puebla, Mexico

Abstract: Glutamate is one of the most important neurotransmitters in the inner ear, like many other transmitters, exerts its pleiotropic roles by means of multiple receptor proteins. Activation or changes in the expression of metabotropic glutamate receptors (mGluRs) have been related to a variety of important ontogenic events, such as neuronal migration and the formation of synaptic circuitry. The purpose of this work was to study the presence and physiological role of mGluRs during embryonic development of the chicken's vestibular organ (Gallus domesticus). We performed a multiunit extracellular recording from the posterior branch of the vestibular nerve in a preparation isolated from the chicken inner ear of 16, 19 and 21 embryonic days. 1S,

3R-ACPD, a mGluRI and mGluRII agonist (1µm-100µm; n=47) increases the basal discharge of vestibular afferents mainly at E19 and E21 in a dose-dependent way (131±5.6% and 207±1.25%, respectively at 100µm). L-AP4, specific agonist of the mGluRIII (0.1-100µm; n=53) also increased the vestibular afferent discharge, however, the main effect was on E16 (148.7±1.9% at 100µm). The presence of MCPG or MPPG, a mGluRII and mGluRIII antagonist, respectively (1µm-100µm), decreased significantly the spontaneous and agonist evoked electrical activity. Complementarily, RT-PCR experiments revealed the presence of the three receptor groups. mGluRI and mGluRII increases its presence at ages near of hatching while mGluRIII have the most important expression at early stage (E16). Our results suggest that mGluR could modulate the spontaneous activity of the vestibular afferent fibers at age-dependent way in the chicken embryo's inner ear.

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Poster

235. Vestibular Hair Cells, End Organs, and Nerve

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VIEP-BUAP

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Title: Histamine receptor 4 antagonist JNJ7777120 inhibits the sodium currents modulating the discharge of vestibular afferent neurons of the rat

Authors: ***R. VEGA**[1], E. SESEÑA[2], E. SALCEDA[1], E. SALINAS[1], E. SOTO[1];
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Abstract: The histaminergic drugs are some of the most commonly used in the treatment of vestibular disorders. Drugs JNJ7777120 and JNJ10191584 thought to be selective type 4 histamine receptor (H4R) were shown to potently inhibit action potential discharge of the vestibular afferent neurons (VAN) (Desmadryl et al., 2012). However, its mechanism of action on the VANs has not yet been determined, with this aim we studied the voltage dependent ionic currents modulated by JNJ7777120 and the participation of G-protein mediated signaling involved in the JNJ77777120 effect. The action of JNJ7777120 on the VAN ionic currents from the rat were studied using the current and voltage clamp technique. In voltage clamp experiments

JNJ7777120 decreased inward and outward current components, effect that remained in cells pretreated with *pertussis toxin* for 20 hours. The isolated voltage gated sodium current (I_{Na}) showed two components: a transient (I_{Nat}) and a persistent (I_{Nap}), the application of JNJ7777120 produced a concentration dependent inhibition of both components of the I_{Na} with an IC_{50} of 41 nM for the I_{Nat} and 16 nM for the I_{Nap}. The JNJ7777120 produced non-significant changes in both I_{Nat} and I_{Nap} current activation or inactivation parameters. The effects of JNJ7777120 remained even after the use of the G-protein blocker GDP--S and zero GTP in the pipette solution. The use of H4R agonists VUF4430 did not significantly modified the I_{Na}. In current clamp experiments JNJ7777120 decreased the action potential discharge produced by depolarizing current injection, and modified the action potential waveform decreasing the amplitude, the maximum depolarization rate, the maximum repolarization rate and increased the action potential duration. Our results show that JNJ7777120 inhibits the VAN electric activity through the inhibition of the I_{Nat}, I_{Nap}, through a mechanism independent of the H4R, and independent of G protein coupled receptors suggesting that JNJ7777120 directly interacts with the voltage gated sodium channels. Acknowledgements: CONACyT grant CA Neurociencias # 229866; CONACyT grant 167052 to Enrique Soto, grants VIEP-BUAP to Rosario Vega and Enrique Soto, and PIFI 2012-2013 grant.

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Poster

235. Vestibular Hair Cells, End Organs, and Nerve

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Topic: D.07. Vestibular System

Support: NIDCD R01DC012347

Title: Spontaneous and driven activity of vestibular afferent calyces in the extrastriolar zone of the mouse utricle

Authors: ***O. LOPEZ**, V. LUMBRERAS, R. A. EATOCK;
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Abstract: Mammalian vestibular sensory epithelia have distinct concentric zones, which are called the striola (center) and extrastriola (surround) in the utricle and saccule. In the chinchilla utricle, primary afferents from the extrastriola have much more regular spike timing than afferents from the striola (Goldberg, Exp Brain Res 130:277, 2000). We are exploring the difference in spike timing in *ex vivo* preparations of the rodent saccule and utricle, which include the sensory epithelia and afferent nerve fibers. Most of the afferents form both calyceal

postsynaptic terminals surrounding type I hair cells and compact bouton terminals on type II hair cells. In whole-cell recordings from the rat saccule, extrastriolar calyces were more regular than striolar calyces (Songer & Eatock 2013), consistent with the *in vivo* chinchilla data. Here we report findings from calyces of the mouse utricle in the lateral extrastriola (LES), which extends from the line of reversal of hair bundle polarity to the lateral edge of the sensory epithelium. Utricles were excised from CD-1 mice at 10-30 postnatal days (15 ± 1.7 (SE) days, n=12) and maintained at 25-28°C in L-15 medium. The pipette solution was KCl-based and included sulforhodamine 101 to fill the terminal arbors. The calyces belonged to afferents that each made simple calyces around 2-4 type I hair cells plus 3-10 boutons on type II hair cells. Resting potential was −44 ± 5 mV (n=7); resting input resistance was 125 ± 27 MΩ, and capacitance estimated from electronic compensation was 9 ± 2 pF. Sinusoidal stimulation (2-100 Hz) of hair cell bundles evoked postsynaptic responses that were a mix of epsps (n=3), apparently non-quantal responses (n=3), and both (n=1). Evoked spiking showed broad tuning and less precise phase-locking compared to striolar rat calyces. Spontaneous firing rate was 20 ± 5 spikes/s (n=7); correcting for temperature raises the rate into the *in vivo* range for mouse vestibular afferents. Spike timing in these extrastriolar calyces was, however, more irregular than expected from *in vivo* chinchilla utricular data or *ex vivo* rat saccular data: CV = 1.1 ± 0.1, Fano factor = 0.24 ± 0.1 (n=12). Possible factors are species, organ, development stage, and sub-zone within the extrastriola (lateral vs. mixed lateral and medial extrastriola). The underlying mechanism could be different expression of low-voltage activated K channels, which make firing more irregular.

Disclosures: **O. Lopez:** None. **V. Lumbreras:** None. **R.A. Eatock:** None.

Poster

235. Vestibular Hair Cells, End Organs, and Nerve

Location: Hall A

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Program#/Poster#: 235.06/L15

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Title: Voltage-dependent inhibition of N-type calcium current by activation of ORL-1 receptor in vestibular afferent neurons of rat

Authors: *E. SESEÑA MENDEZ[1], A. ORTEGA[2], R. VEGA[2], E. SOTO[2];
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Abstract: The vestibular system is modulated by opioid peptides both at the central and peripheral level. The nociceptin/orphaninFQ peptide (N/OFQ) peptide and its receptor opioid receptor like - 1 (ORL-1) has been found to be expressed in the vestibular nuclei complex, and N/OFQ has been shown to modulate the electrical activity of the medial vestibular nucleus neuron (Sulaiman et al., 1999). However, the expression and effect of N/OFQ has not been studied in the vestibular endorgans. In this work we evaluated the effect of ORL-1 activation in the multiunit discharge of the vestibular afferent neurons in the isolated vestibule and also in the voltage gate calcium current (ICa) recorded in the isolated vestibular afferent neurons (VAN) of the rat. Also immunohistochemical analysis of the ORL-1 expression in vestibular endorgans and vestibular nuclei complex was studied. Extracellular multiunit recording of the ampullary nerve electrical discharge was performed in the isolated vestibule of the rat. Micro-application in the vicinity of the ampulla origin of N/OFQ did not significantly modify the basal discharge of the afferent neurons. Recordings of ionic currents were performed in cultured VAN using the perforated patch clamp technique. The perfusion of N/OFQ decreased the high voltage activated ICa (HVA) without significantly modifying the low voltage activated ICa, (LVA). Inhibition of the HVAICa component was dependent on the concentration used. The specific ORL-1 antagonist UFP101 or the pre-incubation of VANs with pertussis toxin completely occluded the N/OFQ inhibitory action of the Ca2+ current. Else more, the use of ω-ctx-MVIIA (specific N-type Ca2+ channel blocker) also occluded the effect of ORL-1 activation. The effect of the N/OFQ on the HVAICa was also reverted by a pre-pulse to 80 mV. Evidence from confocal microscopy analysis of immunorreactivity to the ORL-1 receptor indicate that it is not expressed in the vestibular endorgan. Studies of ORL-1 expression within the vestibular nuclei complex is under way. These results showed that ORL-1 activation inhibits the N-type ICa in the VANs in a voltage-dependent manner. Since activation of the N/OFQ did not modify the discharge rate of vestibular afferents, we thought that ORL-1 mediates a pre-synaptic modulation of the neurotransmitter release form the vestibular afferent terminals, inhibiting N-type calcium current at the synapse with the vestibular nuclei neurons, constituting a target to negatively modulate the afferent gain of the vestibular nuclei neurons.

Disclosures: **E. Seseña Mendez:** None. **A. Ortega:** None. **R. Vega:** None. **E. Soto:** None.

Poster

235. Vestibular Hair Cells, End Organs, and Nerve

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CRCNS/BMBF 01GQ1407

Title: Dynamic diversity of horizontal canal afferent neurons in Xenopus laevis tadpoles

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Abstract: Spike time variation in information transmission plays a fundamental role for neural coding of sensory stimuli. The large dynamic bandwidth of naturally occurring head motion poses a particular challenge for the vestibular system to faithfully encode these stimuli as spike trains transmitted by afferent neurons to central vestibular circuits. Tadpoles of *Xenopus laevis* provide an outstanding opportunity to investigate how natural head kinematics are encoded in vestibular afferent discharge modulation. In this study we present our findings of the fundamental anatomic and physiologic characteristics of these neurons from *in-vitro* preparations of specimens at mid-larval stage. At this developmental stage vestibular afferents exhibited physiologic characteristics comparable to adult amphibian specimens, and extracellular recordings from single horizontal semicircular canal afferents at rest and during sinusoidal rotation revealed a heterogeneous population that exhibited both phasic and tonic dynamics. Phasic afferents exhibited low resting rates (~0.8 sp/s) and irregular spontaneous discharge (CV >1). During sinusoidal rotation at frequencies > 0.5 Hz, these fibers exhibited discharge volleys that were phase-advanced by ~45° (re: head velocity), independent of the stimulus magnitude. Afferents that exhibited more tonic dynamics had higher resting rates (≥8 sp/s) and more regular interspike intervals (CV≤0.4). The firing pattern of these afferents during sinusoidal rotation was modulated approximately in phase with peak head velocity. The cristae were also probed for cellular features that may be associated with afferent physiologic heterogeneity, and were found to contain a distinct group of afferent dendrites that were immuno-positive for Kv1.1, a low-voltage activated potassium channel that facilitates highly transient discharge patterns. These fibers exhibited particularly large axonal (~4 μm) and corresponding somatic (~18 μm) diameters and projected to the crista epithelium isthmus. The presence of a heterogeneous population of vestibular afferents with complementary morpho-physiological features corresponds to a comparably diverse population of central vestibular neurons and complies with the necessity to encode the large range of head motion dynamics. Using band-limited rotational stimuli derived from measurements of head kinematics during natural swimming in these animals will yield deeper insight into how the components of natural stimuli are encoded and transmitted to central vestibular circuits.

Disclosures: **K. Gensberger:** None. **C. Gravot:** None. **L.F. Hoffman:** None. **M. Paulin:** None. **H. Straka:** None.

Poster

235. Vestibular Hair Cells, End Organs, and Nerve

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Topic: D.07. Vestibular System

Support: CIHR

Title: Parallel channels with different timescales encode self-motion information in the vestibular system

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Abstract: Most sensory neurons transmit information about the sensory world through firing action potentials; however, deciphering their neural code (e.g., rate versus temporal code) has intrigued many neuroscientists for decades. To this end, we took advantage of the vestibular otolith afferents which are well-defined anatomically and physiologically and encode easily characterized sensory stimuli (i.e., linear self-motion) to higher-order brain areas. Interestingly, a hallmark of these neurons is the heterogeneity in the variability of their spontaneous discharge (from regularly to more irregularly spiking afferents), which makes them particularly suitable for exploring whether spike-timing plays a role in self-motion processing. Here, we investigated how sensory information is processed in the otolith afferents by recording from utricular fibers in alert macaques while stimulating each unit along its preferred direction during translations with broad band (0-15 Hz) Gaussian noise linear accelerations. We found an increase in the response gain for both regular and irregular afferents as a function of the stimulus frequency; the gain enhancement was more prominent for irregular units. Interestingly, despite a large difference in gain, responses of both afferent types were similarly coherent with the stimuli except for very low frequencies (≤1Hz) at which regular units displayed slightly higher coherences. These findings suggest that while highly sensitive irregular afferents are more advantageous for transient and dynamic stimuli, the regular units can provide accurate information when the stimulus is less dynamic (e.g. static tilt). Finally, to investigate whether spike timing plays a role in the encoding of linear motion we recorded neuronal responses to repetitions of the same stimuli (i.e., frozen noise). In response to frozen noise stimuli, irregular units exhibited coherence in their responses at frequencies beyond those contained in the stimuli indicating higher temporal resolution in their activity. Further quantification revealed that irregular afferents outperformed regular units in discriminating stimuli using faster timescales signifying that information is contained in the fine temporal structure of their spike trains. In contrast,

regular units operate over relatively longer timescales and use a rate coding scheme to encode the stimuli. Our findings suggest that regular and irregular afferents function as two parallel channels with different but complementary coding strategies to encode linear self-motion.

Disclosures: **M. Jamali:** None. **J. Carriot:** None. **M.J. Chacron:** None. **K.E. Cullen:** None.

Poster

235. Vestibular Hair Cells, End Organs, and Nerve

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Title: Spontaneous discharge in mammalian vestibular afferents modelled as Gamma-censored Poisson processes: associations with response dynamics

Authors: L. HOFFMAN[1], K. PULLAR[2], *F. E. SCHWEIZER[3], M. PAULIN[2];
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Abstract: Vestibular primary afferent neurons exhibit broad heterogeneity in spontaneous discharge interspike interval (ISI) distributions, which have been previously modeled by Gamma distributions with shape (k) and scale (θ) parameters. The Gamma model implies a fast Poisson process underlying spontaneous activity, whereby spikes are triggered by integrating Poisson events. However, the temporal characteristics of ISI distributions are inconsistent with simple Gamma models, requiring a temporal offset parameter (d). This parameter is fundamentally different from k and θ in that it represents a fixed offset, not a parameter of a formal stochastic process. Our goal was to generalize the Gamma model to account for temporal offsets in ISI distributions within an underlying stochastic model. The Gamma-censored Poisson process (GCP) is the waiting time for k events in a Poisson process plus 1 event in another, independent Poisson process. In our model, the Gamma process functions to 'gate' events in the second process until a Gamma event occurs, enabling the next Poisson event to occur and the Gamma process is reset. We derived analytical forms of offset Gamma (OG) and GCP processes and fitted these to spontaneous discharge ISIs from several hundred chinchilla horizontal and superior cristae afferents that represented broad ISI heterogeneity. This enabled us to compute parameters of OG (k,θ,d) and GCP (k, θ, τ) models directly from ISI data. Kullback-Liebler divergences (representing differences between probability distributions) were computed between fitted models and ISI distributions. GCP models fit at least as well as OG models for all data and provided better fits for a large fraction of afferents. The associations between parameters of each subcomponent and the broad ISI heterogeneities were explored through regression analyses of

model parameters and spontaneous ISI CV. We found that the Gamma subcomponent k was closely associated with ISI CV for afferents with CV≤0.1 (r^2=0.99); this correlation deteriorated among those with CV>0.1 (r^2=0.67). The parameter τ of the GCP model's Poisson subcomponent was more closely associated with ISI CV for afferents with CV>0.1 (r^2=0.88) than among those with CV≤0.1 (r^2=0.44). We also explored the association between model and evoked discharge parameters to elucidate how underlying factors of spontaneous discharge may be correlated with dynamic response characteristics. The GCP model implies that vestibular afferent spontaneous discharge reflects a Poisson process gated by a separate Gamma process (sum of Poisson processes) that may be associated with synaptic events and dendritic integration, respectively.

Disclosures: **L. Hoffman:** None. **K. Pullar:** None. **F.E. Schweizer:** None. **M. Paulin:** None.

Poster

235. Vestibular Hair Cells, End Organs, and Nerve

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Support: The Garnett Passe and Rodney Williams Memorial Foundation

Title: Age-related effects of vestibular stimulation on gait

Authors: ***V. TUNG**, T. BURTON, S. QUAIL, A. J. CAMP;
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Abstract: Background: Maintenance of posture and balance are multi-factorial with contributions from proprioceptors, vision and the vestibular system. During natural ageing, loss of balance and subsequent falls become more prevalent. Here we investigate the effect of a simple, non-invasive vestibular stimulus on gait pattern and motor coordination of mice across the murine lifespan. Methods: All experiments were approved by the University of Sydney Animal Ethics Committee. C57/Bl6 mice of ages 1 (n = 3), 10 (n = 7), 13 (n = 6) and 27 (n = 8) months were trained to perform a task of walking 30 cm directly and with no assistance to a darkened goal box. Petroleum jelly was then applied to the hind-feet of the mice, which were then returned to the starting point to perform the task. The petroleum jelly residue remaining as mice walk towards the goal box was used to analyse walking patterns. Mice were then given 1 minute of rest and petroleum jelly was reapplied before they were placed in a custom-built rotator that delivered an angular acceleration from 0 to 3Hz over 20s. Mice were retested immediately after the stimulus. After testing, graphite powder was used to darken the petroleum jelly footprints for analysis. An ellipse was fitted to the walking path of mice, before and after the vestibular stimulus, with the minor axis of the ellipse used to quantify the degree to which the

walking path of each mouse deviated from the direct path between the starting point and the goal box. Results: The angular acceleration (vestibular stimulus) did not show an overall relationship when minor axis length was compared across all ages ($p > 0.05$). Rather, the effect developed after 10 months of age and continued through to 27 months of age. For example, while the gait pattern of 1-month-old mice was not affected by angular acceleration (pre-stimulus: 6.90 ± 1.66 mm vs. post-stimulus: 6.64 ± 1.85 mm) the deviation from the direct path was significantly increased in all other age groups following the stimulus (10-month-old, 24.98 ± 5.93 mm vs. 27.74 ± 6.23 mm, $p < 0.05$; 13-month-old, 23.789 ± 6.85 mm vs. 28.61 ± 8.71 mm, $p < 0.05$; and 27-month-old, 21.70 ± 5.00 mm vs. 30.28 ± 14.13 mm, $p < 0.05$). No differences were seen between the older age groups. Conclusion: A vestibular stimulus of low frequency and duration has minimal effect on the gait pattern of young 1-month-old mice. However, at ages 10 months and above, mice noticeably deviated from the most direct path to complete the task, suggesting impaired motor coordination and recovery from the vestibular stimulus.

Disclosures: V. Tung: None. **T. Burton:** None. **S. Quail:** None. **A.J. Camp:** None.

Poster

236. Vestibular Central Physiology, Anatomy, and Behavior

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Program#/Poster#: 236.01/L20

Topic: D.07. Vestibular System

Title: Efferent vestibular neurons show homogeneous discharge output and heterogeneous synaptic input profile in mice *in vitro*

Authors: *M. A. MATHEWS[1], R. WIJESINGHE[1], A. MURRAY[2], V. TUNG[1], A. J. CAMP[1]; [1]USYD, Sydney University, Australia; [2]Columbia Univ., New York City, NY

Abstract: Purpose: Our sense of balance is fundamental to our ability to interact with our environment, yet we still know little about the central control of the peripheral balance system. Here we confirm the location, and characterize the output and input properties of efferent vestibular nucleus (EVN) neurons *in vitro*. **Methods:** All procedures were approved by the Animal Ethics committee of the University of Sydney. *Immunohistochemistry:* Transverse serial sections (40μm) were sliced through the 4-week-old mouse brainstem and labelled with antibodies against CGRP (n=7), and ChAT (n=4). Retrograde labelling using fluorogold injected into the posterior semicircular canal of ChAT-Cre tdTomato mice was also used to confirm the location of EVN neurons. *Electrophysiology:* Transverse slices (200μm) were used to characterize intrinsic action potential (AP) and discharge properties of visualized EVN neurons (n=54) in whole-cell current-clamp mode. Synaptic input profiles of visualized EVN neurons were assessed in whole-cell voltage-clamp mode (n=23). Miniature excitatory and inhibitory postsynaptic currents were identified using pharmacological blockade. **Results:** CGRP and

ChAT immuno-positive neurons were identified dorsolateral to the genu of the facial nerve. Spontaneous firing EVN neurons (n=16) show a significantly deeper afterhyperpolarisation when compared with non-spontaneous (n=38) neurons ($p<0.001$). EVN neurons display slower action potential kinetics including rise time ($p<0.001$) and half-width ($p<0.01$) when compared with neighbouring medial vestibular nucleus neurons. In response to both hyperpolarizing and depolarizing steps all EVN neurons respond with a short bust of high frequency APs at the cessation of the inhibitory stimulus or the onset of an excitatory stimulus. This burst is superimposed on a (6.7 ± 7.8 mV) afterdepolarization amplitude that is completely abolished by the selective T-type calcium channel blocker 3,5-dichloro-N-[1-(2,2-dimethyl-tetrahydro-pyran-4-ylmethyl)-4-fluoro-piperidin-4-ylmethyl]-benzamide (TTA-P2). All EVN neurons show combinations of excitatory and inhibitory synaptic input profiles. EVN neurons are either exclusively inhibitory (n=10) or excitatory (n=5), or predominantly excitatory with: GABA(A)-ergic inputs (n=2), glycinergic inputs (n=1), or combination of inhibitory inputs (n=5). **Conclusion:** E-group vestibular neurons are homogeneous in their discharge output and heterogeneous in their synaptic input profiles suggesting that central control of peripheral vestibular structures may be related more to the source of the input these neurons receive**.**

Disclosures: M.A. Mathews: None. **R. Wijesinghe:** None. **A. Murray:** None. **V. Tung:** None. **A.J. Camp:** None.

Poster

236. Vestibular Central Physiology, Anatomy, and Behavior

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Topic: D.07. Vestibular System

Support: University of Otago Research Grant

Title: Changes in c-Fos expression in the striatum following electrical stimulation of the peripheral vestibular system

Authors: *L. STILES[1,2], Y. ZHENG[1,2], R. M. NAPPER[3,2], P. F. SMITH[1,2];
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Abstract: Connections between the peripheral vestibular system and the basal ganglia have been studied sporadically in the past; however, the results of these studies are conflicting. As both regions are well known to be involved in the processing of movement and dysfunction of both systems produces movement disorders, in recent years there has been increasing interest in how these regions might be connected and how vestibular signals affect striatal function. Immediate early gene proteins like c-Fos can be used as a marker of neuronal activation in neurons, as their

genes are activated following cellular stimulation. The aim of this study was to investigate whether electrical stimulation of the peripheral vestibular system caused an up-regulation of the c-Fos protein in the striatum. Male Wistar rats (weighing between 300 g and 400 g) were randomly allocated into either stimulation or sham groups. The animals were anesthetised using urethane (1.5 g/kg, i.p.) and the round window of the inner ear was exposed using a retro-auricular surgical approach. A stainless-steel bipolar electrode was placed into the round window as the stimulating electrode. Stimulated rats received unilateral electrical stimulation at 100 Hz at the lowest possible current to produce vestibular nystagmus specific to the animal (between 300 and 400 μA) for 10 minutes. Sham animals underwent the entire surgical procedure but received no electrical stimulation. Ninety minutes post-stimulation the animals underwent cardiac-perfusion with saline followed by 4% paraformaldehyde. The brains were dissected out, post-fixed, frozen and stored at -20° C until sectioning. Serial 40 μm sagittal sections throughout the striatum were cryosectioned using a random, systematic sampling design. c-Fos immunolabelling was performed and the immunostaining was quantified using the optical dissector fractionator method of stereology to count positive cells. Preliminary results show an increase in the number of c-Fos positive cells in the striatum of rats receiving vestibular stimulation compared with sham-treated rats. This increase following electrical vestibular stimulation suggests that the peripheral vestibular organs send signals to the striatum, which then activate the cells to cause activation of the c-fos gene. The striatum is the main input site to the basal ganglia and the connections between the vestibular system and striatum may have clinical implications in the treatment of basal ganglia disorders and other movement disorders.

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Poster

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Support: RGC Grant 762313M

Title: Role of G-protein-coupled serotonin receptor on synaptic plasticity of excitatory transmission in the vestibular nucleus of rats

Authors: ***Y.-S. CHAN**[1], L. HAN[1], Y. H. LI[1], C. W. MA[1], D. K. Y. SHUM[2];
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Abstract: Serotonergic projections from the raphe nucleus are known to innervate the medial vestibular nucleus (MV) but the contribution of serotonin in the vestibular system remains

fragmentary. Acute administration of serotonin (5-HT) to the MV of P14 rats impaired the performance in rota-rod task, balance beam test and negative geotaxis. Whole-cell patch-clamp recording in MV neurons of brainstem slice preparations showed increase in the amplitude and frequency of spontaneous excitatory postsynaptic current (EPSC) with 5-HT treatment. Among G-protein-coupled 5-HT receptors that modulate synaptic plasticity in excitatory circuitries, we found 5-HT_2 receptor (Gq-coupled) and 5-HT_7 receptor (Gs-coupled) expressed in neurons of the vestibular nucleus. We then pursued the roles of these receptors in MV neurons. Treatment of MV neurons with agonist of the 5-HT_7 receptor induced reduction in the amplitude of evoked-EPSC but not with agonist of the 5-HT_2 receptor. With the use of combinations of single and dual agonist/ antagonist that target the Gs- and Gq-coupled 5-HT receptors, we further found that the suppression of NMDAR-dependent long-term depression in MV neurons was mediated by the 5-HT_7 receptor but not by the 5-HT_2 receptor. Aside from the PKA-dependent signaling cascade, downstream cross-talk with the PKC-dependent pathway was involved. Taken together, our results reveal that Gs- but not Gq-coupled serotonin receptors mediate the expression of long-term plasticity in excitatory synapses within the vestibular nucleus, and suggest their involvement in the expression of vestibular behaviors.

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Poster

236. Vestibular Central Physiology, Anatomy, and Behavior

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Support: Korea Ministry of Science, ICT & Future Planning (NRF-2013R1A2A2A04014796)

Korea Ministry of Education (2010-0020163)

Title: Neural mediation in vestibular nucleus: habituation and sensitization of horizontal semicircular canal-related neurons by galvanic vestibular stimulation

Authors: *G. KIM[1], S. LEE[1], K.-S. KIM[2], E. JEON[2];
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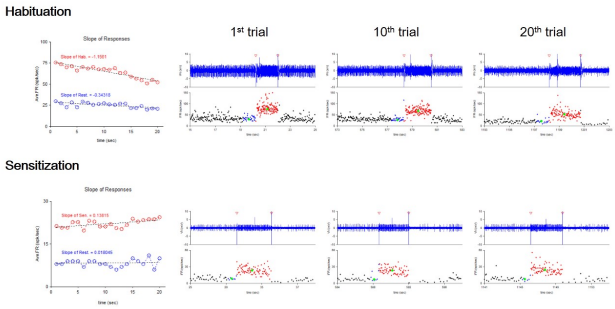
Abstract: Habituation is observed in neuronal responses while sensitization also occurs by the stimulation as applied for habituation. Vestibular system is known as a sensory system with dynamic processes of habituation and sensitization. To see how these processes occur in vestibular system, we recorded the neuronal responses of habituation and sensitization in

vestibular nucleus (VN). We obtained 62 neuronal activities responding to a series of repeated stimuli, composing of multiple sets of a 3-sec stimulation and a 60-sec resting period. A horizontally rotatory stimulation was used to identify whether the recording signal was originated from the afferent neuron of the lateral semicircular canal. The slopes of habituation and sensitization were calculated off-line, using averaged neuronal firing rates during GVS. The results showed that the responding slopes of habituated and sensitized units followed a normal distribution with their mean and standard deviation of -0.2 and 0.9, respectively, indicating slightly more habituation. However, a statistical test implied that the neuronal habituation and sensitization occurred in a similar amount of neurons (p=0.13, binomial test). Based on our data, we hypothesize the neural mediation by habituation and sensitization in VN can be explained by homeostatic process, which balances the strength (slope) between the habituated and sensitized units. The observed neuronal mediation suggested the modified neural information (habituation) was compensated by its opposite process (sensitization) through regulating their new threshold level after a learning process.



Disclosures: **G. Kim:** None. **S. Lee:** None. **K. Kim:** None. **E. Jeon:** None.

Poster

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NIH NEI 5R01EY002007-34

Title: Discharge properties of vestibular neurons during horizontal eye movements in the goldfish

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Abstract: Second order vestibular neurons transform motion-related sensory inputs into respective commands for gaze stabilization and posture control. We have morpho-physiologically characterized these neurons with single-unit recordings and determined their functional role during VOR and oculomotor velocity storage integration in goldfish. Vestibular neurons in the descending octaval nucleus were recorded during visuo-vestibular interactions in alert fish. Sinusoidal optokinetic stimulation demonstrated eye velocity sensitivity whereas visual suppression of the VOR determined head velocity sensitivity. Based on the firing patterns, two main types were found: EIIHI, the most abundant (n=19), and EIHII (n=10). Small proportions of pure HI (n=3) or HII (n=1) were also recorded. Velocity storage signal was tested by long (≥40s) optokinetic steps which showed the build-up firing during the charging of the velocity storage mechanism and the discharge that ensued the optokinetic afternystagmus. Similarly, long head velocity steps resulted in an immediate increase and persistence of the firing rate as a correlate of the velocity storage mechanism with a long time constant (>10s). Visuo-vestibular combinations of long steps resulted in an instant discharge of the velocity storage due to the interaction of the visual and vestibular signals. Intracellular biocytin injection of identified horizontal semicircular canal vestibular neurons demonstrated two main patterns of connectivity within the VOR circuitry. Some vestibular neurons with a disynaptic excitatory input from the ipsilateral horizontal semicircular canal and a contribution to horizontal eye movements had ipsilateral projections to abducens nucleus, area II and intrinsically to vestibular neurons. Other vestibular neurons projected contralaterally to abducens nucleus, area II and descending octaval nucleus. Tract tracing studies with biocytin verified this connectivity. Our study demonstrates the signal processing and connectivity between three hindbrain nuclei responsible for the control of horizontal eye movements. We propose that EIIHI are the excitatory and inhibitory neurons of the VOR reflex arc and EIHII neurons represent a particular class of vestibular neurons that are targeted by three control areas. These areas are the cerebellum, the contralateral vestibular nucleus (as part of the commissural inhibitory system) and the EIIHI neurons as part of an ipsilateral interneuronal inhibition. The pivotal role of the descending octaval nucleus neurons on the control of the VOR reflex arc and storage integrator demonstrates the basic blueprint of eye movement circuits.

Disclosures: A.M. Pastor: None. **P.M. Calvo:** None. **R.R. de la Cruz:** None. **H. Straka:** None. **R. Baker:** None.

Poster

236. Vestibular Central Physiology, Anatomy, and Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 236.06/L25

Topic: D.07. Vestibular System

Support: CIHR

Title: Selective encoding of unexpected head tilt by the vestibular nuclei but not by the vestibular afferents

Authors: *I. MACKROUS, J. CARRIOT, K. E. CULLEN;
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Abstract: During daily activities, our sensory system is simultaneously activated by self-generated and external events. The distinction between the sensory inputs that are the result of our own action or changes in the external world is essential to ensure perceptual stability and efficient motor control. Recently, it has been shown that the sensory signals carried by the vestibular-nerve afferent encode similarly head motions, regardless of whether they are passively experienced or actively generated. This was shown for passive sinusoidal rotations/translations in the horizontal plane, implying that the vestibular periphery provides faithful information about rotational and linear head movements. Conversely, neurons in the first central stage of processing (i.e., vestibular nuclei) robustly encode passively applied head rotation/translation in the horizontal plane but their responses are attenuated during comparable self-generated head motion. However, natural head movements are not restricted to one plane and create more complex vestibular stimuli because of the presence of the gravity. Therefore, we investigated whether gravity is included in the coding of head motions in order to distinguish between self- versus externally- generated head motion at the periphery and at the central levels. Vestibular afferents and vestibular nuclei neurons responses were recorded in alert macaques during passive and active head-on body tilts. As previously shown in the horizontal plane, the afferents similarly encoded passive and active tilts. Conversely, responses related to actively-generated tilts were significantly attenuated (relative to passively applied tilts) in vestibular nuclei neurons. Moreover, this attenuation was comparable to that observed for active versus passive head translations (67 vs 74%, $p>0.05$). Our findings indicate that the neural coding of self-motion requires an elegant computation of an internal model of active head motion that accounts for gravity at the vestibular nuclei level but such computation does not influence peripheral coding of self-motion.

Disclosures: I. Mackrous: None. **J. Carriot:** None. **K.E. Cullen:** None.

Poster

236. Vestibular Central Physiology, Anatomy, and Behavior

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Topic: D.07. Vestibular System

Support: NIH T32-DC011499

K08 DC-013571-01A1

Title: Reticular formation neurons with vestibular inputs respond to hindlimb movement: comparisons between decerebrate and conscious cats

Authors: *D. M. MILLER, D. J. MILLER, G. H. BOURDAGES, L. A. COTTER, B. J. YATES, A. A. MCCALL;
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Abstract: Multisensory integration of vestibular and proprioceptive afferent information within the central nervous system (CNS) is critical to postural regulation. We recently demonstrated in decerebrate cats that hindlimb somatosensory inputs converge with vestibular inputs on neurons in multiple CNS locations that participate in balance control. However, responses of CNS neurons to stimuli can be exaggerated or modified in decerebrate animals. The goals of the present study were to characterize in conscious animals the convergence of limb somatosensory and vestibular inputs onto pontomedullary reticular formation (PMRF) neurons, and to determine if the responses of these neurons to limb movement differed from those in decerebrate animals. PMRF neurons were targeted for study because of their well-established role in control of posture and locomotion. Single unit recordings were obtained from the PMRF in decerebrate and conscious cats while systematically moving the ipsilateral hindlimb through ramp-and-hold movements in the rostral-caudal plane and also while rotating the animals in vertical planes to stimulate the vestibular endorgans. Overall, we isolated 55 PMRF neurons whose activity was modulated by hindlimb movements: 34 in the decerebrate preparation and 21 in conscious cats. We confirmed that hindlimb somatosensory inputs converge with vestibular inputs onto PMRF neurons of the conscious cat. Furthermore, responses to hindlimb movement were similar in conscious and decerebrate animals. Most PMRF neurons in both preparations signaled the movement of the hindlimb in either the flexion or extension directions, but not limb position (74% and 76% of neurons in decerebrate and conscious animals, respectively). Since decerebration did not alter the pattern of responses of PMRF neurons to limb movement, supratentorial areas do not appear to play a critical role in shaping these responses. PMRF neurons that receive convergent limb and vestibular inputs may mediate reticulospinal reflex responses that maintain balance in response to externally applied (exafferent) postural perturbations.

Disclosures: D.M. Miller: None. **D.J. Miller:** None. **G.H. Bourdages:** None. **L.A. Cotter:** None. **B.J. Yates:** None. **A.A. McCall:** None.

Poster

236. Vestibular Central Physiology, Anatomy, and Behavior

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Topic: D.07. Vestibular System

Support: NIH Grant DC012536

Title: Anatomical and physiological characteristics of vestibulospinal neurons in zebrafish

Authors: *M. W. BAGNALL[1], K. L. HEISEY[2], R. ROBERTS[2];
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Abstract: Sensory information about head movement and orientation with respect to gravity is encoded by vestibulospinal neurons. In mammals, these neurons (also known as Deiters' neurons in the lateral vestibular nucleus, or LVN) send axons exclusively to the ipsilateral spinal cord, with long projections that can reach lumbo-sacral levels. About two-thirds of vestibulospinal neurons increase firing for head tilts to the ipsiversive side, while the rest encode tilts to the contraversive side. Currently, very little is known about the nature of synaptic inputs to vestibulospinal neurons *in vivo*, nor the functional organization of their outputs in spinal cord. Here we show that in larval zebrafish, vestibulospinal neurons exist in a small, ~10 neuron cluster directly under the ear, and that they project ipsilaterally to the caudal end of the spinal cord, similar to the Deiters' population in mammals. Morphology of individual axons reveals that they extend collaterals in each spinal segment. We have also made whole-cell electrophysiological recording from vestibulospinal neurons *in vivo*; neurons are capable of high sustained firing rates, and receive barrages of spontaneous excitatory synaptic events. In voltage clamp, spontaneous excitatory post-synaptic currents exhibit stereotypical amplitudes and temporal profile in a given neuron, suggesting the convergence of multiple fibers each with a characteristic, history-independent amplitude. This frequency independent transmission is likely to arise from vestibular afferents. At these ages, only the anterior otolith (utricle) is a plausible source of information to these neurons, simplifying the analysis of their coding strategies. We conclude that in morphology and physiology, vestibulospinal neurons in zebrafish appear highly homologous to those in mammals, and we plan to explore their genetic specification, spinal cord targets, and functional contribution to righting behaviors.

Disclosures: **M.W. Bagnall:** None. **K.L. Heisey:** None. **R. Roberts:** None.

Poster

236. Vestibular Central Physiology, Anatomy, and Behavior

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Topic: D.07. Vestibular System

Support: NIDCD Grant R01-DC002390

CIHR

Title: Plasticity within vestibulo-spinal reflex pathways: implications for use of vestibular prostheses

Authors: *D. E. MITCHELL[1], C. C. DELLA SANTINA[2], K. E. CULLEN[1];
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Abstract: From performing a complex dance routine to calibrating simple reflex behaviors, motor learning is required for the accurate execution of movements. Linking synaptic plasticity with motor learning requires understanding how synaptic efficacy influences behavioral responses. The vestibular system plays a vital role in the generation of vestibulo-spinal reflexes, which work to maintain our posture in response to unexpected self-motion. These reflexes are mediated by a subclass of neurons in the vestibular nuclei, termed vestibular-only (VO) neurons which receive input from the vestibular nerve and in turn send projections to the spinal cord. *In vitro* studies have revealed that repetitive stimulation of the vestibular nerve induces long-term depression at the first central vestibular synapse (McElvain et al., 2010). Whether this type of plasticity occurs in awake behaving animals and, if so, how it contributes to vestibulo-spinal reflexes remains unknown. In order to address these questions, rhesus monkeys were implanted with stimulating electrodes in the horizontal semicircular canals, allowing the temporally precise stimulation of the vestibular nerve during recordings of VO neuron activity. We found that behaviorally relevant patterns of vestibular nerve activation substantially decreased responses of VO neurons receiving direct input from the vestibular nerve (i.e., type I VO neurons), while the responses of afferents remained constant. Thus, plasticity was induced at the synapse between vestibular afferents and these neurons in awake behaving animals. Activation of the vestibular nerve also caused a decrease in evoked head movements, although the magnitude of this decrease was significantly less than that of the neurons. We also recorded from neurons that contribute to the commissural vestibular pathways (type II VO neurons). Surprisingly, regardless of the observed substantial decrease in their input described above (i.e., type I VO neurons), commissural neuron responses did not change following activation of the vestibular nerve. These results suggest that rapid plasticity occurs within the vestibular commissural pathways to compensate for a decreased weight of the first central vestibular synapse and optimize behavioral performance. We speculate that, during natural head motion in everyday life, the first central vestibular synapse does not undergo plasticity to the same extent as during the stimulation we applied here. Nevertheless, plasticity at this synapse in coordination with complementary changes at other sites within the vestibulo-spinal circuitry likely guides the fine tuning of this motor behavior.

Disclosures: D.E. Mitchell: None. **C.C. Della Santina:** None. **K.E. Cullen:** None.

Poster

236. Vestibular Central Physiology, Anatomy, and Behavior

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Title: Pulsed infrared stimulation of the vestibulo-sympathetic reflex (VSR) pathway

Authors: *G. R. HOLSTEIN[1], S. M. RAJGURU[4], G. P. MARTINELLI[2], V. L. FRIEDRICH, Jr.[3], R. D. RABBITT[5];
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Abstract: Studies in humans and experimental animals have demonstrated that the vestibular system influences the sympathetic nervous system control of blood pressure (BP). Linear acceleration, which stimulates the otolith organs, causes transient changes in BP that are attenuated in patients with bilateral vestibular dysfunction. Activation of the otoliths by off-vertical-axis rotation produces increases and decreases in muscle sympathetic nerve activity (SNA) that are in-phase with the head-up and head-down tilt components of the stimulus, respectively. As in humans, linear acceleration and head-up tilt increase SNA and raise BP in experimental animals, while nose-down stimulation decreases both. Numerous studies in experimental animals report that SNA increases during electrical stimulation of the vestibular nerve. We previously demonstrated in rats that there is a direct projection from the caudal vestibular nuclei (VNc) to the rostral ventrolateral medulla (RVLM) that provides a route for vestibular system modulation of neurons controlling SNA. We also demonstrated that this pathway can be activated using tilt or sinusoidal galvanic vestibular stimulation (sGVS), and that activated VSR neurons in both VNc and RVLM accumulate c-Fos. In the present study, pulsed infrared stimulation (pIRs; 1863nm, 100-250pps, 250μs) was directed through the round window toward the utricular macula in rats using customized optical fibers. Changes in BP and heart rate during pIRs were measured to characterize VSR activity. Eye movements evoked by vestibular stimulation were also measured using a video-based eye tracking system (ISCAN Inc, Woburn, MA) and a custom MATLAB program used for analysis. Rats were perfused with mixed aldehydes 90 min after the pIRs and the brainstems were sectioned serially. Sets of sections were used for multiple label immunofluorescence studies of VNc neurons. Activated neurons were identified using c-Fos protein immunodetection, and maps of activated VSR neurons were compared with those obtained using sGVS. This study demonstrated that pIRs can be used to

activate utricular hair cells and central neurons of the VSR with high spatial and temporal specificity.

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Poster

236. Vestibular Central Physiology, Anatomy, and Behavior

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Topic: D.07. Vestibular System

Support: NIH grant DCD R01 0120600

Title: Sound-evoked responses of abducens neurons in rats

Authors: *H. ZHU[1], J. HUANG[1], X. TANG[1], C. STEWART[2], Y. YU[1], J. LIPPINCOTT[1], W. ZHOU[1];
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Abstract: Sound-evoked vestibular myogenic potentials in the sternocleidomastoid muscles (cVEMPs) and in extraocular muscles (oVEMPs) have been widely used in clinics to assess vestibular function. Both cVEMP and oVEMP are currently interpreted as tests of the otolith function. However, there is accumulating evidence challenging this prevailing view. Our recent single unit studies of rat vestibular afferents have demonstrated that acoustic stimulation (clicks and short tone bursts) not only activates the otolithic afferents, but also the semicircular canal afferents (Zhu et al, 2011). Consistent with the single unit recording results, our intra-axonal labeling studies have provided anatomical evidence that sound sensitive afferents innervate horizontal and anterior canal cristae as well as saccular and utricular maculae (Zhu et al., 2014). These new results suggest that semicircular canal contributions to the VEMP should be taken into consideration. Since the oVEMP is mediated by the vestibulo-ocular reflex (VOR) pathway which consists of the peripheral vestibular afferents, the vestibular nuclei, and the abducens nucleus, the current set of experiments examine sound activation of the secondary vestibular nucleus neurons and abducens nucleus neurons of Sprague-Dawley rats. In a previous study, we have reported that non-canal- vestibular nucleus neurons exhibit different frequency tuning response from the Type I and Type II vestibular nucleus neurons (Zhu et al., 2013). In the present study, we have further examined abducens neuron responses to clicks and short latency tones that were delivered into either ear under pentobarbital anesthesia. Preliminary analysis shows that contralateral clicks evoke short-latency (~2.6ms) excitatory responses in abducens neurons, indicating sound-activation of the lateral semicircular canal. Ongoing studies will

elucidate the neural mechanism underlying the oVEMP and provide critical information for the development of more discriminating clinical oVEMP tests.

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Poster

236. Vestibular Central Physiology, Anatomy, and Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 236.12/L31

Topic: D.07. Vestibular System

Title: Exploratory activity, motor coordination, and spatial learning in mice subjected to hindlimb unloading

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Abstract: In view of its effects on vascular parameters, microgravity induced by space flight has been shown to induce oxidative stress and cause neurochemical and metabolic alterations in several brain regions, particularly those linked to the cerebellum such as the vestibular nuclei, the brainstem reticular nuclei, and the precerebellar structures. To mimic microgravity, young adult mice were exposed to hindlimb unloading by tail suspension or not (control group) over a 21-day period and evaluated, after a 15-day period of recovery, for exploratory activity (open-field, elevated plus-maze, and emergence tests), motor coordination on the stationary beam, the coat-hanger version of the suspended bar test, and the rotorod, as well as spatial learning in the Morris water maze to detect possible long-term behavioural alterations. At the end of the behavioural study, animals were killed and brain as well as forelimb and hindlimb muscles removed for measuring regional cytochrome oxydase revealed by histochemistry as a good index of cellular metabolic activity. Both groups of mice increased their body weight in an equivalent manner. No effect was observed in the exploratory activity tests as well as in the Morris water maze, whereas the hindlimb unloaded group of mice were impaired in the stationary beam and the rotorod. Cytochrome oxidase activity decreased in several precerebellar structures as well as regions involved in the planning and control of movement such as the posterior parietal area and the substantia nigra. The present results confirmed a durable effect of microgravity on vestibulo-cerebellar and motor pathways, and muscle function.

Disclosures: C. Strazielle: None. **G. Harle:** None. **J. Frippiat:** None. **R. Lalonde:** None.

Poster

237. Spinal Cord Processing: Anatomy and Physiology

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Topic: D.08. Pain

Support: NIH NINDS R01-NS080954

Stanford Neuroventures

Title: Optogenetic tools for perturbing spinal neural circuits

Authors: *A. J. CHRISTENSEN[1], S. IYER[1], S. VYAS[1], A. FRANCOIS[2], G. SCHERRER[1], K. DEISSEROTH[1], S. L. DELP[1];
[1]Electrical Engin., [2]Stanford Univ., Stanford, CA

Abstract: Spinal neural circuits convey sensory and motor information, perform computation, and are implicated in the development of pathologies such as chronic pain. These circuits are formed by a heterogeneous mixture of neuron types - understanding how each of these types contributes to overall circuit function is a major goal of spinal cord neuroscience. Although optogenetic techniques would greatly facilitate this effort, until now such studies have been stymied by a lack of experimental tools for light delivery to the spinal cord. Here, we overcome the various anatomical and physical constraints that have previously prevented the development of these tools, and show that light can be robustly delivered to the spinal cord of awake, intact mice, over long periods of time without restricting animal locomotion. The methods innovations in this work eliminate a major roadblock in sensory systems neuroscience, and may enable a wide range of studies to elucidate circuits underlying pain, touch, itch and movement.

Disclosures: A.J. Christensen: None. **S. Iyer:** None. **S. Vyas:** None. **A. Francois:** None. **G. Scherrer:** None. **K. Deisseroth:** None. **S.L. Delp:** None.

Poster

237. Spinal Cord Processing: Anatomy and Physiology

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Support: NIH NINDS

Rita Allen Pain Scholar

American Pain Society

Virginia Kaufman Foundation

Competitive Medical Research Fund

American Diabetes Association

Title: Identification of excitatory neuron populations that form the dorsal horn mechanical pain circuit

Authors: *C. PEIRS[1,2], S.-P. WILLIAMS[1,2], X. ZHAO[1,2], J. GEDEON[1], R. SEAL[1,2];
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Abstract: Mechanical hypersensitivity, of which the neuronal circuits are poorly understood, is a widespread and intractable symptom of neuropathic pain for which there is a lack of effective therapy. During tactile allodynia, activation of the sensory fibers, which normally detect touch elicit pain. We recently showed that the vesicular glutamate transporter 3 (VGLUT3), a protein that packages glutamate into secretory vesicles for regulated neurotransmission, is required for expression of mechanical hypersensitivity after inflammation or nerve injury. Mice lacking VGLUT3 also have reduced response to acute, intense mechanical stimuli, but have a normal response in all other somatosensory behaviors including innocuous touch. VGLUT3 thus appears to have a very specific and important role in the circuitry underlying mechanical pain. Our recent data support a model in which VGLUT3 expression in the dorsal horn is required for establishing the neural circuit that transmits mechanical pain in the setting of nerve or tissue damage. We also found that loss of VGLUT3 prevent excitatory postsynaptic currents (EPSCs) in lamina I nociceptive specific neurons generated by polysynaptic A-fiber under pharmacological disinhibition. Here, we use Designer Receptors Exclusively Activated by Designer Drugs (DREADD), a modified GPCR that can be activated by its exogenous ligand Clozapine-N-oxide (CNO) to control the activity specifically in restricted populations of cre-expressing dorsal horn cells. Combining pain behavior tests and immunostaining in wild-type and VGLUT3 knock-out mice, we report the critical role of VGLUT3Cre cells in central mechanical pain circuits. From there, we identified intrinsic properties, sensory inputs and neuronal outputs of VGLUT3Cre neurons using transynaptic tracings and electrophysiological recordings. We took advantage of the highly specific role of VGLUT3 to further identify cells that belong to the neuronal pathway underlying mechanical hypersensitivity. Finally, we manipulated these identified cell populations to determine their relationship to the critical VGLUT3Cre neurons.

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Poster

237. Spinal Cord Processing: Anatomy and Physiology

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Support: NIH AR056288

Ashston Foundation

Title: Painful cervical facet joint injury alters spinal dorsal horn excitatory and inhibitory synapse numbers and astrocytic GLT-1 that is sustained

Authors: *M. E. ITA[1], N. CROSBY[2], B. BULKA[2], B. WINKELSTEIN[2];
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Abstract: The spinal facet joints are a common source of pain. Traumatic facet joint injury in the cervical spine can induce a host of spinal responses characteristic of central sensitization that are also associated with persistent pain. Although spinal hyperexcitability, glial activation and excitatory synaptogenesis have been reported at day 7 after painful facet trauma, it is unknown whether such spinal modifications remain at later times. This study investigated the relationships between pain, spinal synapse numbers, and spinal astrocytic expression of the glutamate transporter, GLT-1, 14 days after facet trauma. Male Holtzman rats underwent either a facet joint stretch or sham surgery (n=5/group); mechanical hyperalgesia was measured for 14 days. At day 14, immunohistochemistry measured co-localization of the pre-synaptic structural protein, bassoon, with each of the excitatory post-synaptic marker, homer1, or the inhibitory post-synaptic marker, gephyrin, separately to quantify excitatory and inhibitory synapses in the dorsal horn. GLT-1 was also co-localized with and normalized to GFAP to quantify astrocytic GLT-1. Hyperalgesia was induced after injury ($p<0.001$). Painful injury induced a significant increase ($p<0.047$) in the number of excitatory synapses in the dorsal horn and a significant decrease ($p<0.003$) in the number of inhibitory synapses. Both total GLT-1 expression and astrocytic GLT-1 ($p<0.047$) increased in the dorsal horn after painful injury. Further, when normalizing astrocytic GLT-1 to total GFAP expression, there is still an increase ($p=0.039$), suggesting that the increase in co-localized GLT-1 and GFAP is not simply due to increased GFAP immunoreactivity after painful injury. The shift in synapse distribution after injury supports the notion that structural plasticity in the spinal cord is sustained after painful facet trauma and likely contributes to the maintenance of joint pain. The increased astrocytic activation is consistent with other reports, whereas the increase in spinal GLT-1 is opposite prior work with this model showing decreases at day 7 in spinal GLT-1 using western blot. However, intrathecal inhibition of GLT-1 has been reported to attenuate neuropathic and inflammatory pain, suggesting that increased GLT-1 in the current findings may contribute to pain. Additional work is needed to define the mechanistic role of GLT-1 in maintaining joint-mediated pain, as well as the relationships between astrocytic responses and synaptogenesis. Nonetheless, these findings implicate long-term modifications of spinal plasticity in pain maintenance after even a transient joint trauma that produces chronic pain.

Disclosures: **M.E. Ita:** None. **N. Crosby:** None. **B. Bulka:** None. **B. Winkelstein:** None.

Poster

237. Spinal Cord Processing: Anatomy and Physiology

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Topic: D.08. Pain

Support: NIH NINDS R01-NS080954

Stanford Bio-X Neuroventures

Title: Optogenetic interrogation of mammalian mechanosensory and nociceptive circuits

Authors: ***S. M. IYER**[1], A. J. CHRISTENSEN[2], S. VYAS[1], S. VESUNA[1], A. FRANCOIS[3], C. RAMAKRISHNAN[1], K. DEISSEROTH[4], G. SCHERRER[5], S. L. DELP[6];
[1]Bioengineering, [2]Electrical Engin., [3]Anesthesiology, Perioperative, and Pain Medicine, Mol. and Cell. Physiol., [4]Bioengineering, Psychiatry and Behavioral Sci., [5]Anesthesiology, Perioperative, and Pain Medicine, Mol. and Cell. Physiology, Neurosurg., [6]Bioengineering, Mechanical Engin., Stanford Univ., Stanford, CA

Abstract: Sensory and nociceptive stimuli are processed by complex neural circuits in the periphery, spinal cord, and brain. Here, we discuss recent efforts we are undertaking to improve our understanding of how the first two steps of this processing occur. We first discuss our recent application of a novel system to deliver light to the spinal cord for optogenetic neuromodulation (for detailed discussion of system methods, see companion abstract: A.J. Christensen, ⋯, S.L. Delp, et al.). We use this system to control many populations of dorsal horn neurons in freely moving animals. We specifically express opsins in defined classes of spinal dorsal horn neurons, and characterize the effects of optogenetic activation of these circuits in freely moving mice on mechanical and thermal measures of pain, and on operant behavior. We also extend previous results in which we demonstrated optogenetic control of primary afferent nociceptors through transdermal illumination in mice injected with AAV6, examining the effects of optogenetic stimulation of the central terminals of primary afferent nociceptors expressing ChR2.

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Poster

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Topic: D.08. Pain

Support: CIHR

CRC

Anne and Max Tanenbaum Chairs

Title: Unitary EPSCs at single primary afferent-lamina I neuron synapses show predominant role of GluN2B- and GluN2D-containing NMDA receptors

Authors: *G. M. PITCHER, A. S. MORRISSY, L. GARZIA, M. D. TAYLOR, M. W. SALTER;
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Abstract: Subunit composition of postsynaptic NMDARs plays a crucial role in excitatory synaptic transmission and plasticity in the CNS. In the spinal cord, NMDARs are expressed in lamina I of the superficial dorsal horn where nociceptive primary afferent information is integrated and relayed to the brain. Because NMDAR subtypes have distinct functional properties and are hypothesized to have differing physiological/pathological roles, a major question has been the specific GluN2 subunit composition contributing to NMDAR function at a given synapse. Here, to elucidate NMDAR subtype at single synapses, we carried out voltage-clamp recordings of lamina I neurons (n=81) in adult rat spinal cord slices and evoked unitary excitatory post-synaptic currents (uEPSCs; Vh: +60 mV) by stimulating a single primary afferent monosynaptic connection to the neuron recorded. uEPSCs exhibited consistent latency, amplitude, and clear stimulation threshold. Potential contributions of different GluN2 subtypes to unitary responses were investigated by analyzing decay time constants and charge transfer of the NMDAR component of uEPSCs using unsupervised hierarchical clustering to determine any relationship between NMDAR kinetics in the 81 recordings. Cluster analysis identified 3 groups and independence of the groups by multivariate anova analysis revealed that charge transfer and decay were indeed different across the 3 groups ($p < 0.0005$) suggesting that NMDAR uEPSCs at individual primary afferent-lamina I synapses derive from NMDARs with distinct kinetic properties. In one group of synapses (32%) the mean decay time constant of the NMDAR uEPSC component was fast (50 ms) and the charge transfer small (0.4 pC). A second subpopulation (18%) had a component with a long decay time constant (1218 ms) and large charge transfer of 6.1 pC. The majority of synapses (50%) had a NMDAR component with an intermediate decay time constant of 296 ms and charge transfer of 1.9 pC. Furthermore, the GluN2B antagonist Ro25-6981 reduced selectively intermediate decay uEPSCs while the GluN2D antagonist DQP-1105 reduced selectively uEPSCs with long time decay. Lamina I glutamatergic synapses are therefore not uniformly identical in terms of NMDAR kinetic properties but occur with fast, intermediate, or slow deactivation kinetics consistent with that of GluN2A-, GluN2B-, or

GluN2D-containing NMDARs, respectively. GluN2B/2D abundance suggests NMDAR subunit composition favoring participation of slow deactivation kinetics and temporal summation, and therefore identifies GluN2B/2Ds as potential molecular targets for treatment of pain associated with prolonged excitation at lamina I synapses.

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Poster

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Stanford Bio-X NeuroVentures

National Science Foundation

Title: Optical and computational tools for analyzing somatosensory circuits

Authors: *S. VYAS, A. J. CHRISTENSEN, S. M. IYER, C. RAMAKRISHNAN, K. DEISSEROTH, S. L. DELP;
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Abstract: Optical imaging techniques to observe neural activity in anesthetized mice present a compelling opportunity for the analysis of dorsal horn circuits in response to sensory stimuli. Here, we discuss viral methods to express genetically encoded calcium indicators (GECIs), such as GCaMP6f in the dorsal horn of the spinal cord through intraspinal injection and transsynaptic retrograde spread following intrasciatic injection, and report on the expression levels and degree of signal-to-noise achieved. We then discuss methods to enable direct imaging of neural activity in anesthetized preparations. These methods involve the placement of a coverslip superficial to the spinal cord, and the placement of a clamping system to enable reduction of motion artifacts during imaging. We present preliminary imaging results from animals that have received such an imaging implant. We also discuss methods to process and analyzing the resulting data, focusing on two questions: 1) identification of specific neural sub-graphs that are formed in response to particular patterns of sensory input, 2) analysis of how these sub-graphs contribute to the transition from sensory input to read-out of this information at the spinal cord and brain. These tools may provide for a system to assess how sensory stimuli are represented in the spinal cord, and how this information is transferred to the brain.

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Poster

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Title: A machine learning methodology for the selection and classification of spontaneous spinal cord dorsum potentials allows disclosure of structured (non-random) changes in population neuronal activity induced by nociceptive stimulation

Authors: ***P. RUDOMIN**[1,2], M. MARTIN[3], J. BEJAR[3], E. CONTRERAS-HERNÁNDEZ[1], D. CHAVEZ[1], G. ESPOSITO[3], U. CORTES[3], S. GLUSMAN[1];
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Abstract: Fractal analysis of spontaneous cord dorsum potentials (CDPs) generated in the lumbosacral spinal segments of the anesthetized cat has revealed that these potentials are produced by ongoing structured (non-random) neuronal activity. Previous studies aimed to disclose the changes produced by nociceptive stimulation on the functional organization of the neuronal networks generating these potentials used predetermined templates to select specific classes of spontaneous CDPs. Since this procedure was time consuming and required continuous supervision, it was mostly limited to the analysis of two types of CDPs (negative CDPs and negative-positive CDPs), thus excluding potentials that could reflect activation of other neuronal networks of presumed functional relevance. Here we present a novel procedure based on machine learning that allows the semi-automatic, efficient and unbiased selection of a variety of spontaneous CDPs with different shapes and amplitudes that is used to build a dictionary by grouping the CDPs in coherent classes. The reliability and performance of the method was evaluated by examining the changes in the probabilities of generation of several types of spontaneous CDPs induced by the injection of small amounts of capsaicin in the plantar hind paw of the anesthetized cat. The results obtained with our selection method allowed detection of spontaneous CDPs that are assumed to represent the activation of functionally coupled sets of dorsal horn neurons that acquire different and structured configurations in response to

nociceptive stimuli. A relevant characteristic of our method is its capability to identify a basic dictionary of CDP classes that show statistical stability through different experimental manipulations. These dictionaries are also able to confirm (and to extend) the expert knowledge, characterizing changes in the CDPs activity and identifying patterns in raw data recorded under normal and pathological situations that would remain undetected with the use of predetermined templates.

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Poster

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JSPS KAKENHI Grant 24590736

Title: Chasing morphological changes of neuronal processes in the spinal dorsal horn in an inflammatory pain model by using two-photon microscopy

Authors: ***S. MATSUMURA**[1], W. TANIGUCHI[2], K. NISHIDA[1], T. NAKATSUKA[3], S. ITO[1]; [1]Med. Chem., Kansai Med. Univ., Hirakata, Japan; [2]Dept. of Orthopaedic Surgery, Wakayama Med. Univ., Wakayama, Japan; [3]Pain Res. Center, Kansai Univ. of Hlth. Sci., Kumatori, Japan

Abstract: Long-term *in vivo* imaging by two-photon microscopy has recently enabled analysis of structural synaptic plasticity in adult neural circuits, such as the structural dynamics of neocortical neurons in the normal and in the injured adult brain. While the pain system in the spinal cord is phylogenetically primitive and easily exhibits behavioral changes such as hyperalgesia in response to inflammation, there is no report on structural changes of dendrites in the dorsal horn, as done in the hippocampus and neocortex, because of spinal cord movements caused by respiration and heart beats and because of the difficult surgical access to the dorsal horn as compared with that to the brain. With our past experience of *in vivo* patch-clamp recordings, we succeeded in establishing experimental procedures to prepare the spinal cord sufficiently for chasing morphological changes of neuronal processes *in vivo* by using two-photon microscopy. Here we demonstrated inflammation-induced structural changes in dendrites in the spinal dorsal horn of two transgenic mice, one expressing enhanced green fluorescent

protein in dorsal spinal neurons and the other expressing yellow fluorescent protein specific to the nervous system over the course of several hours. Structural changes such as the formation of spine-like structures and swelling of dendrites were observed in the spinal dorsal horn within 30 min after the multiple-site injections of complete Freund's adjuvant, a chemical irritant, to a leg; and these changes continued for 5 h. Both AMPA and NMDA receptor antagonists, and gabapentin, a presynaptic Ca2+ channel blocker, completely suppressed the inflammation-induced structural changes in the dendrites in the spinal dorsal horn. The present study first demonstrates by *in vivo* two-photon microscopy imaging that structural synaptic plasticity occurred in the spinal dorsal horn immediately after the injection of complete Freund's adjuvant and may be involved in inflammatory pain. Furthermore, acute inflammation-associated structural changes in the spinal dorsal horn were shown to be mediated by glutamate receptor activation.

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Poster

237. Spinal Cord Processing: Anatomy and Physiology

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Title: The paired-domain transcription factor Pax2, as a possible nuclear marker for adult GABAergic spinal dorsal horn neurons

Authors: *P. SZUCS[1,2], L. L. LUZ[4,5], F. DORA[1,2], M. SIVADO[1,2], E. KOKAI[1,2], Z. MESZAR[3]; [1]MTA-DE-NAP B-Pain Control Res. Group, Debrecen, Hungary; [2]Dept. Physiol., [3]Dept. Anatomy, Histology and Embryology, Univ. of Debrecen, Fac. of Med., Debrecen, Hungary; [4]Inst. of Mol. and Cell Biol., Porto, Portugal; [5]Dept. Exptl. Biol., Univ. of Porto, Fac. of Med., Porto, Portugal

Abstract: Confirmation of the inhibitory nature of biocytin labelled interneurons in the spinal dorsal horn is technically challenging. The most accepted method, demonstration of vesicular inhibitory amino acid transporters (e.g. VGAT) in axon terminals of the neuron, has several pitfalls. The method requires sufficient labelling of the axon in recorded neurons and,

furthermore, the demonstration of the fact that the VGAT positive axon terminals are indeed connected to the neuron to be identified. An ideal and more convenient way to identify inhibitory neurons, even in case of partially labelled neurons, would be to use somatic or nuclear markers. Several lines of evidence support the model where differentiation of GABAergic interneurons in the dorsal cord depends on the paired-domain transcription factor Pax2, with several other factors (including Lhx1 and Lhx5) helping to activate and maintain Pax2 expression in these cells. Pax2 as a marker has already been used to identify future GABAergic neurons in development studies and recently it was also utilized to identify neuronal groups labelled with intrauterine electroporation in the postnatal period. In this study we systematically tested the expression of Pax2 in adult rodent spinal dorsal horn neurons to quantify the overlap with the inhibitory population defined by the presence of GAD2 or VGAT in the soma and terminals, respectively. We found that Pax2 labels the somata of inhibitory neurons in the adult rodent spinal dorsal horn with good reliability making it a putative marker for individually labelled inhibitory neurons.

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Poster

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Support: ANR JCJC

USIAS

Title: Control of nociceptive responses by spinal cholinergic tone after peripheral neuropathy

Authors: *M. CORDERO-ERAUSQUIN[1], M. MEDRANO[1], D. DHANASOBHON[1], I. YALCIN[1], R. SCHLICHTER[2];
[1]CNRS, Strasbourg, France; [2]CNRS - Univ. de Strasbourg, Strasbourg, France

Abstract: Endogenous acetylcholine (ACh) is an important modulator of nociceptive sensory processing in the spinal cord. While a basal tone of spinal ACh seems to modulate the threshold for nociceptive responses, this tone appears to be disrupted in neuropathic animals. However, the underlying network and mechanisms are unknown. The aim of this study was to elucidate the spinal cholinergic tone in naïve and neuropathic animals, through behavioral experiments and *in vivo* electrophysiology. Our behavior experiments confirm the existence of a spinal cholinergic tone modulating mechanical nociceptive responses in naïve mice, involving nicotinic but also muscarinic receptors. While this tone was thought to be completely lost in neuropathic animals,

our results demonstrate that it is still present, although with an altered efficacy. We then performed *in vivo* extracellular recordings in the mouse dorsal spinal cord to unravel what neurons were affected by the cholinergic tone and how. We could distinguish two populations of neurons responding to nociceptive stimulation, one of which was modulated by the topical application of cholinergic antagonists in naïve animals. In neuropathic animals, responses to peripheral stimulation as well as to cholinergic antagonists were modified in both populations. Our study therefore provides important insights into the spinal cholinergic system in nociception. In addition, it provides novel data about the exact nature of the plasticity occurring after neuropathy. Answering these questions should enable the identification of novel therapeutic targets for the treatment of chronic pain.

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Poster

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Title: Intradermal serotonin enhances warming-evoked responses of superficial dorsal horn neurons in the mouse

Authors: *T. AKIYAMA[1], M. NAGAMINE[2], M. I. CARSTENS[2], E. CARSTENS[2];
[1]Dermatol. & Anat. and Cell Biol., Temple Univ., Philadelphia, PA; [2]NPB, UC Davis, Davis, CA

Abstract: Itch is often triggered by warming the skin in patients with itchy dermatitis, but the underlying mechanism is largely unknown. We presently used electrophysiological methods to investigate if intradermal injection of pruritogens (histamine or serotonin) enhances warming-evoked responses of mouse superficial dorsal horn neurons. Under pentobarbital anesthesia, a laminectomy exposed the lumbar spinal cord for single-unit recording in C57BL/6 mice. Once a mechanosensitive unit was isolated, we tested the unit responsiveness to light brushing, followed by pinching, and subsequently thermal stimuli (37°C) to the hindpaw. Then, either histamine (50 µg/µl; 1 µl injection volume) or serotonin (50 µg/µl; 1 µl injection volume) was injected. Unit responses to application of the same thermal stimuli were determined 5-min, 10-min, 15-min, and 30 min postinjection. At the conclusion of the recording, an electrolytic lesion was made. Recordings were made from a total of 85 units located primarily in the superficial dorsal horn. Of 85 characterized units, 35 (41%) responded to brush and pinch of the hindpaw receptive field

(wide dynamic range=WDR), and 50 (59%) to pinch only (high threshold=HT). 20/85 (24%) responded to warm stimuli. 16/30 (53%) responded to histamine. 14/51 (27%) responded to serotonin. In serotonin-responsive units, mean warming-evoked responses were significantly greater following intradermal injection of serotonin compared to pre-injection. Responses evoked by same warming stimulus were not affected following intradermal injection of serotonin in serotonin-insensitive units, or following intradermal injection of histamine that either did or did not increase neuronal firing. Serotonin-responsive superficial dorsal horn neurons may be involved in warming-evoked itch.

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Poster

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Title: Activity-dependent calcium dynamics in spinal cord lamina I neurons

Authors: *E. K. HARDING[1,2], M. W. SALTER[1,2];
[1]Hosp. For Sick Children, Toronto, ON, Canada; [2]Univ. of Toronto, Toronto, ON, Canada

Abstract: Lamina I neurons of the spinal cord are critical for the integration and relaying of nociceptive information from the periphery to the brain. However, little is known about the function of voltage-gated calcium channels (VGCCs) in these neurons. Calcium is an integral second messenger that initiates activation of gene transcription that leads to potentiation of neuronal output. Here, we sought to understand how the activity of lamina I neurons changes calcium concentration in cellular compartments. We made current-clamp recordings from the soma of lamina I neurons, which were loaded via the patch pipette with the calcium indicator

Oregon Green Bapta-1 (OGB1) and the reference fluorophore Alexa Fluor-594 (AF-594). Using this method we were able to perform simultaneous two-photon calcium imaging and current-clamp recordings. We first defined the threshold for visualization of calcium responses in the somata of lamina I neurons. 5ms current injections were able to induce single action potentials, which resulted in robust rises in OGB-1 fluorescence (quantified as ΔG/R). We found that these rises in calcium to single action potentials were visible in the somatic cytosol (peak ΔG/R = 0.09 +/- 0.02, n = 6 neurons), dendrites (peak ΔG/R = 0.2 +/- 0.02, n = 20 neurons), and dendritic spines (peak ΔG/R = 0.07 +/- 0.01, n = 7 spines from 3 neurons). We found that the nucleus of these neurons also showed a calcium rise in response to a single action potential (peak ΔG/R = 0.03 +/- 0.01, n = 6 neurons). However, we found that that both the rise and decay times of the nuclear response were significantly slower (rise time; somatic cytosol = 45ms +/- 11ms, n = 6 neurons; nucleus = 238ms +/- 52ms, n = 6 neurons; dendrite = 35ms +/- 3ms, n = 20 neurons; dendritic spine = 46ms +/- 9ms, n = 7 spines from 3 neurons) (decay time; somatic cytosol = 4.3s +/- 1.2s, n = 6 neurons; nucleus = 21s +/- 15s, n = 6 neurons; dendrite = 2.2 +/- 0.4s, n = 20 neurons; dendritic spine = 1.4s +/- 0.2s, n = 7 spines from 3 neurons). We found that all calcium responses were prevented by the addition of tetrodotoxin to block action potential generation. Bath application of 100μM cadmium significantly reduced the calcium rise in all measured compartments (somatic cytosol = 106% +/- 4%, n = 3 neurons; nucleus = 84% +/- 4%, n = 3 neurons; dendrite = 86% +/- 3%, n = 3 neurons), indicating that the calcium rise due to action potential firing is dependent on VGCCs. These findings suggest that when a lamina I neuron fires single APs, this opens VGCCs and elicits calcium entry into the dendritic arbour and nucleus. This activity-dependent rise in nuclear calcium could be capable of encoding changes that alter gene transcription to upregulate excitability.

Disclosures: E.K. Harding: None. **M.W. Salter:** None.

Poster

237. Spinal Cord Processing: Anatomy and Physiology

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Topic: D.08. Pain

Title: Regulation of lipids in the spinal cord reflects activation of microglia after sciatic nerve transection

Authors: X. DONGMIN[1], *T. OMURA[2], N. MASAKI[3], H. ARIMA[1], M. HANADA[1], T. BANNO[1], M. SETOU[3], Y. MATSUYAMA[1];
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Abstract: Background Sciatic nerve transection (SNT) induces massive transcriptional changes in the motor neuron, DRG as well as in the spinal cord. Lipids, such as PUFA-containing phosphatidylcholines (PUFA-PCs) release arachidonic acid (AA) or docosahexaenoic acid (DHA) which plays an important role in the central nervous system (CNS). However, little is known about the PUFA-PCs in the spinal cord after SNT and how the release of PUFA-PCs are associated with glial cells which are the essential cells in CNS. Methods To elucidate is the regulation of lipid within the spinal cord after peripheral nerve injury, we studies lipid regulation of the spinal cord in C57BL/6JJmsSlc mice after SNT. We used matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) to analyze the distribution of arachidonic acid-containing phosphatidylcholines (AA-PCs) and docosahexaenoic acid-containing phosphatidylcholines (DHA-PCs). Immunohistological analysis was performed to quantify the glial cells in the spinal cord after SNT. Results Seven days after, SNT [PC (16:0/20:4)+K]+ was increased in the ipsilateral ventral and dorsal horns of the spinal cord. The remaining PUFA-PCs species showed no significant difference 7 days after SNT. The temporal analysis of the [PC (16:0/20:4)+K]+ revealed significantly higher regulation in the spinal cord from day 3 to day 28 after SNT. Furthermore, immunohistochemistry analysis demonstrated that both the spatial and temporal expression patterns of Iba1 positive microglial cells, but not GFAP positive astrocytes resembled the expression of [PC (16:0/20:4)+K]+. Conclusions Our study suggest that the increased expression of [PC (16:0/20:4)+K]+ is related with the activated microglial cells, and the expression of [PC (16:0/20:4)+K]+ could be considered as a characteristic feature in response to nerve injury.

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Poster

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Title: Diode laser nociceptive primary afferent-selective activation of spinal dorsal horn neurons

Authors: ***J. ZHANG**[1], D. J. CAVANAUGH[2], A. I. BASBAUM[3], M. I. NEMENOV[4,5]; [1]Univ. of Utah, Salt Lake City, UT; [2]Neurosci., Univ. of Pennsylvania, Philadelphia, PA; [3]Anat.,

Univ. of California San Francisco, San Francisco, CA; [4]Lasmed LLC, Mountain View, CA; [5]Anesthesia, Stanford Univ., Stanford, CA

Abstract: Neuropathic pain can be triggered by activity of diverse primary afferent pathways, including mechano-insensitive and mechano-heat nociceptors. To what extent these afferents differentially contribute to mechanical allodynia and spontaneous pain is not clear. Selective activation of these afferents for experimental analysis, however, is difficult, as these afferents terminate at different skin depths. We recently documented that a diode laser fiber-type selective stimulation (DLss) allowed homogeneous activation of epidermal and dermal C or Aδ nociceptive fibers in rodents and in patients with painful neuropathy. Here, in a mouse model we used DLss combined with *in vivo* extracellular recordings to study the differential input from C or Aδ fiber to spinal heat-responsive neurons in superficial and deep dorsal horn (including presumptive laminae I and V projection neurons). We focused on heat-responsive afferents that innervate the hindpaw. The response properties of these spinal cord neurons to DLss were compared to their responses to conventional Peltier contact thermode stimulation, in mice pretreated with intrathecal vehicle, or capsaicin to ablate TRPV1+ afferents. Protocols for the C or Aδ laser stimuli were based on stimulus-induced withdrawal in normal, awake animals. We found that all spinal cord neurons that responded to the contact thermode stimulus also responded to DLss. This included all nociceptive-specific and wide dynamic range neurons in both laminae I and V. The response delays of these neurons to DLss, we conclude reflect the different conduction velocities of C and Aδ afferents and firing rates correlated both with the receptive field temperature and the relative laser energy applied. The input to the spinal cord neurons was highly convergent: all cells received input from C fiber stimulation, and ~75% of the cells in lamina I and all in lamina V received input from Aδ fibers. Although our initial search strategy was biased to mechanosensitive neurons, in lamina I we recorded one cell that only responded to C and Aδ laser stimuli, but not to noxious contact heat or mechanical stimuli. Unexpectedly, in capsaicin-treated mice, we recorded two heat-insensitive cells (one in lamina I and one in lamina V) that responded to DLss-generated Aδ fiber activation. These findings establish the utility of DLss activation of afferents to study convergent C and Aδ input to the spinal cord. The DLss is also useful in the analysis of nociceptive inputs that cannot be engaged with more conventional heat stimuli.

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Poster

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Topic: D.08. Pain

Title: An exploratory analysis of the variation in firing properties among rat spinal dorsal horn neurons responding to knee joint movement in the monosodium iodoacetate sensitized rat

Authors: *J. A. MIRANDA[1], E. PITT[2], K. GORE[2], M. WHITLOCK[2], H. REES[1];
[1]Pfizer Neurosci. and Pain, Cambridge, United Kingdom; [2]Res. Statistics, Pfizer Clin. Res., Cambridge, United Kingdom

Abstract: Classification of the physiological responses of neurons can aid in revealing the underlying mechanisms of neural processing, particularly when combined with pharmacological investigations. In spinal cord sensory processing neurons, this is often done using subjective criteria based on assumptions about peripheral inputs to these integrating cells. Such classification approaches risk introducing bias, resulting in an incomplete picture of the neural network of interest. We previously established a preclinical physiological assay to test pharmacological modulation of spinal neuron responses to knee joint movement in Sprague Dawley rats. Animals were injected with 1mg of monosodium iodoacetate into the left knee joint and peripheral sensitization was allowed to develop over 14-17 days. In an effort to support the 3Rs (Replacement, Reduction and Refinement) aspect of animal experimentation and make the most of all animal data collected, we have cataloged all data collected from this assay. After repeated runs of the assay, including positive and negative controls, we have accumulated action potential firing profiles for over 200 cells. Here we present our analyses aimed at understanding the variation in firing properties among those cells using multivariate statistical methods on factors such as ongoing activity, phasic firing responses, tonic firing responses, afterdischarge, stimulus-response profiles and the interrelationship among these factors. The preliminary conclusion is that clear-cut subjective classification of response types may not be justified given the high level of heterogeneity among cells in this dataset. Further analysis with a larger sample size is continuing with a goal to provide a clearer picture of knee joint pain and movement processing in the rat spinal cord.

Disclosures: **J.A. Miranda:** A. Employment/Salary (full or part-time):; Pfizer LTD. **E. Pitt:** None. **K. Gore:** None. **M. Whitlock:** None. **H. Rees:** None.

Poster

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Support: NIH Grant R01NS083702

Title: Region-specific organization of mammalian dorsal spinal cord pain circuits

Authors: *W. P. OLSON, J. NIU, A. VYSOCHAN, W. LUO;
Dept. of Neurosci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: It is well established that the organization of motor and autonomic nervous system spinal cord circuits varies based on body region. However, the region-specific variability of the functional organization of somatosensory circuits in the dorsal spinal cord is much less clear. I used an inducible Cre mouse line (MrgprDCreERT2) and an alkaline phosphatase reporter mouse line (RosaiAP) to sparsely label non-peptidergic-type nociceptors. AP staining of whole mount skin and spinal cord tissue reveals the morphology of individual peripheral and central terminal arbors of non-peptidergic nociceptors. Interestingly, while the peripheral arbors of this population show a surprising similarity between body regions, we found clear morphological differences in the central terminal morphology of axially- vs. distally- innervating neurons. Non-peptidergic nociceptors innervating the trunk have exclusively long and thin central terminals in the thoracic spinal cord. In contrast, those that innervate the paws, head, and tail display rounded central terminals in the medial cervical enlargement, medial lumbar enlargement, medulla, and sacral spinal cord. This regional variability in nociceptor morphology has not previously been described and suggests key differences in the organization of pain circuits innervating axial and distal body regions. I am currently investigating potential differences in nociceptor overlap between paw and trunk regions to provide a likely mechanism governing the spatial acuity of different regions (since distal body regions have a heighted spatial acuity for both the light touch and pain systems). In addition, I am testing potential mechanisms that could establish this regional diversity during development.

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Poster

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Title: Characterization of Gbx1 neurons in the mouse spinal dorsal horn

Authors: *K. HAENRAETS[1,2], H. WILDNER[1], S. THERIN[1], H. U. ZEILHOFER[1,2];
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Abstract: Inhibitory interneurons of the spinal dorsal horn play key roles in the processing of sensory information arriving in the CNS from the trunk and extremities. These neurons constitute a highly heterogeneous population. Only recently, techniques became available that allow

linking subtypes of dorsal horn interneurons to specific functions in sensory processing. Here, we focused on a subpopulation of inhibitory dorsal horn interneurons expressing the homeobox gene Gbx1. We found that about half of the neurons expressing the inhibitory marker Pax2 also express Gbx1. Gbx1-positive neurons were concentrated in lamina I - III. Using Gad67^{eGFP} and GlyT2::eGFP reporter lines, we found that 47 ± 5% of Gbx1 neurons in laminae I/II expressed Gad67^{eGFP}, and 74 ± 7% of Gbx1 neurons in lamina III expressed also GlyT2::eGFP. In order to investigate the function of Gbx1 neurons and their integration in pain and itch pathways, we generated a transgenic mouse line expressing the Cre recombinase under the control of the endogenous Gbx1 locus. We ablated Gbx1 neurons unilaterally by intraspinal injection of a Cre-dependent diphtheria toxin fragment A-expressing adeno-associated virus (AAV). Starting at day 5 after AAV injection, Gbx1 neuron-ablated mice exhibited licking and biting directed to the ipsilateral hind limb resulting in hair loss and skin lesions, and flinches of the affected hind limb. Around day 11, the mice became more responsive to mechanical von Frey filament stimulation. At the same time, latencies of withdrawal responses to noxious heat stimulation increased. Injection of a cre-dependent eGFP-expressing AAV into the dorsal horn revealed that not only dorsal horn neurons but also a heterogeneous population of dorsal root ganglion (DRG) neurons expresses Gbx1. About 10% of the DRG neurons innervating the AAV-injected segments expressed eGFP, including cells, which were NF200-immunoreactive, IB4-binding or CGRP-immunoreactive. These neurons may underlie the reduced heat sensitivity observed in our experiments. In order to characterize the integration of dorsal horn Gbx1 neurons in descending pathways, we performed retrograde monosynaptic rabies virus-based tracing experiments to identify neurons presynaptic to Gbx1 neurons. Labeled neurons were found in the raphe nuclei and the reticular formation, in the vestibular nuclei and the cuneate nuclei, the red nuclei and the somatosensory/motor cortex. Our experiments suggest that dorsal horn Gbx1 neurons control the spinal processing of noxious signals. Their activity appears in turn to be controlled by several brain areas including some with hitherto unidentified roles in pain processing or modulation.

Disclosures: K. Haenraets: None. **H. Wildner:** None. **S. Therin:** None. **H.U. Zeilhofer:** None.

Poster

237. Spinal Cord Processing: Anatomy and Physiology

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Topic: D.08. Pain

Support: NSERC DG6044

IASP Early Career Research Grant

Title: Investigating the viability and excitability of adult dorsal horn neurons using a double-sectioning assay

Authors: *A. DEDEK[1], K. FARMER[2], T. BOSNIC[2], M. E. HILDEBRAND[2];
[2]Neurosci., [1]Carleton Univ., Ottawa, ON, Canada

Abstract: The dorsal horn of the spinal cord is an essential processing network within the nociceptive pathway. Hyperexcitability within the dorsal horn contributes to pain hypersensitivity in many chronic pain syndromes. Studying the molecular underpinnings of this hyperexcitability requires the use of adult rats, as behavioural hypersensitivity is most robust and translatable to human conditions in adult animal models of chronic pain. However, studying the electrophysiological properties of adult dorsal horn neurons *in situ* is limited by the sensitivity of these neurons to insults induced by spinal dissection and sectioning. Moreover, the conditions most critical for preserving neuronal viability during these procedures have not yet been identified. Thus, we developed an assay to assess neuronal health in the spinal cord dorsal horn following sectioning for electrophysiology. We sectioned spinal cords at a standard thickness (300µm) and found that it was not possible to quantify neuronal viability using only light microscopy. Thus, we fixed the spinal sections and stained them with a neuronal marker (NeuN) and a cell death marker (Fluoro-Jade B). However, we found that the thickness of the slices prevented marker penetration as well as resolution of individual neurons. We therefore embedded fixed 300µm sections in gelatin and re-sectioned to 40µm to allow for effective staining. Using this assay, we are currently exploring which cytoprotective conditions maximize neuronal viability in the adult dorsal horn. Beyond optimization of spinal slicing procedures, this double-sectioning assay allows us to explore the molecular mechanisms that underlie electrical properties within individual dorsal horn neurons by combining immunofluorescent staining and neuronal recordings.

Disclosures: **A. Dedek:** None. **K. Farmer:** None. **T. Bosnic:** None. **M.E. Hildebrand:** None.

Poster

237. Spinal Cord Processing: Anatomy and Physiology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 237.19/M6

Topic: D.08. Pain

Title: Parabrachial nucleus mediates itch-induced scratching behavior

Authors: *Y. SUN, D. MU, K.-F. LIU, J. DENG, Y. SHI, Q. MAO;
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Abstract: Itching, or pruritus, is defined as an unpleasant cutaneous sensation that serves as a physiological self-protective mechanism to prevent the body from being hurt by harmful external

agents. However, millions of people are suffering from chronic itch, most of which are resistant to current treatment. Based on the responsiveness to antihistamine, itch can be classified into histamine-dependent and histamine-independent itch. Recent studies have demonstrated the molecular mechanism of histamine-dependent and -independent itch, however, the circuitry mechanisms of itch signal processing in brain still remain unknown. By using c-Fos as a neuronal activity marker, we found that parabrachial nucleus in the brainstem is activated by intradermal injection of histamine and chloroquine, which induce histamine-dependent and -independent itch respectively. We thus determined the functional role of parabrachial nucleus in the itch signal processing. Using pharacogenetics to suppress the neuronal activity of parabrachial nucleus, we found that scratching behavior in response to histamine and chloroquine is significantly suppressed. Most of the projection neurons in parabrachial nucleus are glutamatergic neurons, which employs vGluT2 to load glutamate into synaptic vesicles. By stereotaxic injection of AAV-Cre virus into parabrachial nucleus of vglut2f/f mice, we selectively knocked out vGluT2 from glutamatergic neurons of parabrachial nucleus. And we found that the release of glutamate is largely abolished as detected by *in vitro* patch clamp recording. Consistently, we found that blocking the release of glutamate release of parabrachial nucleus decreased the scratching number in both histamine-dependent and histamine-independent itch models. Moreover, the scratching behavior in allergic and chronic itch models is also suppressed. More interestingly, the pattern of scratching is also affected as detected by high resolution scratching behavior recording. In contrast, pain is not affected in animals with vGluT2 deleted from parabrachial nucleus. Our study thus demonstrates that parabrachial nucleus plays an essential role in mediating the itch-induced scratching behavior.

Disclosures: Y. Sun: None. **D. Mu:** None. **K. Liu:** None. **J. Deng:** None. **Y. Shi:** None. **Q. Mao:** None.

Poster

237. Spinal Cord Processing: Anatomy and Physiology

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UQTR research chair in pain neurophysiology

Fondation Chiropratique du Québec

Title: Integration of bilateral nociceptive signals in the human central nervous system: evidence for novel and distinct modulatory mechanisms in the spinal cord and brain

Authors: *N. RUSTAMOV, J. TESSIER, M. PICHÉ;
Dept. of chiropractic, Univ. du Quebec a Trois-Rivieres, Trois Rivieres, QC, Canada

Abstract: The aim of this study was to examine spinal and cerebral integration of bilateral signals arising from the limbs. Two experiments were conducted, in which nociceptive flexion reflex (NFR) and scalp somatosensory evoked potentials (SEP) were evoked by electrical stimulation. In both experiments, the right sural nerve was stimulated at an individually adjusted intensity (120 % of NFR threshold). For experiment 1 (n=25), the contralateral stimulus was applied to the left sural nerve and intensity was adjusted individually to 60, 120 or 140 % of NFR threshold. For experiment 2 (n=18), the contralateral stimulus was applied to the dorsum of the left hand and intensity was adjusted to 60, 120 or 140 % of the pain threshold. In experiment 1, participants were instructed to attend either the right or left stimulus. In experiment 2, participants were instructed to attend the right stimulus only. Right sural SEP were obtained using a subtraction analysis (difference wave): SEP evoked by bilateral stimulation at 60, 120, or140 % of NFR threshold on the left and 120 % of NFR threshold on the right - SEP evoked by left unilateral stimulation at 60, 120, or 140 % of NFR threshold, respectively. These difference waves were compared with right unilateral sural SEP. In experiment 1, NFR amplitude was significantly increased by contralateral stimulation only when the shock was nociceptive (120 and 140 % of NFR threshold, p's < 0.001). However, these effects were not modulated by attention (p = 0.3). In contrast, the amplitude of difference waves was significantly decreased compared with SEP evoked by right unilateral stimulation for all 3 conditions, including 60, 120 and 140 % (p's < 0.001), while attention had no effect (p = 0.8). In experiment 2, the NFR was facilitated by stimulation of the left hand only when the shock was nociceptive (120 and 140 % of pain threshold, p's < 0.001). Similarly to experiment 1, the amplitude of difference waves was significantly decreased compared with the SEP evoked by right unilateral stimulation for all 3 conditions (p's < 0.001). These results indicate that contralateral stimulation facilitates the NFR when the stimulus is concurrent and nociceptive, independently of the attentional focus. Facilitation occurred with contralateral stimulation of either lower or upper limb, suggesting the involvement of descending pathways originating in the brain. In contrast, brain activity specifically evoked by stimulation on the right was decreased by the contralateral stimulus. These results provide evidence for novel and distinct mechanisms for the integration of nociceptive signals in the brain and spinal cord, which may allow unique and adapted protective responses.

Disclosures: N. Rustamov: None. **J. Tessier:** None. **M. Piché:** None.

Poster

237. Spinal Cord Processing: Anatomy and Physiology

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Topic: D.08. Pain

Support: 5T32GM008471-22

5R01DE021996-04

1R01NS088518-01

Title: Characterization of spinal VGF expression and localization following spinal cord injury

Authors: *J. L. COOK, M. S. RIEDL, J. MAXON, A. M. PARR, L. VULCHANOVA; Univ. of Minnesota, Minneapolis, MN

Abstract: VGF is a granin-related neuropeptide precursor whose expression is rapidly and robustly increased in dorsal root ganglion and dorsal horn neurons following peripheral nerve injury. We have demonstrated that intrathecal administration of several VGF peptides derived from the C-terminal region of the precursor results in thermal hyperalgesia and furthermore, that VGF-derived peptides contribute to both the development and maintenance of hypersensitivity after peripheral nerve injury and inflammation. We hypothesized that VGF levels would also increase after spinal cord injury (SCI), another nervous system insult that results in hypersensitivity and inflammation and ultimately in chronic pain. VGF expression was examined by immunohistochemistry at 28 days post-thoracic SCI, a time-point at which mechanical allodynia and thermal hyperalgesia are present. Rostral to the injury,VGF immunoreactivity -(ir) was decreased in the superficial dorsal horn of SCI animals compared to sham controls. At the injury site, VGF-ir was increased throughout the entirety of the gray matter, with highest levels in the dorsal horn and surrounding the central canal. Caudal to the injury, VGF-ir was increased in the dorsal horn of the spinal cord. These changes were seen through six spinal cord segments caudal to the injury site. The VGF derived peptide TLQP-21 has been shown to activate the Complement 3a (C3aR1) receptor. Signaling via C3aR1 is known to activate the mitogen-activated protein kinase family, including ERK, and activated ERK (phosphorylated ERK, or pERK) is increased in microglia after SCI and is associated with hypersensitivity. Following thoracic SCI, the number of Iba1+ cells appeared to be increased at the site of injury, with some cells exhibiting hypertrophic morphology. Iba1+ cells with hypertrophic morphology were also present caudal to the injury in SCI compared to sham animals. pERK-ir was increased at injury level in the dorsal horn and in the gray matter surrounding the central canal, and caudal to the injury in the lumbar cord, SCI animals had increased pERK staining in the spinal dorsal horn. These results suggest that SCI is associated with persistent changes in VGF expression, microglial activation, and ERK phosphorylation at time-points where pain-related behavior has been recorded.

Disclosures: J.L. Cook: None. **M.S. Riedl:** None. **J. Maxon:** None. **A.M. Parr:** None. **L. Vulchanova:** None.

Poster

237. Spinal Cord Processing: Anatomy and Physiology

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 237.22/M9

Topic: D.08. Pain

Support: Stryker Corporation

Title: Dual frequency temporal patterns improve efficacy and efficiency of spinal cord stimulation

Authors: *T. ZHANG[1], J. J. JANIK[5], W. M. GRILL[1,2,3,4];
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Abstract: Spinal cord stimulation (SCS) is an established therapy for treating refractory chronic pain, but fewer than 60 % of patients experience 50 % or greater pain relief, and this success rate has improved little over time. Conventional single frequency SCS (sfSCS) generates both excitation and inhibition in the dorsal horn network, and sfSCS must be applied at a sufficiently high frequency (typically 50 Hz) for SCS-mediated inhibition to overcome SCS-mediated excitation. We hypothesized that SCS could be improved using dual frequency SCS (dfSCS) to disperse SCS-mediated excitation while preserving SCS-mediated inhibition. We simulated the effects of dfSCS on the firing rate of the output neuron in a validated computational model of the Gate Control circuit by delivering two different frequencies of SCS concurrently to distinct groups of dorsal column fibers. We identified dfSCS pairs that were more effective at inhibiting model neuron activity than sfSCS applied at the average of the constituent frequencies and that had average frequencies lower than clinically used 50 Hz (i.e., more efficient). Furthermore, appropriately selected dfSCS frequency pairs continued to inhibit the model spinal sensory neuron even after the GABAergic synapses onto the model neuron were weakened by 50 % to simulate the transition to neuropathic pain. We then measured in urethane anesthesized healthy rats the change in firing rate of spinal sensory neurons produced by dfSCS during concomitant sciatic stimulation. An appropriately selected dfSCS pair generated significantly greater inhibition (i.e., more effective) of spinal sensory neurons *in vivo* than sfSCS applied at each of the two constituent frequencies or at the average of the two constituent frequencies and did so at an average frequency less than 50 Hz (i.e., more efficient). In a subset of experiments, the $GABA_A$ receptor antagonist bicuculline was applied intrathecally prior to SCS to mimic the progression of neuropathic pain. Despite loss of GABAergic inhibition, select dfSCS frequency pairs still produced greater inhibition of spinal sensory neurons *in vivo* than sfSCS applied at the average of the constituent frequencies or at 50 Hz. These computational and experimental results support dual frequency SCS as a novel strategy to improve the efficacy and efficiency of SCS as a therapy for treating chronic pain.

Disclosures: **T. Zhang:** None. **J.J. Janik:** A. Employment/Salary (full or part-time):; Stryker Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property

rights/patent holder, excluding diversified mutual funds); Stryker Corporation. **W.M. Grill:** None.

Poster

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Topic: D.08. Pain

Support: Natural Science and Engineering Research council of Canada (NSERC)

UQTR research chair in pain neurophysiology

Title: Spinal integration of bilateral asynchronous nociceptive signals

Authors: *S. BEN MANAA[1], N. RUSTAMOV[2], S. BOIS[2], J. TESSIER[2], M. PICHE[2];
[1]Dept. de chiropratique, Lab. De Neurophysiologie De La Douleur, Trois Rivieres, QC, Canada;
[2]Dept. de Chiropratique, Univ. du Quebec a Trois-Rivieres, Trois-Rivieres, QC, Canada

Abstract: We recently observed that the lower limb nociceptive flexion reflex (NFR) is facilitated by a concurrent contralateral stimulation. This was observed for stimulation of the contralateral lower or upper limbs, suggesting that facilitation involves descending pathways originating in the brain that modulate spinal circuits. In the present study, we examined whether spinal integration of peripheral and cerebrospinal inputs is modulated when bilateral stimuli are asynchronous. The right and left sural nerves were stimulated in 20 healthy participants at an individually adjusted intensity (120 % of NFR threshold) in five conditions: 1) unilateral right stimulation; bilateral stimulation with 2) no delay 3) a 50-ms delay 4) a 100-ms delay 5) a 500-ms delay. Based on previous results, we anticipated that bilateral stimulation would facilitate the NFR. However, based on the latency of the crossed-extension reflex and reciprocal innervation (agonist/antagonist reciprocal inhibition), we hypothesized that this facilitation would be attenuated or abolished in 50-ms and 100-ms delay conditions. As expected, NFR amplitude was significantly different across the 5 conditions ($F(4,76) = 3.0$, $p = 0.43$; ($\eta p2 = 0.14$). Dunnet's test for multiple comparisons further indicated that NFR was facilitated for the no-delay or 500-ms delay conditions compared with the right unilateral condition ($p = 0.03$ and $p = 0.007$, respectively) while NFR amplitude was not significantly modulated for the 50-ms and 100-ms conditions ($p = 0.2$ and $p = 0.084$, respectively). The present results confirm our hypothesis and indicate that the spinal cord integrates competing inputs from the periphery and from descending modulatory pathways during bilateral stimulation. This study also suggests the existence of spinal circuitry allowing the interaction and regulation of peripheral nociceptive input from both lower limbs. This mechanism may allow adapted protective responses depending on the context.

Disclosures: **S. Ben manaa:** None. **N. Rustamov:** None. **S. Bois:** None. **J. Tessier:** None. **M. Piche:** None.

Poster

237. Spinal Cord Processing: Anatomy and Physiology

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Topic: D.08. Pain

Support: NIH grant NS080889

Title: Properties of cutaneous and muscle afferent synapses onto lamina I projection neurons in the developing rat spinal cord

Authors: ***J. LI**[1], M. BACCEI[2];
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Abstract: Previous work suggests that muscle afferents are more effective at inducing hyperexcitability within spinal cord circuits (i.e. 'central sensitization') compared to cutaneous afferents, and musculoskeletal pain in children is more common than pain of cutaneous origin. However, the reasons for this are still unclear because nothing is known about whether superficial dorsal horn neurons process sensory input from muscle vs. skin differently at the synaptic level. Here we investigated the functional properties of monosynaptic inputs from sensory afferents traveling in the gastrocnemius (GS; predominantly innervating muscle) or sural (largely cutaneous) nerves onto identified lamina I projection neurons (PNs) using *in vitro* patch clamp recordings in an intact neonatal spinal cord preparation. Paired-pulse stimulation experiments demonstrated a significantly lower paired-pulse ratio in response to stimulation of the GS nerve compared to the sural nerve, suggesting a higher probability of glutamate release at muscle afferent synapses onto ascending spinal PNs. Nonetheless, muscle and cutaneous afferent synapses displayed a similar degree of short-term depression following repetitive stimulation and recovered with a similar time course. Long-term synaptic plasticity at these synapses was investigated using a spike timing-dependent plasticity (STDP) protocol involving the pairing of highly correlated presynaptic and postsynaptic firing. A significantly greater percentage of lamina I PNs showed long-term potentiation (LTP) in response to GS nerve stimulation compared to sural nerve activation, which was accompanied by a greater magnitude of LTP at muscle afferent synapses. While the mechanisms underlying the enhanced plasticity of muscle afferent synapses remains to be fully elucidated, it is unlikely to reflect differences in the subunit composition of postsynaptic NMDARs within lamina I PNs, as both the decay kinetics of isolated NMDAR currents and the sensitivity to a NR2B-selective antagonist were similar following GS and sural nerve stimulation. Overall, the present results reveal novel heterogeneity in the properties of primary afferent synapses onto the key output neurons of the developing

spinal nociceptive circuit, which may contribute to the greater ability of muscle afferents to evoke central sensitization.

Disclosures: J. Li: None. **M. Baccei:** None.

Poster

237. Spinal Cord Processing: Anatomy and Physiology

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UQTR research chair in pain neurophysiology

Natural Science and Engineering Reseach Council of Canada (NSERC)

Canadian Institutes of Health Research (CIHR)

Title: Spinal manipulation decreases experimental back pain: specific effects on temporal summation of pain

Authors: *C. RANDOLL[1], N. RUSTAMOV[1], J. TESSIER[1], V. GAGNON-NORMANDIN[1], J. O'SHAUGHNESSY[1], M. DESCARREAUX[2], M. PICHÉ[1];
[1]Chiropractic, [2]Sci. de l'activité Physique, Univ. Du Quebec A Trois-Rivieres, Trois-Rivieres, QC, Canada

Abstract: Several studies have examined the effects of spinal manipulation (SM) on experimental and clinical pain. In spite of some negative results, SM produced hypoalgesic effects for the majority of these studies. However, some studies did not control for potential nonspecific effects. Moreover, there is still limited evidence on how these hypoalgesic effects are produced. The aim of the present study was to examine the hypoalgesic effects of thoracic SM on pain and temporal summation of pain, while controlling for potential nonspecific effects. The study comprised 2 experiments including 16 and 15 healthy participants, respectively. The experimental design included 6 sessions allowing to compare changes in perception or temporal summation for painful vs non-painful electrical stimulation 1) in a control condition 2) after a control light mechanical stimulus 3) after SM. Stimulation was applied locally at the T4 thoracic spine segment, where SM and the control mechanical stimulus were applied. In experiment 1, electrical stimulation was applied as a single 1-ms pulse and perception was rated for each stimulus. In experiment 2, stimulation consisted in a train of five 1-ms pulses delivered at 2 Hz, in order to induce temporal summation. In this case, participants were instructed to rate the strongest sensation for each stimulus train. SM involved articular cavitation while the control mechanical stimulus was a calibrated force of approximately 25 N applied for 2 seconds. In

experiment 1, perception was not significantly altered by SM or the control mechanical stimulus for either the painful (interaction: $F(4,60) = 1.9$; $p = 0.12$) or non-painful (interaction: $F(4,60) = 0.9$; $p = 0.46$) stimulation. In experiment 2, temporal summation was observed for both the painful (main effect: $F(1,14) = 42.0$; $p < 0.001$) and non-painful (main effect: $F(1,14) = 23.7$; $p < 0.001$) stimulation. However, temporal summation was marginally decreased across sessions and blocks (time) for the painful (interaction: $F(4,56) = 2.1$; $p = 0.08$) but not non painful (interaction: $F(4,52) = 0.1$; $p = 0.9$) stimulation. Bonferroni-corrected planned contrasts revealed that temporal summation of pain was significantly decreased after SM compared with baseline ($p = 0.009$) but this effect was not maintained 10 minutes after SM ($p = 0.16$). Planned contrasts also revealed that the control mechanical stimulus did not significantly modulate temporal summation of pain ($p > 0.05$). These results indicate that SM produces transient hypoalgesic effects on temporal summation of pain, while a control mechanical stimulus is ineffective. This suggests that SM-induced hypoalgesia relies in part on a spinal mechanism.

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Poster

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Title: Identification of spinal circuitry for mechanical pain by intersectional genetics

Authors: *B. DUAN[1], L. CHEN[1], M. GOULDING[2], Q. MA[1];
[1]Dana-Farber Cancer Institute, Harvard, Boston, MA; [2]Salk Inst., La Jolla, CA

Abstract: Pain information processing in the spinal cord has been postulated to rely on nociceptive transmission (T) neurons receiving inputs from nociceptors and Aβ mechanoreceptors, with Aβ inputs gated through feed-forward activation of spinal inhibitory neurons (INs). Here, we used intersectional genetic manipulations to identify these critical components of pain transduction. Marking and ablating different populations of spinal excitatory

and inhibitory neurons, coupled with behavioral and electrophysiological analysis, showed that excitatory neurons expressing somatostatin (SOM) include T-type cells, whose ablation causes loss of mechanical pain. Inhibitory neurons marked by the expression of dynorphin (Dyn) represent INs, which are necessary to gate Aβ fibers from activating SOM+ neurons to evoke pain. In addition, our findings using other Cre lines that mark distinct subsets of SOM lineage neurons showed that these neurons represent a heterogenous population of excitatory neurons that transmit distinct forms of mechanical pain.

Disclosures: **B. Duan:** None. **L. Chen:** None. **M. Goulding:** None. **Q. Ma:** None.

Poster

238. Visceral Pain

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Support: Department of Veterans Affairs Merit Grant BX001195

Title: Effects of early life stress vary across paradigm: a comparative analysis of sex-specific alterations in viscerosomatic pain

Authors: *D. PRUSATOR[1], B. GREENWOOD-VAN MEERVELD[2];
[2]Oklahoma Ctr. for Neuroscience, Dept. of Veterans Affairs, [1]Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK

Abstract: Early life stress (ELS) serves as a risk factor for the development of functional pain disorders such as irritable bowel syndrome (IBS) in adults. The long-term effects of ELS are multifaceted and complex to study in patients, therefore the mechanisms leading to long-term consequences of ELS remain poorly understood. Although rodent models have been developed to mirror different forms of ELS experience, the use of predominantly male animals has led to limited information related to sex-related differences in the persistent effects of ELS on pain behaviors in adulthood. In the present investigation, we test the hypothesis that the context or nature of ELS experience may interact with sex-differences to influence the development of chronic pain. In this study, three rodent models mimicking different facets of adversity were used to investigate the long-term effects of ELS on pain perception in rats. As neonates, male and female rat pups were exposed to either maternal separation (MS), limited nesting (LN), or a classical conditioning paradigm, odor-attachment learning (OAL). Following maturation to adulthood, visceral sensitivity and somatic sensitivity were assessed at ~postnatal day 90 via visceromotor response to colorectal distension or von Frey probe, respectively. Our study demonstrates that visceral and somatic sensitivity are increased in male rats exposed to neonatal MS or LN, whereas female rats were unaffected compared to controls that were left undisturbed.

In the OAL model, only females exhibited visceral hypersensitivity in response to unpredictable odor-shock conditioning, but not somatic hypersensitivity. Males were seemingly unaffected following OAL exposure. Our overall analysis suggests that the development of heightened pain behaviors in adulthood is not only influenced by individual ELS experiences, but also by sex-differences. Specifically, this study indicates that male and female animals may exhibit vulnerability or resilience for the development of heightened pain perception based on the specific type of ELS experienced.

Treatment Group		**Colonic Sensitivity**		**Somatic Sensitivity**
		(% of control at 60 mmHg)		**(Withdrawal Threshold, % of control)**
		40mmHg	**60mmHg**	
Control	**Male**	**63.0±7.4**	**100±8.1**	**100±4.5**
	Female	**64.4±12.5**	**100±12.3**	**100±7.7**
MS	**Male**	**84.9±9.6****	**149.1±15.9****	**73.8±4.3^{++}**
	Female	**60.7±5.1**	**98.7±5.7**	**89.7±5.0**
LN	**Male**	**102.5±9.3****	**160.2±11.8****	**82.2±4.1^{+}**
	Female	**83.2±9.4**	**117.6±9.9**	**105.6±5.7**
Unpredictable OAL	**Male**	**44.8±13.0**	**88.9±8.7**	**120.0±6.5**
	Female	**80.9±7.4****	**128.3±7****	**98.5±6.1**

***P<0.05, **P<0.01 vs control RM 2-Way ANOVA with Bonferonni Post Hoc Test, +P<0.05, ++P<0.01 vs control Student''s t-test**

Disclosures: D. Prusator: None. **B. Greenwood-Van Meerveld:** None.

Poster

238. Visceral Pain

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Title: Chronic stress-induced lower back pain in a rat trigeminal neuropathic pain model: central mechanisms that contribute to comorbid pain conditions

Authors: J.-L. YANG, S.-P. ZOU, W. GUO, K. REN, *R. DUBNER, R. TRAUB, F. WEI; Dept Neural and Pain Sci., Univ. Maryland Dent. Sch., Baltimore, MD

Abstract: Chronic pain and stress impact on each other and are potentially modifiable risk factors for poor health outcomes. Clinical data have identified an association between perceived stress and various pain syndromes including orofacial and abdominal pain. However, the mechanisms underlying how psychological stress exacerbates pain experiences are poorly understood. In the present study, we investigated the effects of chronic stress on behavioral hypersensitivity in the rat trigeminal neuropathic pain model induced by chronic construction injury to the infraorbital nerve (CCI-ION). Ten days after CCI or sham surgery, male and female Sprague-Dawley rats were subject to 3 day forced swim stress (10 min on the first day, 20 min on the 2nd and 3rd day) and subsequently tested daily for mechanical sensitivity. While CCI-ION induced mechanical hyperalgesia and allodynia to cutaneous stimulation in the dermatome innervated by the injured nerve (V2) and secondary hyperalgesia in the adjacent mandibular division (V3), they were not exacerbated by forced swim stress. To determine if secondary hyperalgesia or spreading pain develops out of the orofacial area, we tested mechanical sensitivity in the fore paw, hind paw and lower back (region of referred pain from the lower GI tract). There were no significant changes in mechanical nociception at both fore and hind paws in the CCI+stress group. However, there was persistent mechanical hypersensitivity in the lower back starting one day after the last forced swim. This distal hypersensitivity persisted at least 11 days in female rats but only 7 days in male rats, suggesting that subchronic stress induces spreading lower back pain in the presence of an existing orofacial neuropathic pain. This is comparable to our model of masseter muscle inflammation followed by stress inducing chronic abdominal pain. The current model further demonstrates a potential sex difference. In order to explore involvement of central mechanisms, the selective 5-HT3R inhibitor Y25130 (120 fmol/10ul, i.t.) was administered at 7d after forced swim. Hyperalgesia in the lower back was significantly reduced 1 and 3 h after drug treatment and totally recovered at 5 h. This finding indicates that spinal 5-HT3 receptors are involved in stress-induced low back hyperalgesia after nerve injury, suggesting that 5-HT-dependent descending facilitation mediates comorbid chronic stress-induced gastrointestinal hypersensitivity in an orofacial neuropathic pain model.

Disclosures: **J. Yang:** None. **S. Zou:** None. **W. Guo:** None. **K. Ren:** None. **R. Dubner:** None. **R. Traub:** None. **F. Wei:** None.

Poster

238. Visceral Pain

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 238.03/M16

Topic: D.08. Pain

Support: NIH Grant NS37424

Title: Opposing roles of estradiol and testosterone on stress-induced visceral hypersensitivity in rats

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Abstract: Stress can be both pro- and anti-nociceptive. Acute stress generally attenuates pain (i.e. stress-induced analgesia). In contrast, chronic stress exacerbates acute pain and triggers pain in chronic conditions such as irritable bowel syndrome, temporomandibular disorder, fibromyalgia and others. Female predominance, especially during reproductive years, strongly suggests a role of gonadal hormones in these chronic pain disorders. However, the mechanisms underlying gonadal hormone modulation of stress induced pain hypersensitivity are not well understood. In the present study, we tested the role of estradiol (E2) and testosterone (T) on stress-induced visceral hypersensitivity (SIVH) in male and female Sprague-Dawley rats using a forced swim (FS) paradigm as a subchronic stressor. Visceral sensitivity was assessed by recording the visceromotor response (VMR) to colorectal distention. Following baseline recording, rats were subjected to 3 daily sessions of FS (10 min, 20 min, 20 min; 26°C H2O). The VMR was recorded 2,6,10 and 18 days after the last swim session. In female rats FS induced visceral hypersensitivity that lasted 2-3 weeks. In contrast, in males, visceral hypersensitivity was apparent only at 2 days post stress. SIVH was not observed in ovariectomized rats, suggesting a facilitating effect of female gonadal hormones. Intrathecal administration of the estrogen receptor (ER) antagonist ICI-182,780 30 min prior to each FS attenuated the visceral hypersensitivity in intact female rats, further supporting a facilitatory role of E2. The pronociceptive effect of E2 in SIVH was also observed in males; E2 injection every 4 days (which mimics the cyclic changes of estrogen level in intact female rats) dramatically increased visceral hypersensitivity at all timepoints through 18 days. E2 without stress had no effect on the VMR in males. In contrast to E2, Testosterone was found to be protective/antinociceptive. Gonadectomy increased the duration and magnitude of SIVH compared to intact males. Implanting testosterone capsules in females attenuated SIVH, overcoming the facilitation by E2.

Serum levels of E2 in injected males and T in capsulated females were within the physiological range of intact females/males, respectively. Western blot analysis in male tissue revealed the level of the NMDA receptor subunit GluN1 in dorsal spinal cord was increased after E2 injection. In addition, FS decreased spinal mGluR2 expression in E2 injected males. These data suggest that estradiol facilitates and testosterone attenuates stress-induced visceral hypersensitivity by modulating spinal glutamate receptor expression independent of sex.

Disclosures: **Y. Ji:** None. **X. Hu:** None. **R.J. Traub:** None.

Poster

238. Visceral Pain

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Topic: D.08. Pain

Support: NIH Grant NS37424

Title: Stress epigenetically regulates spinal NMDA and mGluR3 expression modulating visceral sensitivity in female rats

Authors: D.-Y. CAO[1,2], *G. BAI[1,3], Y. JI[1,3], R. J. TRAUB[1,3];
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Abstract: Irritable bowel syndrome (IBS) is a chronic pain condition that is more prevalent in women. It is associated with visceral hypersensitivity that is often exacerbated by stress. However, the underlying mechanisms are not completely understood. We previously reported that spinally administered histone deacetylase (HDAC) inhibitors blocked estrogen-induced visceral hypersensitivity by increasing metabotropic glutamate receptor 2 (mGluR2) activity. In the present study we investigated mechanisms underlying stress-induced visceral hypersensitivity focusing on epigenetic modulation of ionotropic and metabotropic glutamate receptors in the spinal cord. Intact female Sprague-Dawley rats were stressed by 3 daily sessions of forced swim (FS). The magnitude of the visceromotor response (VMR) to noxious colorectal distention increased significantly 1 day after the last FS compared with the pre-stress baseline. This was reversed by intrathecal injection (30 nmol, i.t.) of the NMDA receptor antagonist APV (approximately 50% reduction in the VMR, similar to non-stressed rats). Stress also increased NMDA receptor GluN1 protein and mRNA expression in the dorsal spinal cord compared to naïve rats. Intrathecal injection of the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA; 40 μg/day, 30 min before each FS) blocked the stress-induced increase in the VMR and GluN1 mRNA. APV still attenuated the VMR by ~50%. SAHA also increased mGluR3 mRNA

compared with vehicle pre-treated, stressed rats. The mGluR2/3 antagonist LY341495 (20 nmol, i.t.) following SAHA pretreatment reversed the SAHA-evoked block of the stress-induced visceral hypersensitivity, but did not alter FS-induced visceral hypersensitivity in the absence of SAHA. These results suggest that repeated psychophysical stress epigenetically regulates glutamate receptor expression in the spinal cord altering visceral sensitivity by increasing excitatory receptors; there is an increase in GluN1 subunit expression although the full composition of upregulated NMDA receptors is unknown. Spinal HDAC inhibition modulates stress-induced visceral hypersensitivity via multiple mechanisms: preventing the GluN1 upregulation and increasing mGluR3 expression. These data suggest that increasing histone acetylation may be a potential approach to relieving visceral pain induced or exaggerated by stress, a contributing factor to pain in IBS patients.

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Poster

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Title: Voluntary exercise can prevent or reverse comorbid urogenital and mood disorders resulting from early life stress in male mice

Authors: *I. FUENTES, A. N. PIERCE, R. WANG, J. A. CHRISTIANSON;
Univ. of Kansas Med. Ctr., Kansas City, KS

Abstract: Chronic prostatitis patients are commonly co-diagnosed with or suffer symptoms of interstitial cystitis/painful bladder syndrome. This overlap in symptomology complicates proper diagnosis and hinders the development of effective treatment strategies. Common within this cohort of patients is a history of early life stress or adversity, which has been shown to disrupt proper functioning of the hypothalamic-pituitary-adrenal (HPA) axis. Voluntary exercise has

been shown to reverse many of the symptoms attributed to HPA axis dysfunction, both in clinical and preclinical studies. Here we are investigating the therapeutic potential of voluntary wheel running in either preventing or reversing urogenital and anhedonic behaviors and associated molecular changes in a male mouse model of neonatal maternal separation (NMS). Mice were born in-house and underwent NMS from postnatal day 1 to 21. Mice given an early exercise intervention (-Eex) were pair-housed in cages equipped with running wheels at 4 weeks of age, mice given a late exercise intervention (-Lex) were tested for baseline behaviors at 8 weeks of age and were then singly housed in cages equipped with running wheels. Sedentary controls for each group (-Esed and -Lsed, respectively) remained in their home cages. NMS-Esed and NMS-Lsed mice displayed perigenital mechanical allodynia, increased micturition rates, and anhedonic behavior; all of which were not observed in either NMS-Eex or NMS-Lex mice. Dysregulation of the HPA axis was observed by increased histological evidence of mast cell degranulation in the bladder and prostate of NMS-Esed and NMS-Lsed mice and central gene changes within the hypothalamus, amygdala, and hippocampus. Exercise decreased mast cell degranulation in both groups of NMS mice, as well as increased the levels of brain-derived neurotrophic factor (BDNF) mRNA in the hippocampus of both naïve and NMS mice. Interestingly, a correlation between anhedonic behavior and urogenital sensitivity was observed only in sedentary NMS mice, suggesting that these comorbidities are mechanistically linked. Also, NMS mice ran shorter distances than naïve mice, suggesting that reward pathways may be disrupted following NMS. Taken together, these data suggest that NMS in male mice may provide a novel pre-clinical model for studying commonly co-diagnosed urogenital and mood disorders and that voluntary exercise may provide an excellent therapeutic option for increasing limbic regulation of the HPA axis.

Disclosures: **I. Fuentes:** None. **A.N. Pierce:** None. **R. Wang:** None. **J.A. Christianson:** None.

Poster

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NIH T32HD057850

Title: Investigating comorbidities in an early life stress model of chronic urogenital pain in mice

Authors: O. K. ELLER[1], A. N. PIERCE[1], I. M. FUENTES[1], P. T. O'NEIL[1], R. WANG[1], G. O. DUSSOR[2], *J. A. CHRISTIANSON[1];
[1]Anat. and Cell Biol., Univ. of Kansas Med. Ctr., Kansas City, KS; [2]Sch. of Behavioral and Brain Sci., The Univ. of Texas at Dallas, Dallas, TX

Abstract: Urogenital pain patients often suffer from comorbid symptomology involving pain in adjacent and distant locations in the body. Many of these symptoms are induced or exacerbated by acute stress, which has been associated with dysfunction within the hypothalamic-pituitary-adrenal (HPA) axis. We have developed a mouse model of neonatal maternal separation (NMS) that exhibits evidence of increased urogenital sensitivity and dysfunction, likely due to disrupted limbic regulation of the HPA axis. Here we are investigating whether these mice also exhibit behavioral or molecular evidence indicative of fibromyalgia and migraine. Mice were born in-house and underwent NMS from postnatal day 1 through 21. At 8 weeks of age, mice were evaluated for forepaw mechanical sensitivity using graded von Frey monofilaments. NMS mice displayed significantly reduced mechanical withdrawal thresholds compared to naïve mice, indicating forepaw mechanical allodynia. Mice were then placed in cages equipped with running wheels (exercised, -Ex), or remained in their home cages (sedentary, -Sed), to evaluate the efficacy of voluntary exercise to reverse mechanical allodynia. At 12 weeks of age they were retested for forepaw mechanical sensitivity and NMS-Ex mice had significantly higher mechanical withdrawal thresholds than NMS-Sed mice and their own baseline measurements. Interestingly, naïve-Sed mice displayed significant mechanical allodynia compared to naïve-Ex mice. Mice were sacrificed and the dura mater was dissected and processed for either whole mount histological staining with acidified toluidine blue to visualize mast cell contents or for protein isolation and Western blotting with antisera to protease activated receptor 2 (PAR2). Bilateral hippocampi were also dissected and processed for mRNA isolation and real-time PCR to detect mRNA levels of glucocorticoid receptor (GR) and exons I, IV, and IX of brain-derived neurotrophic factor (BDNF). NMS-Sed mice displayed a significant increase in histological evidence of mast cell degranulation and a trend towards increased PAR2 protein levels. Exercise reversed these observations in NMS mice and increased BDNF exons IV and IX and GR mRNA levels in the hippocampus of both NMS and naïve mice. These data suggest that NMS in mice results in wide-spread increases in sensitivity, and could be used as a model for commonly observed comorbidities, including interstitial cystitis, vulvodynia or chronic prostatitis, fibromyalgia, migraine, and depression, for the development of potential therapeutics, including the use of voluntary exercise to strengthen limbic regulation of the HPA axis.

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Poster

238. Visceral Pain

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Topic: D.08. Pain

Support: NIH NICHD HD081709

NIH NIDDK DK100368

Title: Somatic complaint in dysmenorrhea and other visceral pains

Authors: R. ZUCKERMAN[1], J. KANE[2], R. L. SILTON[3], *K. M. HELLMAN[2,1], F. F. TU[2,1]; [1]Univ. of Chicago, Chicago, IL; [2]OB/GYN, NorthShore Univ. HealthSystem, Evanston, IL; [3]Psychology, Loyola, Chicago, IL

Abstract: Increased self-report of somatic complaint, somatization, is a risk factor for many chronic pain conditions including dysmenorrhea, painful bladder syndrome, and irritable bowel syndrome. Individuals with high levels of somatic complaint have demonstrated sensory amplification, widespread bodily sensitivity and indicators of central sensitization, suggestive that the central nervous system plays an important role in visceral pain. Dysmenorrhea, in contrast, has been largely explained as the result of enhanced endometrial tissue breakdown and accompanied exaggerated release of prostaglandins, which support a role for peripheral factors. In the present study, we aimed to characterize peripheral vs. central contributions. We examined the relationship between somatic, psychological and peripheral factors, and dysmenorrhea compared to non-menstrual visceral pain using an online questionnaire (n=1012). We performed a dominance analysis to dissociate somatic complaint, bodily pain complaint, the effect of hormonal contraception, age, menstrual pain, psychological factors and non-menstrual pelvic pain in a regression model. Whereas 11% of the variance in the intensity of menstrual pain was accounted for by peripheral factors such as heavier periods and hormonal contraception, 19% of the variance was associated with somatic complaint, bodily pain, and the intensity of non-menstrual pelvic pain. In contrast, 7% of the variance in the magnitude of other visceral pelvic pain was associated with menstrual factors, while 37% of the variance was associated with somatic complaints and bodily pain. Notably, anxiety and depression were not significant factors because of their collinearity with somatic complaint. Anxiety and depression were correlated with somatic complaint (R2= 55%) and bodily pain (R2=36%). Thus, the effects of anxiety and depression in dysmenorrhea and other visceral pains, often reported in other studies, could be mediated by increased somatic sensitivity. Given that somatic complaint and bodily pain explain significantly more variance than menstrual history factors in dysmenorrhea, this suggests central nervous system dysfunction needs further investigation in this presumed peripherally maintained pain state.

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Poster

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Topic: D.08. Pain

Support: NIH NICHD HD081709

NIH NIDDK DK100368

Title: Neural correlates of sensory amplification in dysmenorrhea and other visceral pain conditions

Authors: K. E. DILLANE[1], S. HARTE[2], K. L. POLNASZEK[3], F. F. TU[1,4], *R. L. SILTON[3], K. M. HELLMAN[1,4];
[1]Ob/gyn, Northshore Univ. HealthSystem, Evanston, IL; [2]Anesthesiol., Univ. of Michigan, Ann Arbor, MI; [3]Loyola Univ. Chicago, Chicago, IL; [4]Ob/gyn, Univ. of Chicago, Chicago, IL

Abstract: Increased painful and non-painful somatic complaint is reported in dysmenorrhea and other visceral pain conditions. We hypothesized that women with dysmenorrhea and other visceral pain conditions have increased sensory amplification that could mediate painful and non-painful somatic complaint. To test this hypothesis, we developed a rapid visual task that could be measured with EEG. Women with dysmenorrhea (n = 12) and healthy controls (n = 6) participated in a passive viewing task that involved watching an annular checkerboard alternating at 12.5 Hz between a positive and negative contrast at 5 different levels of brightness. After each 20-second trial at each level of brightness, participants were asked to rate the unpleasantness of the visual stimulus. During this task, electroencephalography (EEG) was recorded using a 32 channel EEG cap. Evoked activity was measured by performing spectral analysis and measuring the amplitude of the first harmonic (25 Hz). Women with dysmenorrhea reported significantly higher levels of somatic complaint and unpleasantness during the visual task than healthy controls. Increasing brightness during the task was associated with increased evoked EEG activity in parietal and occipital cortical regions ($p<0.001$). Women with dysmenorrhea had less of an increase in parietal activity in response to increasing brightness than healthy controls ($p<0.001$). To specifically examine the role of sensory amplification, we also analyzed a cohort of women who reported higher visual unpleasantness ratings (>10 on a 0-20 scale) compared to the other women in the study. In this cohort, changes in brightness only increased responsiveness in occipital cortical regions. In contrast, the other participants had increased evoked activity in frontal, central and parietal regions. Thus, a wide-spread attention-control network may contribute to suppressing visual unpleasantness. Our results imply that in some women with dysmenorrhea or other visceral pains disorders, network activity that suppresses sensory amplification is awry. Utilization of this rapid EEG task can be used to further examine the mechanisms of sensory amplification in future studies.

Disclosures: **K.E. Dillane:** None. **S. Harte:** None. **K.L. Polnaszek:** None. **F.F. Tu:** None. **R.L. Silton:** None. **K.M. Hellman:** None.

Poster

238. Visceral Pain

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Topic: D.08. Pain

Support: NIH NICHD HD081709

NIH NIDDK DK100368

Title: The effects of depression, dysmenorrhea, and sensory amplification on resting state brain activity

Authors: ***K. BRANDSTATT**[1], K. E. DILLANE[2], K. M. HELLMAN[2,3], F. F. TU[2,3], R. L. SILTON[1];
[1]Loyola Univ. Chicago, Chicago, IL; [2]OB/GYN, NorthShore Univ. HealthSystem, Evanston, IL; [3]Ob/gyn, Univ. of Chicago, Chicago, IL

Abstract: Depression, dysmenorrhea and sensory amplification/somatization are often comorbid and are known to effect brain activity. Our prior research has suggested that the combination of dysmenorrhea and increased somatic complaint are associated with the presence of other visceral pain conditions. In this preliminary analysis (n=17), we examined spectral analysis of resting state electroencephalography (EEG) activity in women with varying levels of menstrual pain. Four periods of resting state activity with eyes closed (60 seconds each) and four periods with eyes open (60 seconds each) were recorded with a 32-channel EEG system. Relative power was used to identify frequency bands altered in relationship to depression, dysmenorrhea, and sensory amplification. Depression was associated with decreased relative frontal alpha and delta activity, but increased frontal gamma activity (p's <0.05). Depression was also associated with more left alpha activity in parietal cortical regions ($p=0.015$), but not right. Dysmenorrhea was associated with decreased relative frontal theta but increased frontal and temporal gamma activity. No significant findings emerged in relation to sensory amplification. Since increased gamma activity is known to be associated with increased local blood-oxygenation, metabolic activity and excitatory activation, frontal cortical regions appear to be more engaged during resting state in depression and dysmenorrhea. Since depression and dysmenorrhea are associated with visceral pain in women and are related to frontal cortical activation, these findings support the idea that changes in central nervous system could contribute to risk for visceral pain. In contrast, our lack of ability to find resting state changes related to sensory amplification suggests that sensory

amplification is mediated by an alteration in a dynamic process rather than the network activity engaged in the resting state.

Disclosures: **K. Brandstatt:** None. **K.E. Dillane:** None. **K.M. Hellman:** None. **F.F. Tu:** None. **R.L. Silton:** None.

Poster

238. Visceral Pain

Location: Hall A

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Topic: D.08. Pain

Support: NIH AR47410

Title: Alterations in expression of aspartate aminotransferase and glutaminase in drg neurons: comparison between rat models of arthritis and inflammatory bowel disease

Authors: ***K. E. MILLER**[1], Z. ZHANG[1], B. R. BOLT[1], S. DAS[1], R. JOHN[1], M. B. ANDERSON[1], C. KIM[1], K. TYLER[2], B. GREENWOOD-VAN MEERVELD[2];
[1]Dept Anat. & Cell Biol, Oklahoma State Univ. Ctr. Hlth. Sci., Tulsa, OK; [2]Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK

Abstract: Glutamate synthesis in neurons occurs by two enzymes, aspartate aminotransferase (AST) and glutaminase (GLS). Our previous studies examined alterations in expression of AST and GLS in rat dorsal root ganglion (DRG) neurons during several somatic and visceral injury/inflammation models including unilateral adjuvant-induced arthritis (AIA) and TNBS-induced colitis resembling that seen in inflammatory bowel disease (IBD). We have noted that there are similar alterations in AST and GLS expression in the DRG with these two models. The purpose of the current study, therefore, was to compare the temporal alterations of AST and GLS in the AIA and IBD models. The L4 DRG was examined for the AIA model, whereas the S1 DRG was studied for the IBD model. Temporally, there is a biphasic expression pattern observed in both models. Increased production of AST and GLS (25-40%) in the DRG occurs within 24-hr of AIA or colonic inflammation and is sustained for 1-2 days. By day 4, AST and GLS levels return to baseline, but elevate again (20%) in small diameter DRG neurons by 8 days of AIA or colitis. By day 16, AST and GLS levels are near baseline in the AIA model, but remain elevated (5-10%) in small diameter neurons for more than 30 days in the IBD model. This study illustrates a common pattern of expression for AST and GLS in DRG neurons during injury and/or inflammation. A shared blueprint of neurogenic inflammation, multiple inflammatory mediators, and neurotrophic factors may be responsible for the similarities in expression patterns. Elevated AST and GLS levels in DRG neuronal perikarya leads to increased glutamate production in peripheral and central terminals. The hypersensitivity observed in both models,

therefore, may have similar mechanisms involving altered glutamate synthesis and release. Interventional therapies for diminishing altered glutamate synthesis may hold promise for pain relief in both somatic and visceral injury and inflammation.

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Poster

238. Visceral Pain

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Topic: D.08. Pain

Support: NIH AR47410

Title: The role of glutamatergic colonic innervation in trinitro-benzene-sulphonic acid (TNBS)-induced colitis in rats

Authors: S. DAS[1], Z. ZHANG[1], M. B. ANDERSON[1], *K. S. CURTIS[2], B. GREENWOOD-VAN MEERVELD[3], K. E. MILLER[1];
[1]Dept. Anat. and Cell Biol., [2]Dept. Pharmacol & Physiol, Oklahoma State Univ. Ctr. For Hlth. Sci., Tulsa, OK; [3]Hlth. Sci. Ctr., Univ. of Oklahoma, Oklahoma City, OK

Abstract: Inflammatory bowel disease (IBD) is a prevalent visceral disorder characterized by abdominal cramping and pain. Pharmacologic therapy for IBD is limited and, based on our previous studies; this led us to investigate the role of the glutamatergic innervation of the colon during TNBS-induced colitis in rats, a model of IBD. Experimentally, rats received an intracolonic infusion of TNBS and colons and lumbosacral dorsal root ganglia (DRG) were collected and examined at different time points. Inflammation in the colon was robust at day 2 after TNBS infusion and we observed that glutaminase (GLS)-immunoreactive (IR) nerve fibers come into close contact with immune cells, CD161-IR (NK) cells, indicating a neuroimmune interaction during colitis. We sought, therefore, to block both the neuronal and immune components by pretreatment and co-treatment of rats with a glutaminase (GLS) inhibitor, 6-diazo-5-oxo-L-norleucine (DON) or the glucocorticoid (GC), hydrocortisone. Both DON and GC reduced colonic inflammation to control levels. For example, the mRNA for the anti-inflammatory cytokine, IL-10, was reduced at day 2 of colitis, but DON or GC pre-treatment brought IL-10 mRNA back to control levels. We also investigated the effect of DON and GC on phenotypic alterations, e.g., elevated GLS expression, in sacral DRG neurons during TNBS-induced colitis. TNBS treatment increased GLS-IR in sacral DRG neurons at day 2 and this

increase in GLS-IR was attenuated by DON or GC treatment. Our results indicate that TNBS-induced colitis has strong neurogenic and neuroimmune components and that interruption of these components provides substantial reduction in colonic inflammation.

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Poster

238. Visceral Pain

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Topic: D.08. Pain

Support: NIH Grant F31DK104538

Title: Asymmetrical involvement of the left and right central amygdala in bladder pain

Authors: *K. E. SADLER, A. M. TROUTEN, B. J. KOLBER;
Dept. of Biol. Sci., Duquesne Univ., Pittsburgh, PA

Abstract: Hemispheric lateralization is a widely recognized theme in neuroscience. First made popular by identification of brain regions like Broca's and Wernicke's areas, asymmetrical involvement of the two hemispheres is now attributed to many different neural processes outside of language cognition. Lateralization is observed in many species from the most basic invertebrates to humans. Recently this phenomenon has been reported in the central amygdala (CeA) during pain processing in rodents. As a member of the limbic system, the left and right CeA are well positioned to integrate both affective and sensory information that is generated during chronic pain conditions like interstitial cystitis/bladder pain syndrome (IC/BPS). Our lab has previously demonstrated the importance of the right CeA in bladder pain processing using genetic, pharmacological, and optogenetic techniques, however the involvement of the left CeA in these processes is unknown. In this report, we use optogenetics to activate both the left and right CeA in the context of bladder distension to determine each nucleus's contribution to this specific type of visceral pain. Increases in bladder pain-like responses are observed only during activation of the right CeA; activation of the left CeA has no effect on pain-like responses. Immunohistochemical analysis demonstrates equal cellular activation levels and basal neuron densities between the two nuclei, suggesting that another mechanism must be responsible for the observed physiological asymmetries.

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Poster

238. Visceral Pain

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Topic: D.08. Pain

Support: Contract from Medtronic, Inc.

Title: Pudendal nerve stimulation inhibits visceromotor responses to urinary bladder distension during concurrent treatment with acute or chronic baclofen

Authors: *T. J. NESS[1], A. RANDICH[2], B. CLODFELDER-MILLER[2], J. MCNAUGHT[2], C. DEWITTE[2], D. E. NELSON[3], X. SU[3];
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Abstract: Background: Bilateral electrical pudendal nerve stimulation (bPNS) anecdotally reduces pain in humans with pain due to interstitial cystitis (IC) and we have reported that it reduces bladder hypersensitivity in rat models. Concurrent medication use can alter responses to neuromodulation and so prior to the development of a clinical trial, the interaction between use of the GABA-B receptor agonist, baclofen, and acute bPNS was examined. Methods: Bladder hypersensitivity was produced by neonatal bladder inflammation in rats pups coupled with a second inflammatory insult as an adult. A dose-response function for baclofen (1-5 mg/kg i.p.) was established. Then baclofen was administered acutely (1 mg/kg i.p.) or chronically (5 mg/kg i.p. daily for 2 weeks prior to the final experiment). Acute bPNS consisted of bilateral biphasic electrical stimulation (10 Hz, 100 μsec biphasic pulses for 10 min) of the mixed motor/sensory component of the pudendal nerves using embedded hook electrodes. Visceromotor responses (VMRs; abdominal muscle contractile responses to urinary bladder distension, UBD) were used a nociceptive endpoints. Results: Baclofen produced a dose-dependent inhibition of VMRs to UBD. bPNS resulted in statistically significant inhibition of VMRs to UBD in hypersensitive rats that had received acute or chronic i.p. baclofen injections. Conclusions: This study suggests that inhibitory effects of bPNS can be evoked in subjects who are receiving baclofen therapy, thus giving guidance to potential clinical trials for treating episodic pain in IC.

Disclosures: **T.J. Ness:** 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.; Medtronic, Inc. **A. Randich:** None. **B. Clodfelder-Miller:** None. **J. McNaught:** None. **C. DeWitte:** None. **D.E. Nelson:** A. Employment/Salary (full or part-time):; Medtronic, Inc. **X. Su:** A. Employment/Salary (full or part-time):; Medtronic, Inc.

Poster

238. Visceral Pain

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 238.14/M27

Topic: D.08. Pain

Title: Macrophage-derived high mobility group box 1 participates in the development and maintenance of pancreatic pain through the activation of RAGE and CXCR4 in mice with cerulein-induced acute pancreatitis

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Abstract: High mobility group box 1 (HMGB1), a nuclear protein, is passively or actively released from various cells including macrophages, and facilitates inflammatory responses by targeting multiple molecules including toll-like receptor 4 (TLR4) and receptor for advanced glycation endproducts (RAGE). HMGB1 also forms a complex with two molecules of CXCL12, a chemokine, and enhances the activation of CXC chemokine receptor 4 (CXCR4) by CXCL12 through the receptor dimerization. We have reported that HMGB1 participates in processing of somatic and bladder pain via the activation of TLR4 and/or RAGE. Here we investigated the role of HMGB1 in the development and/or maintenance of pancreatic pain accompanying cerulein-induced acute pancreatitis in mice. Repeated i.p. administration of cerulein induced acute pancreatitis characterized by increased serum amylase activity and pancreatic weight, and also referred hyperalgesia in the upper abdomen, an indicator of pancreatic pain. The referred hyperalgesia, but not the pancreatitis symptoms, was prevented by pretreatment with the anti-HMGB1 neutralizing antibody or recombinant human soluble thrombomodulin (rhsTM), known to delete HMGB1. They, even when administered after the establishment of acute pancreatitis, reversed the referred hyperalgesia accompanying the pancreatitis. Similarly, low molecular weight heparin (LMWH), known to inhibit RAGE, or AMD3100, a CXCR4 antagonist, exerted both preventive and therapeutic effects on the referred hyperalgesia accompanying the pancreatitis, while LPS-RS, a TLR4 antagonist, showed only the preventive effect. The protein levels of CXCL12, but not HMGB1, in the pancreatic tissue increased following repeated treatment with cerulein. Minocycline, an inhibitor of the activation of macrophage/microglia, or ethyl pyruvate, known to inhibit the release of HMGB1 from macrophages, suppressed cerulein-evoked referred hyperalgesia, but not the pancreatitis symptoms. Together, our data suggest that macrophage-derived HMGB1 contributes to the development and maintenance of pancreatic pain accompanying cerulein-induced pancreatitis through the activation of RAGE and CXCL12/CXCR4 pathways, and that the HMGB1/TLR4 signals participate only in the developmental process of pancreatic pain.

Disclosures: **Y. Irie:** None. **M. Tsubota:** None. **F. Sekiguchi:** None. **H. Ishikura:** None. **M. Nishibori:** None. **A. Kawabata:** None.

Poster

238. Visceral Pain

Location: Hall A

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Program#/Poster#: 238.15/M28

Topic: D.08. Pain

Title: Zinc deficiency aggravates bladder pain accompanying cyclophosphamide-induced cystitis through the enhanced activity of $Ca_v3.2$ T-type Ca^{2+} channels in mice

Authors: ***T. OZAKI**[1], J. MATSUOKA[1], M. TSUBOTA[1], S. TOMITA[1], F. SEKIGUCHI[1], T. MINAMI[2], A. KAWABATA[1];
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Abstract: The function of $Ca_v3.2$ T-type Ca^{2+} channels is suppressed by zinc and enhanced by zinc chelators and also hydrogen sulfide (H_2S). We have reported that intracolonic (i.col.) administration of N,N,N',N'-tetrakis(2-pyridylmethyl)-ethylendiamine (TPEN), a zinc chelator, or H_2S causes $Ca_v3.2$-dependent colonic pain in mice. In the present study, we asked if the decreased zinc levels affect the bladder pain accompanying cyclophosphamide (CPA)-induced cystitis in mice, considering our recent evidence for the involvement of the increased H_2S generation by the upregulated cystathionine-γ-lyase (CSE) and the enhanced $Ca_v3.2$ activity in the same bladder pain model. In the mice fed on zinc deficient diet [Zn(-) mice] for 14 days, the zinc levels greatly decreased in the plasma, but not bladder tissue, compared with the mice fed on the control diet [Zn(+) mice]. Intraperitoneal administration of CPA at 400 mg/kg, but not 200 mg/kg, induced bladder pain-like nociceptive behavior, referred hyperalgesia and increase in bladder weight, an indicator of bladder edema, in Zn(+) mice. In Zn(-) mice, however, CPA even at 200 mg/kg, as CPA at 400 mg/kg did, caused bladder pain/referred hyperalgesia and bladder edema. Similarly, in mice that received i.p. administration of TPEN at 5 mg/kg, the subeffective dose, 200 mg/kg, of CPA induced the bladder pain/referred hyperalgesia and bladder edema. NNC 55-0396, a T-type Ca^{2+} channel blocker, or zinc chloride, administered i.p. after the development of CPA-induced cystitis, reversed the bladder pain/referred hyperalgesia, but not bladder edema, induced by the subeffective dose of CPA in the Zn(-) mice and in the mice pretreated with TPEN. Finally, TPEN in combination with CPA at 200 mg/kg did not mimic the upregulation of CSE in the bladder caused by CPA at 400 mg/kg. These results suggest that zinc deficiency aggravates CPA-induced bladder pain by enhancing the activity of Cav3.2 T-type Ca^{2+} channels, and bladder edema in $Ca_v3.2$-independent manner.

Disclosures: **T. Ozaki:** None. **J. Matsuoka:** None. **M. Tsubota:** None. **S. Tomita:** None. **F. Sekiguchi:** None. **T. Minami:** None. **A. Kawabata:** None.

Poster

238. Visceral Pain

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 238.16/M29

Topic: D.08. Pain

Title: Bladder pain accompanying cyclophosphamide-induced mouse cystitis involves HMGB1 release upstream of the cystathionine-gamma-lyase/H2S/Cav3.2 pathway in the bladder tissue

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Abstract: High mobility group box 1 (HMGB1), a nuclear protein, is passively released from necrotic cells and actively secreted by certain cells such as activated macrophages, promoting inflammatory and pain signals. The active secretion of HMGB1 is facilitated by histone acetyltransferase (HAT) and suppressed by histone deacetylase (HDAC). Our previous evidence has demonstrated that HMGB1 contributes to the bladder pain in mice with cyclophosphamide (CP)-induced cystitis through the activation of receptor for advanced glycation endproducts (RAGE). On the other hand, we have also reported that endogenous hydrogen sulfide (H2S), generated by the upregulated cystathionine-gamma-lyase (CSE), participates in the bladder pain accompanying CP-induced cystitis via the enhancement of Cav3.2 T-type calcium channel activity. Here we investigated a cross talk between the HMGB1 signals and CSE/H2S pathways in the CP-induced bladder pain. CP caused bladder pain-like nociceptive behavior and referred hyperalgesia accompanying the increased bladder weight, an indicator of bladder edema. The bladder pain/referred hyperalgesia, but not bladder edema, was prevented by deletion of HMGB1 with the neutralizing antibody or with recombinant human soluble thrombomodulin (rhsTM) that sequesters HMGB1 and promotes its degradation. Ethyl pyruvate, known to inhibit the release of HMGB1 from macrophages, also inhibited the CP-induced bladder pain/referred hyperalgesia. DL-propargylglycine (PPG), a CSE inhibitor, administered i.p. before CP treatment, abolished the bladder pain/referred hyperalgesia and partially inhibited the increased bladder weight. CP treatment induced downregulation of HDAC1 in addition to the upregulation of CSE in the bladder tissue. The CP-induced upregulation of the bladder CSE was inhibited by the anti-HMGB1 neutralizing antibody or rhsTM, whereas the CSE inhibition by PPG did not affect the CP-induced HDAC1 downregulation. Thus, in the mice with CP-induced cystitis, HMGB1 is considered to be secreted by macrophages and other cells in the bladder tissue possibly following

its accelerated acetylation attributable to downregulation of HDAC1, and cause the upregulation of CSE and subsequent increased formation of H2S, leading to the Cav3.2-dependent bladder pain.

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Poster

238. Visceral Pain

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Topic: D.08. Pain

Support: MSIP, 2008-0062282

Title: Central connectivity of the bladder afferent terminals in the rat spinal cord

Authors: ***Y. BAE**, S. PARK, A. P. DEVI, J. BAE, W. MAH, Y. KIM, Y. CHO;
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Abstract: Visceral afferents from urinary bladder project to L6 spinal cord and is involved in voiding and nociception. At present, little is known about how the visceral information from urinary bladder is processed in the 1st relay nucleus of the spinal cord. To address this issue, we investigated central connectivity of the bladder afferent terminals and involved neurotransmitters in the superficial dorsal horn (DH) and lateral horn (LH) of the rat L6 spinal cord by retrograde tracing with Cholera toxin B subunit-horseradish peroxidase (CTB-HRP), quantitative ultrastructural analysis and electron microscopic immunogold staining with GABA, glycine and glutamate antisera. CTB-HRP labeled bladder afferent boutons contained clear round vesicles. Most of the labeled boutons also contained large dense core vesicles. Labeled boutons were presynaptic to dendrites and frequently received axoaxonic synapse from pleomorphic vesicles containing endings (p-endings). Most of the labeled boutons showed simple synaptic connectivity with one or two postsynaptic dendrites in the DH. Whereas considerable fraction showed complex synaptic arrangement with 3 or more postsynaptic dendrites in the LH. P-endings frequently showed GABA-immunoreactivity (IR) and/or glycine-IR, suggesting that bladder afferents receive GABAergic and/or glycinergic presynaptic modulation. Fraction of the labeled boutons presynaptic to dendritic spines was significantly higher in the LH than in the DH, suggesting higher synaptic plasticity in the LH than in the DH. These findings suggest unique and different processing of bladder-related visceral information between in DH and LH of the L6 spinal cord.

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Poster

238. Visceral Pain

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Program#/Poster#: 238.18/M31

Topic: D.08. Pain

Support: NIH P20GM103648

Title: Glutamate metabolism and pleural inflammation in heat-killed tuberculosis induced pleurisy

Authors: *Z. ZHANG[1], S. DAS[1], S. GANDHAPUDI[2], M. ANDERSON[1], K. TEAGUE[2], K. E. MILLER[1];
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Abstract: Pleurisy, the inflammation of the visceral and parietal pleura, can make breathing or coughing painful after pulmonary infection. The infection is treated depending on the cause, while pleuritic pain often is not adequately addressed. Parietal and visceral pleura have sensory innervation from thoracic DRG neurons and pleuritic pain is conveyed to the spinal cord by these DRG neurons using glutamate as their neurotransmitter. Glutamate is synthesized by glutaminase (GLS) in DRG neurons. In previous studies, we have shown that increased GLS levels in DRG neurons responding to peripheral inflammation contribute to the pain hypersensitivity in the inflamed skin and colon. The goal of this study is to determine in *tuberculosis*-induced pleurisy: 1) the response of DRG neurons, i.e., GLS levels, to pleural inflammation and 2) the potential anti-inflammatory effects of inhibiting GLS in the pleura. Heat-killed *Mycobacterium tuberculosis* (hk-TB) were injected into the rat pleural cavity, whereas control groups received saline injections. At 1, 2, 4 and 8 days, the pleural cavity was washed and the pleural contents collected. Leukocytes were harvested for FACS analysis and inflammatory cytokines in pleural effusion were analyzed. T1 to T4 DRGs were collected bilaterally and pooled to evaluate GLS expression level using quantitative immunohistochemistry and western blot. A GLS inhibitor, 6-diazo-5-oxo-L-norleucine (DON), was administrated as a pretreatment to evaluate the effect of GLS inhibition on hk-TB-induced pleurisy. Intrapleural injection of hk-TB caused a substantial pleural effusion and leukocyte recruitment in the pleural cavity. The leukocyte population shifted from neutrophils to macrophages as the inflammation progressed. Pretreatment with DON, GLS inhibitor, significantly suppressed the leukocyte infiltration induced by hk-TB. In the DRG, an increase in GLS immunoreactivity was observed at 1-2 days of pleural inflammation. These results demonstrate that chronic pleural inflammation causes elevated GLS expression in the DRG neurons, indicating an increase in glutamate synthesis in the peripheral and central terminals of sensory afferents innervating the pleura. The overproduction of glutamate at peripheral terminals leads to aggravation of inflammation, manifesting as a neuro-immune

interaction at the inflamed tissue. With this information, our goal is to close the knowledge gap between pleural inflammation and pleuritic pain and to identify potential novel anti-inflammatory and analgesic targets for treating pleurisy.

Disclosures: **Z. Zhang:** None. **S. Das:** None. **S. Gandhapudi:** None. **M. Anderson:** None. **K. Teague:** None. **K.E. Miller:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Kemmx Corporation, Sapulpa, OK.

Poster

239. Musculoskeletal Pain

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 239.01/M32

Topic: D.08. Pain

Title: A pan-trk inhibitor with low brain penetration inhibits NGF-induced pharmacological responces, and exerts analgesic effect comparable to Morphine in rat osteoarthritis pain model

Authors: T. YASUHIRO, K. ODA, T. NAGAURA, *K. MITSUI, S. KATSUMATA, Y. HIROTA;
ONO Pharmaceut. Co., Ltd., Osaka, Japan

Abstract: Background and aimsPeripheral tropomyosin receptor kinase (Trk) A, specific receptor for NGF, is a promising target for inhibition of the signaling processes that contribute to the manifestation of chronic pain. The importance of these processes has been highlighted by the anti-NGF antibodies which have demonstrated significant analgesic effects when compared to non-opiate medications in patients with chronic pain. We discovered an orally available small molecule pan-Trk (TrkA, TrkB and TrkC) inhibitor with low brain penetration and evaluated its effects in rat peripheral NGF-induced increase of dermal blood flow, hyperalgesia and the MIA osteoarthritis pain model. Methods The pan-Trk inhibitor (0.1 to 3 mg/kg) was administered orally, 12 hours later NGF (1 μg/site) was injected intradermally into the plantar of hind limb. 3 hours after NGF injection, changes in dermal blood flow by thermal stimulation were measured using laser speckle blood flow imager. For assessment of hyperalgesia, NGF (5 μg/site) was injected intramuscularly into calf. Three hours after NGF injection, pressure hyperalgesia to the plantar of hind limb was evaluated by a Randall-Selitto analgesiometer. The osteoarthritis model was established by MIA (3 mg/site) injection intraarticularly into the knee . The pan-Trk inhibitor (0.1 to 10 mg/kg) was administered orally twice a day from 14 to 21 days after MIA injection. The pain-related behavior was evaluated weight bearing on hind limb using a Linton Incapacitance Tester. Results The pan-Trk inhibitor dose-dependently inhibited NGF-induced increase of dermal blood flow and hyperalgesia, and the dose of 3 mg/kg was maximum effect in both models. Moreover, this compound dose dependently inhibited the pain-related behavior in

the MIA osteoarthritis model, and the analgesic effect at ≥1 mg/kg b.i.d. was comparable to that of morphine at 3 mg/kg. Conclusion The pan-Trk inhibitor showed potent analgesic effects in osteoarthritic pain model comparable to strong opiate. The effective plasma concentration of this compound in osteoarthritic pain model was similar to that at 3 mg/kg; the dose required to significantly inhibit peripheral NGF-induced increase of dermal blood flow and hyperalgesia in rats.

Disclosures: **T. Yasuhiro:** A. Employment/Salary (full or part-time):; full-time, ONO Pharmaceutical co.,LTD. **K. Oda:** A. Employment/Salary (full or part-time):; full-time, ONO Pharmaceutical co.,LTD. **T. Nagaura:** A. Employment/Salary (full or part-time):; full-time, ONO Pharmaceutical co.,LTD.. **K. Mitsui:** None. **S. Katsumata:** A. Employment/Salary (full or part-time):; full-time, ONO Pharmaceutical co.,LTD. **Y. Hirota:** A. Employment/Salary (full or part-time):; full-time, ONO Pharmaceutical co.,LTD..

Poster

239. Musculoskeletal Pain

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 239.02/M33

Topic: D.08. Pain

Support: IASP Early Career Research Grant

Title: Cav3.2-expressing low-threshold C fibres in human hairy skin contribute to cooling allodynia - a non-TRPV1- and non-TRPM8-dependent phenomenon

Authors: ***D. A. MAHNS**, M. S. SAMOUR, S. S. NAGI;
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Abstract: Background: It is generally agreed that cold allodynia is a consequence of impaired (Aδ-fibre-mediated) central inhibition of C-nociceptive inputs. However, it is also known that C polymodal nociceptors are not activated at innocuous low temperatures. We recently showed that another form of allodynia, namely tactile allodynia, in which the peripheral driver is typically ascribed to large myelinated afferents, can be mediated by low-threshold C mechanoreceptors, termed C-tactile fibres (CTs). In the current study, we investigated whether this, or a related, C-fibre class contributes to cooling allodynia. Methods: In 30 healthy and 3 chronic-pain subjects, a series of normally innocuous, localized, thermal stimuli were applied to the skin overlying a painful tibialis anterior muscle (induced by infusion of 5% hypertonic saline). The effects of thermal stimulation on muscle pain were observed prior to and following conduction block of myelinated fibres by compression of sciatic nerve. Furthermore, intradermal capsaicin, menthol and TTA-A2 were used for desensitization of TRPV1, TRPM8 and T-type calcium (Cav3.2) channels respectively. Results: Prior to muscle pain, all thermal stimuli were reported as non-

painful regardless of whether myelinated fibres were conducting or not. During muscle pain, dynamic skin cooling (32°C→20°C) evoked significant and reproducible increases in the overall pain intensity (allodynia). This increase was short-lived and locked to the dynamic phase of cooling with pain levels returning to baseline during sustained cooling. Dynamic warming (32°C→39°C) had no effect on pain levels. Cooling allodynia persisted following nerve compression and TRPV1 and TRPM8 desensitization, but was abolished by localized Cav3.2 blockade. In clinical subjects, C-fibre-mediated allodynia was observed without the need for experimental pain-producing manipulations. Conclusion: Cooling allodynia represents a non-TRPV1- and non-TRPM8-dependent phenomenon, which is mediated by low-threshold Cav3.2-expressing C fibres. Further investigations are underway to examine the effect of Cav3.2 suppression on tactile allodynia in experimental and clinical pain conditions.

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Poster

239. Musculoskeletal Pain

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Topic: D.08. Pain

Support: NIH Grant RO1AR060364

NIH Grant RO1AR064251

Title: Spinal microglial activation in a murine surgical model of osteoarthritis is driven by joint damage

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Abstract: Osteoarthritis (OA), the most common form of arthritis, is a leading cause of chronic pain. Mechanisms of chronic pain associated with OA are poorly understood. We characterized a murine surgical model of OA, in which joint damage progresses slowly over 16 weeks after destabilization of the medial meniscus (DMM). In association with joint damage, mice develop progressive mechanical allodynia, which is maintained for 16 weeks. Behavioral changes indicative of chronic pain, along with accelerated joint damage, first appear 8 weeks after DMM surgery. The aim of this study was to characterize molecular and cellular changes that may be associated with the persistence of pain. We assessed fractalkine (CX3CL1) release by DRG neurons as well as microglial activation in the dorsal horn (DH) over 16 weeks following DMM. We also investigated whether joint protection could attenuate these changes by using *Adamts5* null mice, which are protected from developing OA and mechanical allodynia. Methods: DMM

or sham surgery was performed in the right knee of 10-week old male wild type or *Adamts5* null C57BL/6 mice. At 4, 8 and 16 weeks post surgery, L3-L5 DRG cells from DMM, sham or age-matched naïve control mice were harvested and cultured for 4 days; supernatants were collected for CX3CL1 ELISA. For immunohistochemistry, mice were perfused transcardially with paraformaldehyde, and the spinal column decalcified prior to cryosectioning. To assess microglial activation in the DH, L4 spinal sections were immunostained with Iba1 antibody and the number of activated microglia was quantified based on the morphology of Iba1 immunoreactive microglia, using established methods. Results: In wild-type mice, 4 weeks after DMM, DRG cultures released similar amounts of CX3CL1 as naïve controls. However, at 8 and 16 weeks post-DMM, DRG cells released more CX3CL1 than controls. There were also increased numbers of activated microglia in the DH at 8 and 16 weeks post-DMM compared to sham and age-matched naïve controls. In contrast, 8 weeks after DMM in *Adamts5* null mice, cultured DRG cells did not release increased levels of CX3CL1, and microgliosis in the DH was not detected. Conclusion: In the DMM surgical model of OA, microglial activation occurs in the DH during the chronic pain phase. The temporal correlation of CX3CL1 release with microglial activation suggests that CX3CL1 may contribute to microglial activation. The reduction in microglial activation in *Adamts5* null mice compared to wild-type mice suggests that protecting the joint and preventing peripheral sensitization can attenuate central sensitization.

Disclosures: P.B. Tran: None. **R.E. Miller:** None. **S. Ishihara:** None. **R.J. Miller:** None. **A. Malfait:** None.

Poster

239. Musculoskeletal Pain

Location: Hall A

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Program#/Poster#: 239.04/M35

Topic: D.08. Pain

Title: Antinociceptive effects of ONO-2952, a TSPO antagonist, on acidic saline-induced mechanical hyperalgesia in rats

Authors: *S. KATSUMATA[1,2], K. MITSUI[3], Y. EZAKI[4];
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Abstract: Acidic saline-induced hyperalgesia is recognized to be a model of fibromyalgia. We evaluated effects of ONO-2952, a TSPO antagonist, on mechanical hyperalgesia in this model and TSPO occupancy in several brain regions to clarify relationship between the effective dose and occupancy rate. Male Crl: CD (SD) rats (5 weeks old) were used. Hundred μL of saline at pH 4.0 was injected twice, 5 days apart, into left gastrocnemius muscle of rats. Saline was

injected for control group. Vehicle (0.5% methylcellulose, b.i.d.), ONO-2952 (0.1, 1 and 10 mg/kg, b.i.d.), duloxetine (30 mg/kg, q.d.) or pregabalin (20 mg/kg, b.i.d.) were orally administered for 6 days from 7 days after second injection (Day1) to Day6. On Day 7, each solution was administered once in the morning. Vehicle was administered to control group. Withdrawal thresholds of right and left paw were determined in Randall-Selitto test at just before and 2 hours after first administration on Day1 and Day7. After evaluation, hippocampus, midbrain and pons and medulla were dissected. Homogenates were prepared to determine specific binding of ^{3}H-PBR28 for the calculation of TSPO occupancy in each region. On Day1, paw withdrawal thresholds (PWTs) of both paws in control group were significantly lower than those of normal group. PWTs in ONO-2952 or duloxetine group were not different from those in control group. PWTs after administration in pregabalin group were significantly higher than those of control group. On Day7, PWTs of both paws in control group were significantly lower than those of normal group. PWTs in ONO-2952 and duloxetine group both before and after administration were significantly higher than those of control group. Left PWT before administration and left and right PWTs after administration were significantly higher than those of control group. ONO-2952 occupied TSPO in 3 regions with about 30% at 0.1 mg/kg, 80% at 1 mg/kg and 95% at 10 mg/kg. There was no significant difference in specific binding of ^{3}H-PBR28 in each region. Thus, ONO-2952 inhibited acidic saline-induced mechanical hyperalgesia with repeated oral administration at the doses of 0.1, 1 and 10 mg/kg in rats. The effects were exerted with more than 30% occupancy of TSPO in the brain and reached maximum at 80% occupancy. These results suggest that ONO-2952 would be an efficacious treatment for fibromyalgia.

Disclosures: **S. Katsumata:** A. Employment/Salary (full or part-time):; ONO Pharmaceutical CO., LTD. **K. Mitsui:** A. Employment/Salary (full or part-time):; ONO Pharmaceutical CO., LTD. **Y. Ezaki:** A. Employment/Salary (full or part-time):; ONO Pharmaceutical CO., LTD..

Poster

239. Musculoskeletal Pain

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 239.05/M36

Topic: D.08. Pain

Title: Systemic administration of the bioflavonoid rutin reduces fibromyalgia-like pain in hypoestrogenic rats

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Abstract: Fibromyalgia (FM) is a musculoskeletal syndrome characterized by chronic widespread pain, tenderness to palpation and various concomitant symptoms, including affective disorders such as depression. In addition, several surveys have reported that approximately 80 to 90 % of FM patients are women and has been reported that hormonal abnormalities due to an early age-of-onset menopause might play an important role in the altered processing of somatosensory information. Drugs with confirmed efficacy for the treatment of FM produce adverse effects that promote treatment discontinuation. Scientific studies have corroborated analgesic and anti-inflammatory effects of flavonoids. Rutin (quercetin-3-O-rutinoside) is a glycoside of the well known aglycon quercetin. In this study we analyzed the antihyperalgesic and antiallodynic effect of rutin by using the reserpine-induced myalgia model of FM. Eight groups (n=8/group) of female Wistar Rats (200-250 g) were ovariectomized 14 days before reserpine (1 mg/kg, s.c. during 3 consecutive days) injection. An extra healthy control group was added. Rutin was administrated at increasing doses (30-1000 mg/kg, i.p.). Pramipexole (1 mg/kg s.c.) was used as the reference drug. All drugs were evaluated on day 5 after the last reserpine injection. The muscle pressure, tactile response and cold allodynia thresholds were measured before drugs' administration and 30, 60,120, 150, 180, 210 and 240 min after treatments. Analysis by two-way repeated-measures ANOVA revealed that subcutaneous injection of reserpine produce significant diminution in the muscle pressure and tactile response thresholds in 48 % and 70 %, respectively. Whereas, in the cold allodynia test, injection of reserpine increased 8 times the time response in acetone spray test. Rutin, in a dose-dependent manner, reverted the effects of reserpine on muscle pressure (ED30=288 mg/kg), tactile response (ED30=894 mg/kg) and cold allodynia (ED30=929 mg/kg), reaching its maximum effect after one hour. The antihyperalgesic and antiallodynic effects of rutin at 562 mg/kg were similar to that observed with pramipexole. In conclusion, these results give evidence that systemic rutin administration produces analgesic effects in an experimental model of FM supporting the potential of rutin for the FM therapy.

Disclosures: **A. Hernández León:** None. **A. Fernández-guasti:** None. **M. González-trujano:** None.

Poster

239. Musculoskeletal Pain

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 239.06/M37

Topic: D.08. Pain

Support: FAPESP

CNPq

FAEPA

Title: Long term muscle involvement after neonatal pain

Authors: A. B. SIMÕES[1], J. A. ALVES[1], F. J. DIAS[1], E. C. CARMO[2], N. L. B. MACHADO[2], L. S. SANADA[2], *V. S. FAZAN[3];
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Abstract: Nociceptive stimuli applied soon after birth play an important role in the development of pain in adult life. Previous studies in our laboratory have shown that pain in the neonatal period can cause a number of changes both in the central nervous system and the musculoskeletal [1], but experimental substrates for these changes still need investigation. We designed an experimental study to explore whether pain in the neonatal period can cause long term changes in the locomotor system. The pain groups (male and female Wistar rats, N = 10 per group) were stimulated with a needle on the right paw, twice a day, since birth, for 15 consecutive days. The control groups (male and female Wistar rats, N = 10 per group) were stimulated with a cotton swab. Final experiments were performed 180 days after birth. In order to evaluate the motor function of the hind paws, grip strength tests were conducted. All tests were performed by one single observer that was blind to animals' experimental groups (pain or control). Maximum and average (mean of three consecutive measures) grip strength was studied. Lateral (LG) and medial gastrocnemius (MG) muscles, obtained by surgical biopsy, were frozen and stained with the techniques for nictotinamide adenine dinucleotide (NADH) and mitochondrial succinate dehydrogenase (SDH). For muscle morphometry, the number of fibers was counted and their area and diameter were measured. Specific statistic tests were applied and differences were considered significant when $p<0.05$. The grip strength was higher in males compared to females in both, control and pain groups, but smaller on pain groups, in both genders, compared to controls. Preliminary histological results showed, for NADH staining, larger area of the dark fibers on the right LG compared to the right MG, without difference between right LG and left LG. Also, no differences between right MG and left MG were observed. For SDH staining, no significant differences were observed for any type of fibers analyzed (dark, intermediate and light). Our results are suggestive that pain in the neonatal period caused a reduced grip ability that was maintained long term after the painful stimulus was ceased, with a slightly better performance on older females. Increasing the number of animals for the histological studies will help the identification of the type of muscle fibers that are being related to the grip ability alterations observed in this model of pain. [1] Sanada et al., Int J Dev Neurosci. 35:55-63, 2014.

Disclosures: A.B. Simões: None. **J.A. Alves:** None. **F.J. Dias:** None. **E.C. Carmo:** None. **N.L.B. Machado:** None. **L.S. Sanada:** None. **V.S. Fazan:** None.

Poster

239. Musculoskeletal Pain

Location: Hall A

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Program#/Poster#: 239.07/M38

Topic: D.08. Pain

Support: NIH K23 AR060241

Title: Reliability of pressure pain threshold and tolerance determined by an automated pressure system in knee osteoarthritis patients

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Abstract: Introduction: Quantitative sensory testing (QST) is commonly used in pain research however its long-term reliability in chronic pain patients over the course of months and years is not well established. This lack of reliability standards limits the application of QST in longitudinal studies of chronic pain. Here we present six-month QST reliability in patients with knee osteoarthritis (KOA). Methods: QST was performed in KOA patients (n = 23) using a computer-automated pressure delivery system. Discrete pressure stimuli were delivered to the dominant thumbnail following a standardized ascending method of limits protocol. Pressure pain threshold (PPT) and tolerance (PTT) was obtained at three observation points: baseline (T1), 12 weeks (T2) and 24 weeks (T3). Subjects underwent an 8-week intervention with an FDA approved oral or topical KOA medication, with a 4-week washout prior to each QST session. Intraclass Correlation Coefficients (ICC) were calculated using a mixed effects model and type consistency. Correlation between observations were evaluated using Pearson's product-moment correlation coefficient (r). One-way analysis of variance (ANOVA) for repeated measures assessed for differences between observations. Results: Mean PPT was 2.63 ± SE 0.23 kg/cm2, 2.33 ± 0.27 and 2.20 ± 0.21 at T1, T2 and T3, respectively. ANOVA revealed no significant differences in mean PPT between observations ($DF = 2$, $F = 2.97$, $p = 0.062$). The intraclass correlation coefficient (ICC) for all three observations was 0.79. Inter-observation correlation coefficients for T1/T2, T2/T3 and T1/T3 were $r = 0.58$, 0.61 and 0.52 (all $p < 0.01$), respectively. Mean PTT values were 5.85 ± SE 0.35 kg/cm2, 5.70 ± 0.30 and 5.59 ± 0.29 at T1, T2 and T3, respectively. ANOVA revealed no significant differences in mean PTT between observations ($DF = 2$, $F = 0.494$, $p = 0.614$). The ICC for all observations was 0.85. Inter-observation correlation coefficients for T1/T2, T2/T3 and T1/T3 were $r = 0.65$, 0.77 and 0.58 (all $p < 0.01$), respectively. Anxiety scores were not significantly correlated to PPT or PTT at any observation period (all $p > 0.12$). Conclusion: This study showed that QST using an automated stimulus delivery method demonstrated high test-retest reliability across three observation points, conducted over a period of six months in KOA patients. Inter-observation correlations were moderate to strong, while intraclass correlation was substantial in both PPT and PTT. These results suggest that automated QST provides potential value in longitudinal assessment of pain sensitivity in chronic pain patients.

Disclosures: J.R. Scott: None. **K. Phillips:** None. **D.J. Clauw:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified

mutual funds); Co-inventor of the pain testing device used in this study. F. Consulting Fees (e.g., advisory boards); Merck and Company Inc. **S.E. Harte:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-inventor of the pain testing device used in this study.

Poster

239. Musculoskeletal Pain

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 239.08/M39

Topic: D.08. Pain

Support: Fapesp Grant 2012/13910-2

Title: Effects of interferential current on pain relief in people with chronic nonspecific low back pain: a randomized placebo-controlled trial

Authors: *R. LIEBANO[1], J. B. CORRÊA[2], N. T. B. OLIVEIRA[2], L. O. P. COSTA[1], K. A. SLUKA[3];
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Abstract: Background and aims: Low back pain is an important public health problem. Interferential current (IFC) is commonly used for pain relief, but the effects of carrier frequency of the current and its action on pain relief remain unclear. The aim of this study was to evaluate pain over time and after 12 sessions treatment. Methods: A three-arm randomised controlled trial with patient and assessor blinded to the group allocation: IFC 1 kHz (n= 50), IFC 4 kHz (n= 50) and placebo (n= 50). The interferential current was applied three days per week over four weeks. Results: After treatment, there was no statistically significant difference on pain among the groups 1 kHz x placebo -0.9 (mean difference) 95% CI -2.0 to 0.2, 4 kHz x placebo -0.8 (mean difference) 95% CI -0.3 to 1.9, 1 kHz x 4 kHz 0.01 (mean difference) 95% CI -1.1 to 01.0. The estimated number of sessions needed for a 50% decrease in the pain relief score was 3.82 (95% CI: 2.84 to 4.79) in the 1 kHz IFC group, 5.01 (CI: 3.77 to 6.25) in the 4 kHz IFC group, and 6.09 (CI: 4.82 to 7.37) in the placebo IFC group. There was statistically significant differences only in 1 kHz IFC group compared to IFC placebo group ($p = 0.03$). Conclusions: There was no difference between active IFC and placebo IFC in resting pain intensity after treatment. However, when compared to placebo IFC, subjects receiving 1 kHz IFC took less time to achieve a 50% reduction in pain intensity.

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Poster

239. Musculoskeletal Pain

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 239.09/M40

Topic: D.08. Pain

Support: the Ministry of Health, Labour and Welfare Sciences Research Grant

Grant-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Science

Nakatomi Foundation

Title: A model of chronic pain from human prostate cancer cells implanted into the mouse tibia

Authors: *M. ENOMOTO, H. KABURAGI, T. HIRAI, Y. WAKABAYASHI, K. YAGISHITA, T. YOKOTA, A. OKAWA;
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Abstract: Metastatic bone tumors are often found in patients with prostate or breast cancer. Recently developed treatments such as bisphosphonates increase patient survival, but it will also be necessary to control cancer pain to improve quality of life. Experimental rat models of bone metastasis have been developed by injection of cancer cells into long bones (Blouin et al., Clin. Exp. Metastasis 2005). The current study developed a metastatic bone tumor model by implantation of human prostate cancer cells into the mouse tibia. After implantation, tumor progression was followed using microcomputed tomography (microCT). Mice were observed weekly for 4 weeks after implantation for symptoms of chronic pain, changes in sensitivity to cutaneous stimulation. PC3 human prostate carcinoma cells expressing GFP (PC3-GFP cells) were cultured. After one passage, the cells were concentrated and 5 µl of cell suspension ($1X10^5$ or $2X10^5$ cells/µl) was implanted into the tibia of BALB/cSlc-nu/nu mice. Cutaneous hypersensitivity to both tactile and heat stimulation was observed beginning 3 weeks after implantation. microCT demonstrated the presence of osteolytic lesions around the implant site. A large lesion was observed in the tibia with $2X10^5$ cells/µl. After final observations, frozen sections of tibias and L4 dorsal root ganglia (DRG) were obtained and H&E staining and immunohistochemistry were performed. Immunohistochemical evaluation revealed neurofilament positive fibers around the GFP-positive area. Small DRG neurons from the ipsilateral hind leg expressed increased TRPV1 compared to the contralateral control leg. The findings indicate a chronic pain-like state is induced following implantation of human prostate carcinoma cells. Thus, the current mouse model could be useful to elaborate the molecular mechanism of cancer pain due to metastatic bone tumors.

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Poster

239. Musculoskeletal Pain

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Topic: D.08. Pain

Support: CIHR grant MOP102586

Louise and Alan Edwards Foundation grant

Title: Chronic low back pain is mediated by increased NGF in intervertebral discs: Therapeutic effect of active lifestyle and pharmacological interventions in mice

Authors: *M. MILLECAMPS[1], M. SUZUKI[2], Y. SUN[1], A. DANCO[1], A. P. MATHIEU[3], A.-J. CHABOT-DORE[1], B. XUE[1], J. KIM[1], L. S. STONE[1];
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Abstract: Chronic low back pain (LBP) affects up to a third of the global population and is the leading cause of disability world-wide. Intervertebral disc (IVD) degeneration is a major factor contributing to chronic LBP. However, the causal relationships between disc degeneration (DD), axial LBP and radiating leg pain are still under debate. Nerve Growth Factor (NGF) is elevated in painful degenerating human IVDs, where it is thought to promote nociceptive nerve ingrowth and peripheral nerve sensitization. We recently demonstrated pathological increases in sensory IVD innervation in our clinically-relevant SPARC-null mouse model of progressive age-dependent DD-related LBP. The Aims of the current investigation were to a) determine if NGF is upregulated in degenerating SPARC-null mouse IVDs; b) assess how an active lifestyle that decreases LBP symptoms, affects NGF levels; and c) investigate the therapeutic benefit of anti-NGF therapy on behavioral indices of LBP in this model. Methods. Animals were 3-20 month old, male SPARC-null and age-match C57Bl6 Wild Type (WT) mice. Behavior included measures of radiating leg pain (cold allodynia in the acetone test) and axial LBP (grip test and tail suspension assays). DD was assessed using X-ray and T2-MRI imaging. NGF expression was examined in protein extracts from lumbar IVDs using commercially available ELISA kits. The effect of increased physical activity on LBP behavior and disc health was investigated by providing unlimited access to running wheels in home cages and the effect of anti-NGF therapy on LBP behavior was investigated by systemic treatment with an anti-NGF antibody. Results. NGF expression was significantly elevated in IVDs from old SPARC-null mice. At baseline, SPARC-null mice presented behavioral signs of both radiating and axial discomfort. Daily access to running wheels significantly decreased radiating cold allodynia, disc degeneration severity, and NGF content. NGF therapy resulted in a complete reversal of cold allodynia. Conclusions. SPARC-null mice show signs of axial LBP and radiating leg pain associated with intervertebral disc degeneration. NGF is over-expressed in aging degenerating IVDs from

SPARC-null mice. Both increased physical activity and anti-NGF therapy were efficacious against behavioral indices of LBP. These data suggest that NGF over-expression in degenerating IVDs contributes to DD-related LBP, potentially as a result of increased innervation and/or sensitize spinal nerve roots. Furthermore, this study suggests that targeting NGF either through lifestyle changes or pharmacological intervention may be an effective treatment for DD-associated chronic LBP.

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Poster

240. Somatosensory Functional Organization

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 240.01/M42

Topic: D.09. Tactile/Somatosensory Systems

Support: HHMI

NIH grant R01 DE022750

Title: Molecular-genetic tools to study spinal cord anterolateral tract neurons and their projections to the brain

Authors: *S. CHOI, D. D. GINTY;
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Abstract: The perception of pain is critical for animal welfare. We protect ourselves from potentially harmful environments by sensing and avoiding noxious stimuli. Noxious stimuli, including hot/cold temperatures, intense mechanical pressure and chemical irritants, activate the peripheral endings of nociceptive sensory neurons. These, in turn, form synapses upon second-order neurons, including interneurons and projection neurons of the spinal cord. Most pain and temperature information is transmitted from the spinal cord to higher order brain regions through anterolateral tract (ALT) projection neurons whose axons decussate and ascend in the ventrolateral white matter of the spinal cord. A great deal is known about how noxious stimuli are detected by primary sensory neurons in the periphery. However, how pain and temperature information is processed in the spinal cord is less well understood in part because of a lack of tools to interrogate second-order neurons of pain pathways, including ALT neurons. We have developed several mouse molecular genetic tools to visualize and functionally manipulate ALT neurons. First, we generated a knock-in mouse line in which tamoxifen-inducible Cre recombinase (CreERT2) is inserted into the *Robo3* locus, because Robo3 is transiently expressed in commissural neurons of the developing spinal cord, including ALT neurons. Second, we generated a knock-in mouse line in which CreERT2 is inserted into the *NK1R* locus, because a

subset of NK1R-positive neurons is labeled by retrograde tracers injected into target brain regions. Third, we screened transgenic GFP mouse lines from the GENSAT project, and identified lines that exhibit GFP expression in the superficial lamina (Lamina I) of the spinal cord dorsal horn, where a large fraction of ALT neurons reside. These knock-in and BAC-GFP transgenic mouse lines are currently being used to assess morphological and physiological properties of ALT neurons, and to elucidate the roles of these neurons in conveying discriminative and affective somatosensation from the periphery to the brain. This study will advance our understanding of the neural circuits underlying pain perception; particularly how nociceptive information is processed in the spinal cord and propagated to the brain.

Disclosures: S. Choi: None. **D.D. Ginty:** None.

Poster

240. Somatosensory Functional Organization

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 240.02/M43

Topic: D.09. Tactile/Somatosensory Systems

Title: Joint perception task activates motor-related areas in healthy subjects: a functional magnetic resonance imaging study

Authors: *H. HIROYUKI[1], Y. HIRANO[2], C. SUTOH[4], D. MATSUZAWA[3], R. NAGAI[5], E. SHIMIZU[2];
[1]Chiba Univ., Fujiwara, Funabshi-shi, Japan; [2]Res. Ctr. for Child Mental Develop., [3]Chiba Univ., Chiba-city, Chiba, Japan; [4]Chiba Univirsity, Chiba-city, Chiba, Japan; [5]Ichikawa-city Rehabil. Hosp., Ichikawa-city, Chiba, Japan

Abstract: Motor imagery has been shown to activate the brain similarly with active actual movement, and its application is considered to be effective in the rehabilitation for stroke patients to improving their motor function. However, some stroke patients feel quite difficult in imaging the movement of their hands and legs, thus easy tasks are demanded. A previous report revealed that the motor-related brain areas were activated when subjects imagined a joint perception. Although perception task may be application to treatment, the neural mechanism is not clear. Our study investigated the brain activation by comparison of perception task with active and passive movement task using functional magnetic resonance imaging (fMRI, 1.5T, Toshiba). Seven healthy volunteers were recruited. The task consisted of four parts, (i) active movement task (AMT), (ii) passive movement task (PMT), (iii) joint position sense task (passive movement and perceptive question, JPST), and (iv) control (CON). Subjects' right hand was used in the AMT, PMT and JPST. In the AMT, subjects moved their wrist with extension and flexion. In the PMT, an examiner moved subject's wrist comparable with the AMT in frequency and the range of motion. In the JPST, the examiner moved their wrist randomly ranging five

steps of height, and the subjects were asked to answer the position of their wrist in their mind (position number from 0 to 4). No movement was demanded in the CON. Subjects were scanned once a week for performing four tasks. The MRI data were analyzed using MATLAB and SPM8. JPST was shown activation in premotor area and supplementary area. The JPST was similar activation with AMT. No activation was observed in the CON in any motor-related areas. Considering the observed activation in the JPST, it is suggested that the prediction of the joint movement can activate the related motor brain areas comparable with the motor imagery. In conclusion, JPST can be effective in the rehabilitation of stroke patients who cannot move the affected limb.

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Poster

240. Somatosensory Functional Organization

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Topic: D.09. Tactile/Somatosensory Systems

Support: NRF 2011-0027921

Title: Neural activation induced by the perception of sticky tactile stimuli

Authors: *J. YEON[1], J. KIM[2], J. RYU[3], J.-Y. PARK[3], S.-P. KIM[1];
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Abstract: Tactile perception is generally divided into three dimensions from the view of psychophysics: rough/smooth, hard/soft, and sticky/slippery. Among them, sticky perception is the most controversial one; not only specific features that arouse sticky feelings are not well understood, but also, to our best knowledge, the sticky/slippery dimension has not yet been investigated in focus. Since the sticky perception has rarely been explored up to date renders itself as one of the fundamental dimensions in tactile perception, we set our study goal to finding the spatial neural activation patterns during processing of sticky senses. 12 participants (5 females; Mean age = 24.58) participated in the study. 8 different sticky silicone stimuli made with different concentration levels of silicone hardener (5, 6, 7, 8, 9, 10, 20 and 30% consistency) were prepared. Lower levels of hardness provided stickier silicone stimuli. A sham stimulus was prepared using an acrylic panel, providing no sticky sense. Before scanning, a behavioral test was done with all sticky silicone stimuli. Participants touched each stimulus with the right index finger and determined whether it was sticky or not. During scanning, 6 sticky

stimuli (5, 6, 7, 8, 10, 30% consistency) and sham stimulus were provided. Participants put their right index finger on a given stimulus when heard a "Ready" sound and detached the finger with a beep sound. The behavioral test results revealed that the stimuli with 5, 6, and 7% consistency were perceived to be 'sticky', whereas other 5 stimuli were 'non-sticky'. In the fMRI data analysis, basal ganglia including corpus callosum, bilateral caudate tail and caudate body as well as right dorsolateral prefrontal (DLPFC) areas were activated ($p<0.005$, unc.) for 'Sticky (5, 6, 7%) - Sham' contrast. In comparison, for 'Non-sticky (8, 10, 30%) - Sham' contrast, only basal ganglia area including right caudate tail and left caudate body was activated ($p<0.005$, unc.). The activation of the right DLPFC may imply that the sticky stimuli were treated as aversive ones as a sticky stimulus could be believed to signal that the skin tissue was stretched and thus induce a feeling of potential damage, a lacerated wound. The right DLPFC is also known to be activated in rewarding situations and might reflect rewards associated a pain relief during sticky sense processing, which could occur when participants detached their finger from a sticky stimulus and felt as escaping from the aversive stimulus. Our results may suggest a specific characteristic of neural processing underlying the perception of sticky tactile stimuli.

Disclosures: **J. Yeon:** None. **J. Kim:** None. **J. Ryu:** None. **J. Park:** None. **S. Kim:** None.

Poster

240. Somatosensory Functional Organization

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 240.04/M45

Topic: D.09. Tactile/Somatosensory Systems

Title: Writing lies and feelings of alleviation caused by hand soap: Neural underpinnings of the Macbeth effect

Authors: C. DENKE[1], M. SCHAEFER[2], H.-J. HEINZE[2], *M. SCHAEFER[1];
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[2]Dept. of Neurology, Otto-von-Guericke Univ. Magdeburg, Magdeburg, Germany

Abstract: The Macbeth effect describes a link between physical cleansing and moral transgressions. Behavioral experiments showed that guilt induced by transgressions can be reduced by cleaning the body. This Macbeth effect has been explained by an embodiment of moral cognitions. Here we aimed to explore the neural underpinnings of the Macbeth effect by means of an fMRI experiment. Thirty-five participants were prompted with short scenarios. Subsequently they were asked to put themselves into the shoes of the protagonist and behave in either a moral incorrect way (writing a lie on a clipboard) or acting in a moral correct way (writing a true message on a clipboard). Then the participants had to evaluate the desirableness of hand soap products and noncleansing goods. Behavioral result demonstrated that participants evaluated hand soap products (but not noncleansing goods) more desirable when writing a lie

before (in contrast to writing the truth), thus replicating the Macbeth effect ($t(34) = 2.03$, $p < 0.05$). FMRI results showed that this Macbeth effect was correlated with activation in somatosensory brain areas (in particular the primary somatosensory cortex) during the evaluation phase. We argue that these results strongly suggest that somatosensory brain regions play an important role for embodied moral cognitions.

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Poster

240. Somatosensory Functional Organization

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Topic: D.09. Tactile/Somatosensory Systems

Support: IBS-R015-D1

Title: Spatiotemporal neuronal and hemodynamic integration in sensorimotor cortex of mice upon external stimuli

Authors: *E. BAEG[1], J. SIM[1], S. BAE[2], H. RYU[3], M. SUH[1,2,3];
[1]CNIR, Inst. For Basic Sci. (IBS), Suwon, Korea, Republic of; [2]Biol. Science, Sungkyunkwan Univ., Suwon, Korea, Republic of; [3]Biomed. Engineering, Sungkyunkwan Univ., Suwon, Korea, Republic of

Abstract: We investigated the spatiotemporal neuronal dynamics of sensorimotor cortex to study macroscopic cortical facilitation and suppression that is modulated by external stimuli. The coupling between neuronal activities and hemodynamic change was addressed to understand the degree to which vascular response is coupled to neuronal activation. Voltage sensitive dye (VSD) was applied over large regions of the mouse cortex to directly image the neuronal membrane potentials in sensorimotor cortex. Additionally, single units and local field potentials were recorded in order to observe the transition from depolarizing membrane potential to resulting activities of neuron. Cortical blood volume change was accessed using intrinsic optical signal (IOS). One or two adjacent whiskers of slightly anesthetized or awake mouse were stimulated simultaneously or serially at various inter-stimulus intervals. A single brief whisker deflection induced highly distributed depolarizing cortical sensory responses in VSD signal, which started in the barrel cortex and subsequently spread out (<8ms) to the whisker motor cortex. Facilitated depolarizing responses were observed to the stimulation of two adjacent whiskers when deflected simultaneously. Short delay (<5ms), given between deflections of the two whiskers, however, made slower and weaker depolarizing response, representing suppressive process. Single units in barrel cortex also showed facilitated responses when the two whiskers deflected simultaneously. However, obvious increase of blood volume was not

observed in motor cortex, although apparent dynamic volume changes in barrel cortex according to applied deflections. Cortical network or function is likely to be the result of the relative strength of cortical conductance and of the temporal relationship in space and time, with the support of non-linearly acting hemodynamic responses.

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Poster

240. Somatosensory Functional Organization

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Topic: D.09. Tactile/Somatosensory Systems

Support: NINDS P01NS057228

Title: Muscle proprioceptors and their central connections in the adult rodent

Authors: H. M. GABRIEL, J. A. VINCENT, *P. NARDELLI, A. S. DEARDORFF, R. E. W. FYFFE, T. C. COPE;
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Abstract: Despite the increased use of rodents to study the development and function of spinal sensorimotor circuits, data on the central trajectory and connectivity of physiologically identified muscle proprioceptors is limited in the adult rodent. A complete account of the peripheral encoding along with the central connectivity of rodent muscle proprioceptors is critical to understanding the manner in which proprioceptive feedback influences rodent spinal circuits. In the present study, intraxonal records were obtained of tricep surae proprioceptive afferents *in vivo* from adult female wistar rats. Following the collection of intra-axonal data, 12 of these afferents (4 Ia, 4 group II, and 4 Ib) were labeled with Neurobiotin for detailed anatomical analysis of their central trajectory and terminal distribution. Afferent responses during isometric twitch contractions were used to distinguish muscle spindles (1a/group II) from tendon organs (Ib). Befitting with their role as dynamic sensors, Ia afferents achieved much higher peak firing rates, and displayed larger dynamic index (DI) values in response to varying stretch amplitudes and velocities. Group II afferents achieved much lower peak firing rates, lower DI values, and unlike Ia afferents frequently fired on the release from stretch. These firing properties are consistent with those obtained in the cat (Matthews 1972), and more recently in the rat (De-Donker et al., 2003, Bullinger et al., 2011). Interestingly a large proportion of our Ib afferents also generated robust firing in response to muscle stretch in the physiological range, but displayed lower peak firing rates. Overall, each afferent type showed a characteristic central morphology and trajectory that was consistent with observations in the cat. Terminal arborizations for 1a and group II afferents were limited to lamina V//VI, lamina VII, and Lamina

IX. While 1b afferent collaterals were limited to lamina VI and LVII. The highest level of variability between afferent types was the characteristic density and distribution of synaptic contacts within each target lamina. These distinguished areas of termination of muscle afferent fibers in the rat revealed some variance with the terminal organization of synaptic contacts found in the cat. These data, for the first time in the adult rodent, demonstrate the central trajectory, terminal distributions, and peripheral encoding of muscle proprioceptors, and provide valuable insight into their organization and functional contribution to spinal sensorimotor circuits. Additionally, these results suggest that the findings, so far only available in the cat, are generalizable to other mammalian species. .

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Poster

240. Somatosensory Functional Organization

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 240.07/M48

Topic: D.09. Tactile/Somatosensory Systems

Title: Neural encoding of saltatory tactile velocity in human glabrous hand using fMRI

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Abstract: BACKGROUND: Among the functions of the somatosensory system, processing information about the location and velocity of tactile stimuli on the body surface is presumed essential for the development and maintenance of fine motor control of the hand. However, limited data exist on the cortical representation of moving tactile stimuli on the glabrous skin of the digits in humans. OBJECTIVE: The aim of this study was to map the relation between saltatory pneumotactile stimulation at 3 velocities on the glabrous hand and cerebral BOLD activations in neurotypical adults. METHODS: Participants - 15 neurotypical, right-handed adults, age 19-30 years. A Galileo somatosensory tactile array was programmed to deliver punctate (50 ms duration, 9 ms rise/fall) pneumotactile sequences through acetyl TAC-Cells (6 mm ID) placed on the glabrous skin of the right hand, including p1, p2 segments of D3 (middle finger); p1, p2, p4 segments of D2 (index finger), and p4, p1 of D1 (thumb). Programmed time delays between individual TAC-Cells resulted in a saltatory velocity sequence traversing the tips of D2, D3 through the basal phalyngeal segments to the distal phalaynx of the thumb. A randomized-balanced block design (40 s) included the following 5 conditions: Saltatory velocities @ 25, 45, and 105 cm/sec, simultaneous TAC-Cells ON, and all cells OFF. An MPRAGE sequence (1 mm isotropic, TE=30ms, TR=2400ms) was followed by 3 functional

scans using a 3T Siemens Skyra (32-ch head coil). Functional images: T2*-weighted EPI sequence, 36 slices (1.7x1.7x.2.0mm, TE=24ms, TR=2500ms, FOV=220mm). Using SPM12, 990 acquired brain volumes/subject were realigned, and smoothed with an isotropic Gaussian kernel (FWHM=6 mm). RESULTS: Significant BOLD responses were localized to the contralateral hemisphere, including digit (D1, D2, D3) representations within BA 3b of S1 and a second BOLD ROI in the left supramarginal gyrus. The spatial extent of the evoked BOLD response is highly dependent on saltatory tactile velocity with the largest response apparent at 25cm/s. Resultant peak-level BOLD response statistics for S1: 25 cm/s [MNI (mm)= -34, -27, 72; T=8.09, p<.001], 45 cm/s [MNI (mm)= -36, -27, 72; T=6.89, p<.001], and 105 cm/s [MNI (mm)= -36, -27, 72; T=6.79, p<.001]. Peak-level BOLD response statistics for supramarginal gyrus: 25 cm/s [MNI (mm)= -56, -22, 20; T=6.66, p<.001], and 45 cm/s [MNI (mm)= -63, -22, 20; T=5.42, p<.001]. CONCLUSIONS: fMRI BOLD results suggest the presence of a scalable cortical network (localized to contralateral BA 3b and supramarginal gyrus), which encodes saltatory pneumotactile velocity on the glabrous hand in humans. Supported by: Barkley Trust Foundation (Barlow)

Disclosures: H. Oh: None. **R. Custead:** None. **S.M. Barlow:** None.

Poster

240. Somatosensory Functional Organization

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Topic: D.09. Tactile/Somatosensory Systems

Support: Harvard-MIT Joint Research Grants Program in Basic Neuroscience

NSF pre-doctoral fellowship

F31 NIH training grant

Title: Vagal sensory neuron subtypes that differentially control breathing

Authors: *D. E. STROCHLIC[1], R. B. CHANG[2], E. K. WILLIAMS[2], B. D. UMANS[2], S. D. LIBERLES[2];
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Abstract: Breathing is essential for survival, and under precise neural control. The vagus nerve is a major conduit between lung and brain required for normal respiration. Here, we identify two populations of mouse vagus nerve afferents (P2ry1, Npy2r), each a few hundred neurons, that exert powerful and opposing effects on breathing. Genetically guided anatomical mapping revealed that these neurons densely innervate the lung and send long-range projections to different brainstem targets. Npy2r neurons are largely slow-conducting C fibers, while P2ry1

neurons are largely fast-conducting A fibers that contact pulmonary endocrine cells (neuroepithelial bodies). Optogenetic stimulation of P2ry1 neurons acutely silences respiration, trapping animals in exhalation, while stimulating Npy2r neurons causes rapid, shallow breathing. Activating P2ry1 neurons did not impact heart rate or gastric pressure, other autonomic functions under vagal control. Thus, the vagus nerve contains intermingled sensory neurons constituting genetically definable labeled lines with different anatomical connections and physiological roles.

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Poster

240. Somatosensory Functional Organization

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Topic: D.09. Tactile/Somatosensory Systems

Support: German Research Foundation DFG; CR 479/1-1

Swedish research council 2010-2607

Title: Hair follicle density and the perception of touch in humans

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Abstract: Introduction C-tactile afferents are unmyelinated mechanoreceptors in the human hairy skin that are involved in the processing of pleasant and erotic touch. They are optimally activated for a slow, light stroking of the skin. The C-tactile afferents are an analog to the C-fiber low-threshold mechanoreceptors (C-LTMRs) found in animals. Association between hair movement and C-LMTRs was suggested in early studies in rodents, cats and monkeys. Recent histological studies in rodents using genetic techniques to stain C-LTMRs have found that the nerve endings are located around hair follicles. The location of the C tactile nerve endings in human skin is unknown. We aimed at exploring the relationship between the number of hair follicles and perceived eroticism and pleasantness of a gentle brush stimulus in healthy human adults. Method Thirty-six healthy, adult participants took part in the study (23 female; age 26.22 ± 5.7 years). The participants received tactile stimulation with velocities optimized for C tactile activation (1, 3, 10 cm/s) and suboptimal for C tactile afferent activation (0.3 and 30 cm/s) on the

dorsal side of the forearm in a highly standardized fashion. For each stimulus, participants rated the perceived eroticism, pleasantness and intensity. Hair follicle count was performed on a 1cm2 area of the body part, using the cyanoacrylate skin stripping method. Additionally, all participants completed the Social Touch Questionnaire (STQ), as well as a questionnaire regarding partnership and sexual activity. Results There was a significant positive correlation between the number of hair follicles and the eroticism ratings for the C tactile optimal velocity of 3 cm/s (r_s=0.375, p=0.029). The sample was split into participants with higher and lower sexual activity. Participants with higher sexual activity had a significantly more hair follicles per cm2 than those with lower activity ($t(32)$=-2.308, p=0.028). Additionally, the high sexual activity group had higher scores on the STQ, indicating that this group is more positive to social touch. Conclusion These preliminary findings indicate a connection between C-tactile afferents and hairs in the human perception of erotic touch.

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Poster

240. Somatosensory Functional Organization

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Topic: D.09. Tactile/Somatosensory Systems

Support: Marie Curie Integration Grant FP7-PEOPLE-2012-CIG-334201 (REMAKE)

Italian Ministry of Foreign Affairs

Title: Neural correlates of ankle joint proprioception test: A preliminary fMRI study

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Abstract: Proprioceptive feedback is very important for movement planning and execution; however its role in both healthy subjects and patients with neurological diseases that affect sensorimotor functions is poorly understood. The aim of this work was to investigate the neural basis of proprioceptive input during ankle joint position sense matching tasks using functional MRI. In this ongoing study four healthy subjects were recruited to perform two matching tasks which required a combination of passive and active movements. Task 1 consisted in unilateral matching task, where subjects had to reply a determined position of the ankle joint with the

ipsilateral foot. Task 2 consisted in bilateral matching task where subjects had to reply a determined position of the ankle joint using the controlateral foot. The fMRI experiment was acquired with a block design paradigm (30 sec rest + 30 sec task) while an operator shifted the foot to different inclination values using a custom built MRI compatible device and the subjects matched as described above. In addition, the experiment included three active tasks involving plantar-dorsi flexion movements cued by a metronome set at 0.7 Hz: ipsilateral active task with left foot, right foot and bilateral active task with both feet moved in anti-phase. Feet were firmly strapped into the MRI compatible device that allowed one degree of freedom movement in the sagittal plane. The device was developed in order to minimize head movement during ankle plantar-flexion. The typical motor and sensorimotor network was recruited during the active tasks while areas of the parietal cortex were recruited during both matching tasks. Moreover, unilateral matching task showed activations not only in the controlateral emisphere but also in the ipsilateral one. These results suggest that, with proprioceptive target, also passive movements can elicit activations that are similar to the one produced by active movements. Finally, we found highly disseminated activations patterns for both matching tasks, indicating that subjects required an higher cognitive effort with respect to running simple motor tasks.

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Poster

240. Somatosensory Functional Organization

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Topic: D.09. Tactile/Somatosensory Systems

Support: NIH Grant GM48677

Title: Defining the populations of pruritogen-sensitive neurons in the mouse dorsal root ganglion

Authors: *S. S. ESPINO, R. TEICHERT, B. OLIVERA;
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Abstract: Itch is defined as a sensation in the skin that causes the reflex to scratch. The sensation of itch is detected by nerve endings in the skin whose cell bodies are located in the dorsal root ganglion (DRG) or in the trigeminal ganglion (TG). There are two types of itch namely: histamine dependent and histamine independent. Chloroquine, an anti-malarial drug, defines histamine independent itch. Pharmacologically, itch is a heterogeneous somatosensory modality. Can the same heterogeneity be said for populations of DRG neurons (pruriceptors) that mediate the sensation of itch? To address this question we identified and characterized pruriceptors in the

adult mouse DRG using calcium imaging of the dissociated neurons. Three major populations that are senstitive to the pruritogens histamine and chloroquine were identified in the dissociated DRG neurons of the adult mouse. These populations include cells that respond to histamine only, cells that respond to chloroquine only and another population that respond to both histamine and chloroquine. Combinations of ion channels expressed by these histamine and chloroquine-sensitive cells were also identified. To achieve this goal we used conotoxins that are specific antagonists of various ion channel subtypes. For example using conotoxins κM-RIIIJ and κJ-PlXIVA which selectively inhibit specific voltage-gated potassium channel subtype, we identified subpopulations within the population of histamine and chloroquine-sensitive cells. Using a combination of pharmacological agents and ion channel specific conotoxins, we were able to refine the definition of the populations of cells that are sensitive to histamine and chloroquine in the adult mouse DRG. This fine-tuned definition of the histamine and chloroquine-sensitive populations and the identification of the unique constellation of ion channels and receptors present in these cell populations will be useful in the refinement of our understanding of itch. Furthermore, the ion channels and receptors identified are potential therapeutic targets for the treatment of itch.

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Poster

240. Somatosensory Functional Organization

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Topic: D.09. Tactile/Somatosensory Systems

Support: R01NS055251

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Association of Migraine Disorders

Title: Functional comparison of Cav3.2-expressing and TRPV1-expressing sensory neuron subpopulations

Authors: *D. M. DUBREUIL[1], D. S. KIM[1,2], S. DENOME[1], D. LIPSCOMBE[1];
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Abstract: Diverse subpopulations of somatosensory neurons collaborate to detect and encode thermal, mechanical, and chemical stimuli. Genetic, functional, and anatomical classification schemes have been proposed to distinguish distinct subpopulations, but there have been few analyses of single subpopulations regarding all three criteria. In this work, we seek to understand

the functional contribution of $Ca_V3.2$-expressing and TRPV1-expressing sensory neurons to somatosensation and their synaptic input to the dorsal horn of the spinal cord. $Ca_V3.2$ low-voltage activated T-type calcium channels regulate neuronal excitability by lowering the action potential firing threshold and are thought to increase the sensitivity of low-threshold mechanosensory neurons. In contrast, TRPV1 cation channels are activated by noxious heat and are expressed in high-threshold, thermosensory nociceptors. Previous analyses of isolated dorsal root ganglia neurons have shown that TRPV1 and $Ca_V3.2$ channels are expressed in distinct subsets of sensory neurons, consistent with the hypothesis that they differentially contribute to somatosensation *in vivo*. Using a novel *Cacna1h*-Cre or existing *Trpv1*-Cre driver strain, we have expressed ChR2-EYFP or TdTomato reporter proteins in $Ca_V3.2$- or TRPV1-expressing neurons throughout development. Blue light stimulation of the hindpaws of awake *Cacna1h*/ChR2-EYFP and *Trpv1*/ChR2-EYFP adult mice is sufficient to drive a nocifensive paw withdrawal response, suggesting that $Ca_V3.2$- and TRPV1-expressing sensory neurons both carry nociceptive information; however, prolonged activation of either subpopulation, in the absence of inflammatory mediators, was insufficient to generate hypersensitivity to mechanical or thermal stimuli. Furthermore, by functional analyses, we show that TRPV1 and CaV3.2 are rarely co-expressed in sensory neurons. Fixed sections of isolated dorsal root ganglia from *Trpv1*/TdTomato mice revealed highly overlapping expression of TRPV1 and the proalgesic neuropeptide CGRP. In fixed spinal cord sections, TRPV1-expressing afferents specifically terminate in superficial layers of the dorsal horn (lamina I-II), whereas $Ca_V3.2$-expressing afferents terminate throughout the superficial dorsal horn (lamina I-IV). In order to understand the synaptic function of these subpopulations, we are using whole-cell electrophysiology in acute spinal cord sections. This combined approach of behavioral, functional, and imaging analyses will allow us to form a comprehensive definition regarding the anatomy and physiology of specific genetic subpopulations of sensory neurons in somatosensation and nociception.

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Poster

240. Somatosensory Functional Organization

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Topic: D.09. Tactile/Somatosensory Systems

Title: Peripheral sensory perception of diabetics using Semmes Westein 5.07 monofilament

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Abstract: Gradual loss of sensation in the foot is a major consequence of diabetes mellitus. It is a precursor to foot ulcers, which may necessitate amputation eventually. Early detection of peripheral sensory loss in the foot would help to prevent further complications. In developed countries the ability to perceive 5.07 monofilament is accepted as normal, but in Nigeria the assessment of peripheral sensory perception with 5.07 monofilament method is not common, and its relevance not appreciated. The aim of this study was to compare peripheral sensory perception in patients with diabetes mellitus and those without, using Semmes Westein 5.07 monofilament. The experimental group included 100 patients diagnosed with diabetes mellitus, while the control group comprised 100 subjects without diabetes mellitus, recruited using purposive non-probability sampling technique, with informed consent and ethical approval. Skin sensation was tested on eleven pressure points on the sole and the dorsum of the right foot using 5.07 Semmes Weinstein monofilament in all the subjects. Data were analysed using independent student t-test, and one way ANOVA. Results showed that 71% of subjects with diabetes had intact sensation while 29% had impaired sensation. In control subjects, 96% had intact sensation while 4% had impaired sensation. Subjects with diabetes had significantly lower peripheral sensory perception than the control subjects ($p>0.05$). Age and gender had no influence on subjects' peripheral sensory perception ($p>0.05$). It was concluded that peripheral sensory perception was significantly lower in subjects with diabetes than in apparently healthy control subjects. The use of Semmes Westein 5.07 monofilament is hereby recommended for preliminary diagnosis of peripheral neuropathy in subjects with diabetes mellitus.

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Poster

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VIEP PRODEP BUAP (EM)

Catedra Marcos Moshinsky (EM)

Title: Stochastic resonance in the brain elicited by optogenetic noise-photostimulation

Authors: *E. MANJARREZ[1], P. LINARES[1], N. HUIDOBRO[1], A. MENDEZ-FERNANDEZ[1], B. DE LA TORRE VALDOVINOS[1], I. MENDEZ-BALBUENA[2], A. FLORES[1], R. GUTIERREZ[3];

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Abstract: Stochastic resonance is a phenomenon in nonlinear systems characterized by a response increase of the system induced by a particular level of input noise. This phenomenon occurs in many physical and biological systems. The aim of the present study was to implement a new optogenetic approach based on noise-photostimulation to examine causal features of the generation of stochastic resonance in the *in vivo* mouse brain. In order to apply noise-photostimulation directly to specific neurons within the barrel cortex (layers 2/3, 4 and 5) we used 6 anesthetized Thy1-ChR2-YFP mice (line 18) expressing Channelrhodopsin-2 under the Thy1 promoter. Furthermore, we used other 5 wild-type littermate mice as a control. The periodic input signal consisted of subthreshold mechanical stretching of the mice vibrissae; whereas the noise-photostimulation consisted of optogenetic noisy-light delivered via optic fiber to the brain. We implemented a novel noise-photonics system to apply noise-photostimulation. It included a Wavetek noise generator and a Thorlabs' optogenetics device with a 470 nm blue light-emitting diode source. The area of the brain stimulated with the blue noise-photostimulation was the barrel somatosensory cortex. Stable extracellular unitary recordings of identified neurons from layers 4-5 of the same illuminated barrel cortex were obtained by means of a minimatrix system from Thomas Recording (5 to 7 MΩ). Recorded neurons were selected for their response to vibrissae stretching performed by a Chubbuck mechanical stimulator-transducer. We found that in all transgenic mice the total number of neuronal spikes measured from the peristimulus histogram exhibited an inverted U-like form as a function of the noise-photostimulation level. The maximal increase of the number of spikes for 16 neurons for optimal noise was about 300 % relative to the zero noise condition. We used the nonparametric Friedman test to examine the statistical significance of the total number of spikes, between the three conditions (optimal noise ON, zero noise ZN and high noise HN) in all the mice. The results showed significant differences between the three conditions (Wilcoxon test; $p<0.001$). A subsequent post hoc analysis uncovered significant differences between ZN and ON ($p<0.001$) and between ON and HN ($p<0.001$). Importantly, in wild-type mice, the same levels of noise-photostimulation did not produce any stochastic resonance effects in 17 neurons recorded. To the best of our knowledge, these results are the first demonstration of the phenomenon of stochastic resonance produced by optogenetic noise-photostimulation on the brain.

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Poster

240. Somatosensory Functional Organization

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Topic: D.09. Tactile/Somatosensory Systems

Support: CIHR

Title: Mesoscopic calcium imaging reveals the presence of modules of synchronized activity delimited by strong boundaries and dependent on behavior context

Authors: *M. P. VANNI[1], G. SILASI[2], D. XIAO[2], A. CHAN[2], J. LEDUE[2], T. MURPHY[2]; [1]Dept. of Psychiatry, [2]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Brain function arises from assembly of neurons computing similar features which could operate at very long distance within cortex. Correlation methods previously showed that several clusters of synchronized activity can co-exists within cortex. However, the limits of these clusters and their number were underexplored, especially under the context of behavior modulation and the relationship with the topography of connections between areas. Wide field calcium imaging was performed on mice to explore what rules this parcellation at different mesoscopic scales. Transgenic mice expressing the calcium indicator GCaMP (Emx1-cre;CaMK2a-tTA;TITL-GCaMP6: Ai93 and Ai94) were implanted with a chronic window covering most of the cortex including sensori-motor, cingulate, retrosplenial and visual cortex. Wide-field fluorescence was collected on anesthetized animals or awake head-fixed quiet or performing a task (pulling a lever to get a water reward). The correlations signals between each pair of pixel were calculated to generate seed pixel correlation maps as well as distance parcellation. Seed pixel correlation maps revealed between 2 and 3 main clusters of correlated activity delimitated by stable boundaries (gradient). This spatial organization was confirmed by other parcellation methods such as distance tree, K-means or community structure. Interestingly, the number and structure of these clusters was strongly dependent of the behavior activity for at least, three different conditions: 1) anesthetized or awake quiet, 2) learning a task (pulling a lever) and 3) performing a learned task. Inside each cluster, between 1 and 3 remote islands of correlated activity derived from seed pixel correlation maps revealed the long range topography of connection between areas: between primary visual cortex and extrastriate areas or between primary motor and somatosensory cortex. These results were confirmed with single unit electrophysiology, as well as anatomical tracing (Allen Brain Institute Database) These results showed that within mouse cortex, several functional modules run in parallel and their spatial limits are directly related to behavior. Interestingly, the topography and cortical area boundaries appeared to be a weak constraint for functional clustering. Taken together, this contextual parcellation of mesoscopic calcium signals represents one of the first attempts to link brain function with behavior and could open new avenues in the interpretation of brain imaging data.

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Poster

240. Somatosensory Functional Organization

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Topic: D.09. Tactile/Somatosensory Systems

Title: Electroencephalographic correlates of tactile vs. visual guidance during a tracing task

Authors: P. J. LEE, R. N. HUYNH, *S. N. KUKKE;
Biomed. Engin., The Catholic Univ. of America, Washington, DC

Abstract: Physical therapy for upper extremity motor disorders usually focuses on the practice of visually-guided motor tasks. However, touch also provides a strong input to hand function, and is often weakened in individuals with brain injuries, including cerebral palsy and stroke. The recovery of hand function may therefore benefit from both visuo- and tactile-motor task practice. To explore neural mechanisms by which sensory input can guide movement, we investigated EEG correlates of tactile vs. visual feedback. 7 right-handed, healthy adults (20-22 yrs) were included in this IRB-approved study. Volunteers traced 6 irregular shapes (path length = 45.7±3.6 cm on a 156 cm2 plate) 10 times with the right 2nd digit in 2 feedback conditions. In the tactile-motor (TM) task, movement was guided only by tactile feedback of semi-circular bumps along the path (height = 0.13 cm, spacing = 0.76 cm). In the visuo-motor (VM) task, movement was guided only by vision of dots along the path (dia = 0.13 cm, spacing = 0.76 cm) seen through a circular window (dia = 3 cm) at the fingertip. EEG data were recorded from 28 scalp leads. Cortical activation, and connectivity between regions were quantified by task-related power (TRPow) loss, and coherence (Coh) between pairs of leads, respectively. ANOVA was used to test effects of feedback modality, cortical region, and frequency band (alpha = 8-12 Hz, beta = 13-20 Hz) on TRPow. TRPow loss was greater during the VM vs. TM task. In both tasks, the central, temporal, and parietal regions had more TRPow loss than the frontal and occipital regions, likely due to sensory processing and movement. There was more TRPow loss in the alpha vs. beta band in the VM task, but no difference between frequencies in the TM task. In both frequency bands and both hemispheres, there was more within-hemisphere Coh among frontal leads, and also among posterior leads in TM vs. VM trials. In contrast, there was more within-hemisphere Coh between parietal and temporal regions in VM vs. TM trials. Between-hemisphere Coh was greater in frontal leads in TM vs. VM trials and greater in posterior leads in VM vs. TM trials. There was no difference in trace time between TM and VM trials (t-test, $p = 0.17$). This study provides evidence of cortical activation and connectivity patterns that are specific to the sensory modality used during movement. Our results suggest that inclusion of both touch and vision in motor practice can stimulate a broad range of neural networks. Future studies are needed to test the extent to which repeated exposure to sensorimotor tasks with either feedback modality alone or both combined can lead to adaptive changes in EEG parameters that correlate with behavioral changes.

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Poster

240. Somatosensory Functional Organization

Location: Hall A

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Program#/Poster#: 240.17/N10

Topic: D.09. Tactile/Somatosensory Systems

Support: Swiss National Science Foundation PZ00P3_131731

Swiss National Science Foundation PP00P1_157420/1

Title: Upward propagation of spiking activity across cortical layers

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Abstract: We previously observed a pattern of functional interactions directed upward along the layers of cortical columns in primary sensory cortex (S1) of rats, both in S1 contralateral to whisker stimulation and in the ipsilateral S1. The interactions between the two S1s, however, selectively targeted granular and infragranular layers, suggesting that the upward information flows allow for integration of local and large-scale neocortical processes (Plomp et al. 2014). If each layer functionally drives the one above it, then spiking activity should follow a similar pattern: spikes should propagate mostly upward within columns, not downward. Likewise, after spikes in one S1, spikes in the other S1 should follow first in deeper layers, and then propagate up to higher layers. To test this we determined spiking activity at each cortical layer, both in stimulus evoked activity and at rest. We then calculated the delay with which subsequent spikes occurred in other layers of the same S1, and in layers of S1 in the other hemisphere. We found that spike-to-spike delays systematically increased with upward distance: when a spike occurred e.g. in L5, it took longer for a subsequent spike to occur in L3 than in L4, and again longer for L2 and L1. This increased delay held for spikes in each layer and was observed both in stimulus-evoked and in spontaneous activity. Importantly, spike delays were constant in the downward direction and the observed delays could not be attributed to differences in spike rate per layer. When we time-locked LFPs to the spikes in each layer we found a similar pattern: for spikes in any layer, peaks of the spike-triggered LFP in the layers below preceded the spike, while the peaks in the layers above followed the spike. In addition, spikes in one hemisphere were time-locked to LFPs in the other hemisphere, showing an ongoing coupling between the S1 activities in both hemispheres. The systematic spike delays and spike-triggered LFP results are in line with an information flow from deeper layers to local processing in superficial layers.

Disclosures: G. Plomp: None. **C. Quairiaux:** None. **C. Michel:** None.

Poster

240. Somatosensory Functional Organization

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 240.18/N11

Topic: D.09. Tactile/Somatosensory Systems

Support: EU FP7 Project VERE (No. 257695)

ENIAC Project DeNeCoR (No. 324257)

Title: ECoG based high-gamma somatosensory activity measurement for digit separation

Authors: R. PRUECKL[1], C. KAPELLER[1], K. KAMADA[2], F. TAKEUCHI[2], H. OGAWA[2], *G. EDLINGER[3], C. GUGER[1];
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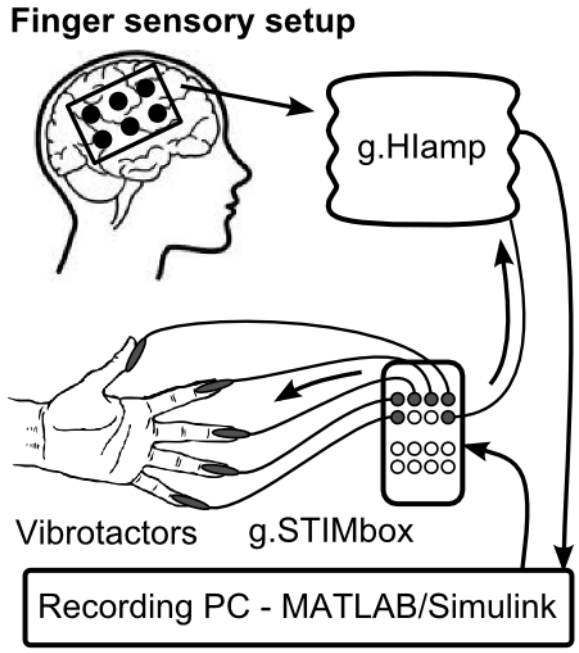
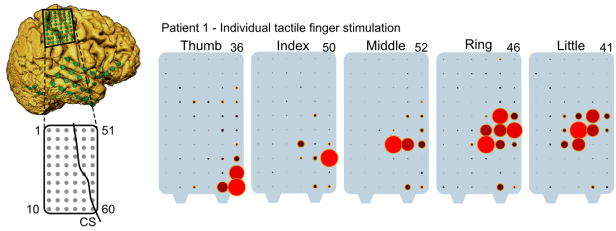
Abstract: This study demonstrates invasive functional mapping of finger-related areas in the human brain in the gamma band and the feasibility of distinguishing the fingers from each other by observing spatial activity changes. Data were collected during a vibrotactile finger stimulation paradigm (see Figure 1) and showed significant cortical activation in the high-gamma range over the contralateral somatosensory cortices (SCX) of two patients who participated. The results are consistent with previous studies that used fMRI in test subjects without implanted electrodes. Activation was detected in the SCX from anterior and inferior for the thumb to more posterior and superior for the little finger (see Figure 2). A previous study states that there is a distance of 2 mm between the ring and the little finger, which is reproduced here and explains the relatively small difference in activation observed between those two fingers. In patient 1 an electrode grid with higher spatial resolution has been used that led to a much better ability to discover the spatial distribution of activation depending on the finger that is stimulated in contrast to a standard ECoG grid. The distances between cortical finger representations, and the fact that fMRI studies currently map the SCX with resolutions below 1x1x1 mm, encourage minimizing the size of the electrodes used in future ECoG studies. As fMRI provides rather high time-constants, only this new approach could increase both the temporal and spatial resolution of study data.





Disclosures: **R. Prueckl:** None. **C. Kapeller:** None. **K. Kamada:** None. **F. Takeuchi:** None. **H. Ogawa:** None. **G. Edlinger:** None. **C. Guger:** None.

Poster

240. Somatosensory Functional Organization

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 240.19/N12

Topic: D.09. Tactile/Somatosensory Systems

Title: Brain mechanisms for the processing of the gentle touch stimuli in patients with anorexia nervosa

Authors: ***M. DAVIDOVIC**[1], L. KARJALAINEN[2], E. WENTZ[2], H. OLAUSSON[3];
[1]Inst. of Neurosci. and Physiol., Gothenburg, Sweden; [2]Gillberg Neuropsychiatry Centre, Inst. of

Neurosci. and Physiology, Univ. of Gothenburg, Gothenburg, Sweden; [3]Dept. of Clin. and Exptl. Medicine, Linkoping Univ., Linkoping, Sweden

Abstract: Introduction Dysfunction in the system for the mental representation of one's own body is believed to be the basis for the body image disturbance, one of the key features of anorexia nervosa (AN). This mental representation is a result of the multisensory integration of visual, tactile, vestibular and proprioceptive inputs. Tactile stimuli can not only disclose dysfunction in this system, but also help to cure it. In this study we aim to investigate brain mechanisms for the gentle touch processing in patients with AN. Gentle stroking over the skin has a quality of not only being descriptive (i.e. gives information about stroking velocity and pressure), but also having an affective component (subjects can experience it as either pleasant or unpleasant). Methods Study includes 25 patients with AN and 25 healthy subjects. All subjects completed a scanning session in magnet camera, in which structural, functional, resting state and diffusion weighted images were collected. During the collection of functional images subjects were gently stroked over their underarm and asked to rate the pleasantness of the stroking. In addition, all subjects have filled a battery of questionnaires, which included Social Touch Questionnaire (STQ) (an instrument that assesses comfort and preferences regarding touch that occurs in social situations). Results AN patients rated skin stroking as less pleasant than healthy subjects. They also scored higher in STQ, which means that their experience of the touch that occurs in social situations is less frequent and less enjoyable. The analysis of functional data shows the significant difference between healthy and AN subjects in the extrastriate body area (EBA). While healthy subjects activate this area during the skin stroking, AN patients show hypoactivation. We also conducted an extensive connectivity analysis between EBA and the rest of the brain, which shows that EBA has central importance in the body image network of the brain. Conclusions Our results are in agreement with the findings in previous studies which investigated visual body perception in patients with AN. Since the system for the perception of our own body is activated by touch, our results open for the application of gentle touch as additional form of therapy in AN.

Disclosures: M. Davidovic: None. **L. Karjalainen:** None. **E. Wentz:** None. **H. Olausson:** None.

Poster

240. Somatosensory Functional Organization

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 240.20/N13

Topic: D.09. Tactile/Somatosensory Systems

Support: NIH Grant R01 NS061963

Title: Retrosplenial interconnectivity with motor and medial frontal cortical areas in the mouse

Authors: *N. YAMAWAKI, G. M. G. SHEPHERD;
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Abstract: The retrosplenial cortex (RSC) appears anatomically linked with medial areas of the frontal cortex via axonal projections in both directions. To characterize this RSC-frontal circuit electroanatomically, we used optogenetic labeling to photostimulate presynaptic axons in brain slices together with whole-cell recordings from pyramidal neurons identified by laminar location and/or retrograde labeling. In the RSC→frontal direction, RSC axons excited pyramidal neurons across multiple layers and projection classes in medial frontal cortex, including RSC-projecting corticocortical neurons and corticospinal neurons. The origin of these RSC axons was traced to both the dysgranular (mainly layer 2/3 and 5A) and granular (mainly layer 5A) regions of the RSC. In the reverse direction, frontal→RSC axons originating from medial motor/frontal areas primarily innervated the dysgranular RSC, especially layer 2/3 pyramidal neurons, including corticocortical neurons with axonal projections back to frontal cortex. Our findings thus indicate (1) that the RSC and medial frontal cortices are extensively yet intricately interconnected through inter-areal projections that excite each other's reciprocally projecting corticocortical neurons; (2) that dysgranular and granular RSC areas are distinct in their laminar patterns of interconnections with frontal cortex; and (3) that multiple projection classes including corticospinal neurons are engaged by these circuits, providing divergent output to downstream networks. These results provide new insight into the cellular organization of synaptic circuits linking the RSC to frontal areas including medial motor cortex and thus from dorsal hippocampal networks involved in memory and navigation to neocortical/spinal networks involved in sensorimotor integration and motor control.

Disclosures: N. Yamawaki: None. **G.M.G. Shepherd:** None.

Poster

240. Somatosensory Functional Organization

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Topic: D.09. Tactile/Somatosensory Systems

Support: Dana foundation grant to LMC

Title: Spatial-temporal functional reorganization of somatosensory area 3b and area 1 of squirrel monkeys after spinal cord injury

Authors: *L. CHEN[1], R. WU[1], L. SU[2], P.-F. YANG[1];
[1]Radiology and Inst. of Imaging Sci., Vanderbilt Univ., Nashville, TN; [2]Duke Univ., Durham, NC

Abstract: Spatial-temporal Functional Reorganization of Somatosensory Area 3b and Area 1 of Squirrel Monkeys After Spinal Cord Injury Ruiqi Wu, Langting Su, Pai-Feng Yang, Li Min Chen **Keywords:** optical imaging; hand; touch; primates; dorsal column **Abstract:** Somatosensory cortices of adult primates reorganize after sensory loss such as that caused by disruptions of dorsal column afferents. Correlated cortical reorganization and behavioral recovery led to the hypothesis that cortical reactivation and reorganization mediate functional and behavioral recovery after spinal cord injury (SCI). However, since parallel reorganizations have been observed in multiple somatic areas, it is not clear how different cortical areas work together to restore or compensate for the loss of function. As a first step to address this question, with the high spatial and temporal resolution afforded by the optical imaging of intrinsic signals (OIS), we quantified the spatial-temporal features of the cortical responses in areas 3b and 1 several weeks after a unilateral dorsal column lesion. Three adult squirrel monkeys were included in this study and were imaged under light anesthesia before and after SCI. Specifically, we measured the areas, magnitudes and temporal profiles of OIS responses to 8Hz vibrotactile stimulation (in 3.5 seconds duration) of individual digits. We found that the magnitudes of OIS signals obtained several weeks after the lesion were significantly decreased in both areas 3b and 1. OIS signals also peaked significantly earlier than those obtained prelesion. The size of activation in area 3b was larger, indicating more diffused responses to stimuli. A similar activation size increase was observed in area 1, but statistically the increase was not significant. Together, these results demonstrated that contralateral area 3b and area 1 underwent similar spatial and temporal reorganization after lesion, which were characterized as weaker, shorter and more diffused responses. These altered response properties may be responsible for the partial recovery of the loss of sensory functions.

Disclosures: L. Chen: None. **R. Wu:** None. **L. Su:** None. **P. Yang:** None.

Poster

240. Somatosensory Functional Organization

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Topic: D.09. Tactile/Somatosensory Systems

Support: NSF IIS-1016998

Title: Thermal Pattern Identification on the Hand

Authors: *L. A. JONES, A. SINGHAL;
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Abstract: Changes in skin temperature are encoded in the responses of cold and warm thermoreceptors at a rate that depends on the baseline temperature of the skin and the rate at

which the temperature is changing. In contrast to the wealth of sensations that are evoked by tactile stimulation of the skin, in response to thermal stimulation there is perceptible warming or cooling and this can be quantified in terms of the intensity and duration of the thermal stimulus. The spatial properties of the thermal stimulus such as its area, shape and location are poorly resolved due to the pervasive effects of spatial summation. With the exception of studies on the effect of the rate of temperature change on thermal thresholds, the temporal aspects of thermal stimulation have not been extensively studied. Previous research has demonstrated that the time to process thermal stimuli within the innocuous range is relatively slow in comparison to other sensory modalities. The objective of the present experiments was to determine whether thermal patterns created by varying the direction, magnitude and rate of temperature change could be reliably identified when presented on either the thenar eminence or the index finger. Three thermal profiles (square wave, step and ramp) were used, each of which had two values to give a total of six patterns. A thermal display based on a Peltier device was used to present the stimuli and the temperature of the display and hand were measured continuously using thermal sensors. Each thermal pattern was presented 8 times to 10 participants who had to identify the stimulus using a visual template of the change in skin temperature. The temperature of the skin tracked that of the thermal display but did not reach the minimum and maximum intensities of the display within the presentation time. Preliminary experiments on the response of the skin to various types of thermal inputs indicated that waveforms such as square waves, sinusoids and triangular waves resulted in very similar changes in skin temperature and so were unlikely to be perceptually distinguishable. The three profiles selected did produce distinct changes in temperature as reflected in the participants' performance. The individual mean scores associated with the six thermal stimuli ranged from 80% to 98% on the thenar eminence and from 81% to 88% on the index finger with overall means of 91% and 84% respectively. The information transfer values for the thenar eminence averaged 2.26 bits and for the finger 1.86 bits. These findings demonstrate that with sufficiently long presentation times, the information processing capabilities of the thermal sensory system may rival those achieved with vibrotactile inputs.

Disclosures: **L.A. Jones:** None. **A. Singhal:** None.

Poster

240. Somatosensory Functional Organization

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Topic: D.09. Tactile/Somatosensory Systems

Support: ESRC Grant R10568

Title: Functional, acoustic and articulatory outcomes of speech training: a multi-modal investigation of native and non-native imitation

Authors: *D. CAREY[1], M. MIQUEL[3], B. EVANS[4], P. ADANK[5], C. MCGETTIGAN[2]; [1]Royal Holloway, Univ. of London, Surrey, United Kingdom; [2]Royal Holloway, Univ. of London, London, United Kingdom; [3]Dept. of Physics, Queen Mary, Univ. of London, London, United Kingdom; [4]Speech, Hearing and Phonetic Sci., [5]Univ. Col. London, London, United Kingdom

Abstract: Flexibility of vocal behaviour is a critical skill in human communication. This flexibility can be placed under strain when acquiring the phonemes of a second language, particularly for adult L2 learners, where native perceptual and articulatory processes may dominate. Yet, to date, relatively little research has explored the articulatory and acoustic outcomes of speech learning in association with functional activation in speech production networks. Here, we used a speech training paradigm combined with fMRI and real-time imaging of the vocal tract (rtMRI) to explore vocal imitation skill. Participants were trained to imitate a (native) front vowel and (non-native) front rounded vowel prior to scanning. Their performance on imitation of these vowels and a similar untrained pair was then measured during approximately 1 hour of task performance, while we measured fMRI of bloodflow responses, rtMRI of vocal tract dynamics and in-scanner acoustic recordings of speech output. Behavioural results showed that acoustic imitations of non-native vowels were less accurate than for the corresponding native vowels, with considerable individual differences in imitation success. fMRI results showed significantly greater activation in somato-motor cortex, IFG and superior temporal cortex when participants produced non-native vowels versus native vowels. Using representational similarity statistics (Kriegeskorte et al., 2008), we were able apply a novel analysis approach, by directly comparing rtMRI of vocal tract articulators to fMRI activity during phoneme production. By generating representational similarity matrices to describe the articulation of different vowel categories, we were able to probe the functional fronto-temporal speech production network to identify regions representing the categorical dimensionality of trained and untrained vowels. These results help to inform our account of speech imitation as a skill, with respect to acoustic, articulatory and neural indices.

Disclosures: D. Carey: None. **M. Miquel:** None. **B. Evans:** None. **P. Adank:** None. **C. McGettigan:** None.

Poster

240. Somatosensory Functional Organization

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Program#/Poster#: 240.24/N17

Topic: D.09. Tactile/Somatosensory Systems

Title: A possible challenge to sensorimotor adaptation

Authors: *B. J. MARTIN[1], T. A. THRASHER[2], C. S. LAYNE[2], B. LEE[2];
[1]Industrial & Operations Engin., Univ. of Michigan, Ann Arbor, MI; [2]health and Human performance, Univ. of Houston, Houston, TX

Abstract: Learning compensation strategies through practice has been demonstrated for a large variety of tasks, including adaptive responses to unpredictable external force perturbations as well as trips and slips. It has been hypothesized that improvement of responses to perturbation is primarily associated with the adaptation of motor commands through the update of an internal model. The evolution of the internal model allows a progression from primarily feedback to primarily feedforward correction of significant perturbation-induced error, as feedback control is too slow to engender effective compensatory movements. The aim of this study was to determine whether a short lead time vibrotactile cue could improve trip recovery and challenge adaptation. A split belt treadmill was controlled to produce a trip by the abrupt stop of one of the belts at the foot loading phase and resume its motion after the first heel strike of the non-trip foot. An algorithm identified all gait events. Trip occurrence was randomized within a series of strides. Two experiments involving two different groups of young adults were designed to compare trip recovery performance resulting from the learning by repetition method and from vibrotactile cuing, respectively. Experiment I evaluated trip recovery learning only from repeated exposure to perturbation. Experiment II investigated the effects of vibrotactile cuing on recovery as a function of the stimulus application location (upper arm, trunk, lower leg) and lead time (250, 500 ms before the trip). To characterize trip recovery kinetic and kinematic responses, seven outcome measures were defined: response step time, maximum response step force, recovery time, maximum trunk flexion angle, maximum trunk flexion velocity, trunk flexion angular dispersion, and maximum whole body center of mass velocity. In Experiment I, trip recovery improved progressively from the 4th to the 8th trial to reach an ‘adapted response’. In Experiment II, from the first cued trial all cued trip recoveries were equivalent to the ‘adapted response’. These results suggest difference in the mechanisms underlying recovery in the cued versus the adapted response to external perturbations. More importantly, their contrast suggests that “immediacy” opposes “adaptive evolution” and the likelihood of a pre-existing generic internal model derived from life experiences. The associated feedforward response may be modulated by peripheral mechanisms.

Disclosures: B.J. Martin: None. **T.A. Thrasher:** None. **C.S. Layne:** None. **B. Lee:** None.

Poster

240. Somatosensory Functional Organization

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 240.25/N18

Topic: D.09. Tactile/Somatosensory Systems

Title: Anatomical basis of spiking correlation in upper layers of somatosensory cortex

Authors: *U. CZUBAYKO[1], G. BASSETTO[3], R. T. NARAYANAN[4], M. OBERLAENDER[4], J. H. MACKE[2,3], J. N. D. KERR[1];
[1]Dept. of Behaviour and Brain Organization, [2]Res. Ctr. Caesar: A Max Planck Inst., Bonn, Germany; [3]Neural Computation and Behaviour, Max Planck Inst. for Biol. Cybernetics and Bernstein Ctr. for Computat. Neurosci., Tuebingen, Germany; [4]Computat. Neuroanatomy Group, Max Planck Inst. for Biol. Cybernetics, Tuebingen, Germany

Abstract: In neuronal populations of the sensory cortex, stimulus responses are shaped by the cortical architecture on anatomical scales from tens of microns to millimeters. In particular, in L2/3 rodent vibrissal cortex we previously observed that whisker deflection evokes pairwise correlations that decrease both with distance between neurons and distance to the center of the whisker-associated column (Kerr, de Kock, Greenberg, Bruno, Sakmann, and Helmchen. (2007). J. Neurosci. 27: 13316-28). One possible explanation for this finding is that these correlations arise from anatomically structured common inputs. L4 spiny stellate (SS) cells send vertical axon fibers to L2/3 that are confined within the borders of the whisker-associated column and neuronal pairs closer together could exhibit greater dendritic overlap. Therefore, for pairs closer to the column center more of this overlap will intersect with SS projections. We tested this hypothesis using 2-photon targeted patching of L2/3 pyramidal pairs in anaesthetized rats to record sub- and suprathreshold stimulus responses followed by anatomic reconstruction of the neurons and barrel field. We found a positive and statistically significant association between correlated AP firing and dendritic overlay inside the whisker-associated column. This effect was strongest for suprathreshold activity evoked shortly after whisker deflection (~20 ms), and decayed rapidly thereafter. It was also robust with respect to the voxel size, determined by the L4 axon reconstructions, used to quantify dendritic overlap. No relationship was detectable for offset responses or spontaneous activity. These results support the notion that the spatially structured correlations observed for short-latency stimulus-evoked spiking arise from anatomically structured feed-forward projections.

Disclosures: U. Czubayko: None. **G. Bassetto:** None. **R.T. Narayanan:** None. **M. Oberlaender:** None. **J.H. Macke:** None. **J.N.D. Kerr:** None.

Poster

240. Somatosensory Functional Organization

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Program#/Poster#: 240.26/N19

Topic: D.09. Tactile/Somatosensory Systems

Title: Variations in corticospinal excitability associated with different modes of hedonic touch

Authors: I. AL-BUHAISI[1], A. REMAUD[3], *F. TREMBLAY[2];
[1]Sch. Rehab Sc, [2]Sch. of Rehabil. Sci., Univ. of Ottawa, Ottawa, ON, Canada; [3]Clin. Neurosci., Bruyere Res. Inst., Ottawa, ON, Canada

Abstract: In real life situations, touch sensations can arise from multiple sources. For example, one may accidentally bump into an object (external) or come in contact with another person (interpersonal). One may also get sensations from intra-personal (self) touch. In recent years, touch sensations have been further categorized on the basis of whether they served a primary discriminative function as opposed to a more hedonic (social) function (see McGlone, et al Neuron 82, 2014). This categorization reflects in large part different neural mechanisms subserving each touch function from periphery to cortex (McGlone 2014). In the present report, we sought to determine whether hedonic touch would lead to a differential modulation in brain excitability when sensations arise from intra vs. inter-personal contact or from contact with an object. Corticospinal excitability was assessed by monitoring changes in MEPs elicited in the first dorsal interosseous (FDI) of the resting left hand in response to right TMS while blindfolded participants (n=11, 24.3± 1.7 years) were subjected to different modes of hedonic touch applied to the dorsum of the hand. The modes consisted in: 1) INTRA, self-induced touch with the right index finger, 2) INTER, touch via the experimenter's finger contact and 3) EXT, external touch via a paint brush. In all modes, the stimulation consisted in a single stroke displacement of ~ 1 cm/s from the base of 3rd knuckle to the wrist line (15 MEPs/mode, randomized sequence). Analysis of MEP modulation revealed that MEPs under the INTRA mode were significantly larger than those in either the INTER or EXT mode (Tukey, post-test, $p<0.05$). Consistent with this, MEP latency was also significantly reduced during INTRA when compared to INTER touch. Interestingly, INTRA (self) touch was rated as the least pleasant by participants (10/11), whereas the brush was rated as the most pleasant (8/10). The increased excitability seen with INTRA-touch likely reflects the more extensive recruitment of sensorimotor areas associated with active movement and bilateral interactions between left and right motor cortices (Ackerley et al, Front Behav Neursc 2012). The fact that it was also rated as the least pleasant mode can be linked with the sensory cancellation phenomena (Chapman and Tremblay, 2015) Scholarpedia, 10(3):7953). The observation that passive modes (INTER and EXT) elicited lesser facilitation might be linked with recent observations showing that passive touch was associated with less intense and more restricted activation in contralateral somatosensory areas than active touch (Ackerley et al 2012).

Disclosures: I. Al-Buhaisi: None. **A. Remaud:** None. **F. Tremblay:** None.

Poster

240. Somatosensory Functional Organization

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Topic: D.09. Tactile/Somatosensory Systems

Support: NIH/NINDS R01NS079288

NIH/NINDS R01NS072171

Title: Intracellular responses induced by transcranial magnetic stimulation

Authors: J. BANERJEE[1,3], M. SORRELL[1,3], P. CELNIK[2], *G. PELLED[1,3];
[1]Radiology, [2]Physical Med. and Rehabil., Johns Hopkins Sch. of Med., Baltimore, MD;
[3]Kennedy Krieger Inst., Baltimore, MD

Abstract: Transcranial Magnetic Stimulation (TMS) is a non-invasive brain stimulation technology that has been shown to induce changes in neural excitability that outlast the period of stimulation by induction of weak electric currents using a rapidly changing magnetic field. TMS has shown preliminary success as a therapeutic tool in the clinic in adult neurological diseases such as stroke, epilepsy, major depression and migraines. Nevertheless, the cellular mechanisms targeted by TMS remain unknown. Therefore, we have sought to investigate the cellular mechanisms affected by TMS by using intracellular electrophysiology and calcium imaging methods. Whole-cell patch-clamp recordings of spiking activity and intracellular steady-state currents were performed in excitatory pyramidal neurons located in the primary somatosensory cortex in live rat brain slices. Excitatory neurons were identified by GFP expression driven by CaMKII promoter. Current-clamp recordings showed that 20 Hz TMS (frequency often used in clinical setting) induced spiking activity in the neurons even at sub-threshold depolarization. Additionally, voltage clamp experiments show that TMS increased the steady-state currents at these sub-threshold voltages. The results together suggest that TMS activates specific voltage gated ion channels leading to changes in neuronal excitability. For imaging experiments, cortical neurons obtained from E15-E16 mouse embryos were co-cultured with cortical glia obtained from P0-P2 mouse pups. The cell cultures were loaded with the calcium indicator fura-2/AM and the percent change in fura-2 ratio at 340/380 nm excitation was calculated from the images. In agreement with the electrophysiology results, the imaging experiments showed that 20 Hz TMS induced increases in intracellular calcium concentrations, indicative of neuronal activity. The above results cumulatively show that TMS affects the excitability of excitatory neurons and also shows increased calcium responses. Understanding the exact mechanism by which TMS alters cellular excitability may help to refine and improve treatment strategies. This work was supported by NIH/NINDS grants: R01NS072171 (G.P.) and R01NS079288 (G.P.)

Disclosures: **J. Banerjee:** None. **M. Sorrell:** None. **P. Celnik:** None. **G. Pelled:** None.

Poster

240. Somatosensory Functional Organization

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 240.28/N21

Topic: D.09. Tactile/Somatosensory Systems

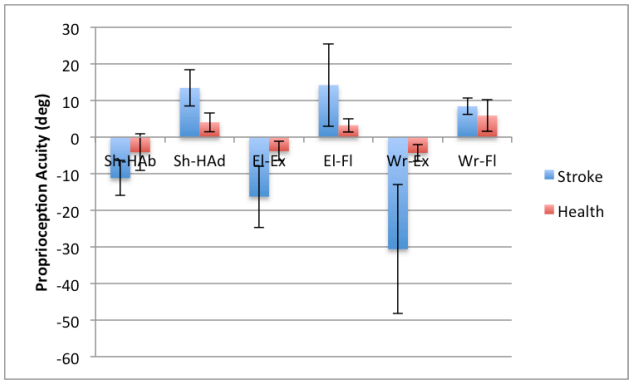
Support: NSF/CBET-0854498

NIDRR H133A140065

Title: Multi-joint proprioception deficits at the shoulder, elbow and wrist post stroke

Authors: *L.-Q. ZHANG[1], D. XU[1], Y. REN[1], H. PARK[2], S. KANG[3], Y. LEE[3], Y.-S. LIN[3]; [1]Rehabil. Inst. of Chicago, Chicago, IL; [2]KAIST, Daejeon, Korea, Republic of; [3]RIC, Chicago, IL

Abstract: It is well known that sensorimotor functions of multiple joints (shoulder, elbow, wrist and hand) of the upper limb are impaired post stroke. In particular, stroke survivors often have sensory deficit, which significantly affects motor control, motor learning, and recovery of functional activities after stroke. Sensory impairment was reported as one of the main predictors for motor recovery of stroke survivors. Past research has been focused on characterizations of single-joint or end-point proprioception and there has been a lack of characterization of multi-joint proprioception. Considering that human functional activities almost always involve multiple joints simultaneously in the upper limb, examination and treatment of multiple joint is needed. Multi-joint and multi-DOF proprioception was investigated in six patients post stroke and six healthy controls using a multi-joint rehabilitation robot called IntelliArm. When selected, the shoulder was moved in horizontal abduction or adduction, and the elbow and wrist was moved in flexion or extension. With the shoulder, elbow or wrist joint randomly selected, the joint was moved at a slow speed of 0.5 degree/sec by the IntelliArm robot, with the subject not looking at the joints. Subjects were asked to push a hand-held switch as soon as he/she felt a joint movement and to tell the operator which joint was moved and in which direction. Proprioception acuity of the horizontal abduction (Sh-HAb) and adduction (Sh-HAd), elbow flexion (El-Fl) and extension (El-Ex), and wrist flexion (Wr-Fl) and extension (Wr-Ex) of stroke survivors showed considerable impairments. Patients post stroke had higher proprioception thresholds at the shoulder, elbow and wrist than their counterparts of healthy controls. Multi-joint sensory characterizations are helpful in understanding sensorimotor deficits post stroke and in planning subsequent impairment-specific neurorehabilitation.



Disclosures: **L. Zhang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Rehabtek. **D. Xu:** None. **Y. Ren:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Rehabtek. **H. Park:** None. **S. Kang:** None. **Y. Lee:** None. **Y. Lin:** None.

Poster

240. Somatosensory Functional Organization

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 240.29/N22

Topic: D.09. Tactile/Somatosensory Systems

Support: Swedish Research Council FF-2013-687

Title: fMRI reveals altered processing of affective touch in heterozygous carriers of a nerve growth factor beta (NGFB) mutation influencing C tactile afferent pathway

Authors: *I. MORRISON, I. PERINI;
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Abstract: In this fMRI study we investigated the effects of a rare nerve growth factor beta (NGFB) mutation on affective touch perception, both for directly-experienced and touch and observation in others. This mutation (R221W) causes a reduction in unmyelinated C fibers, which include C nociceptors and C-tactile (CT) afferents. CT afferents contribute to the processing of hedonic touch, with preferential responses to caressing speeds which are rated as most pleasant (Löken et al, Nat Neurosci, 2009). Homo- and heterozygous carriers with low C-afferent density evaluate affective touch differently than controls, and show cortical-level differences in the processing of such stimuli (Morrison et al, Brain, 2011). The present study further explored behavioral and hemodynamic affective touch variables in a group of healthy heterozygous R221W carriers with milder reductions in C afferent density. We compared 12 heterozygote carriers with age-, gender-, and education-matched controls. CT-optimal vs non-optimal skin stroking speeds (3 cm/s vs 30 cm/s) were contrasted for both experienced and observed touch (see Morrison et al, J Neurosci, 2011). In both groups, posterior/mid insula and parietal opercular cortex responded to CT-optimal touch (3cm/s) vs CT-non-optimal (30 cm/s) touch. However, only the controls showed a selective increase in orbitofrontal cortex activation, suggesting processing of CT-optimal stroking speeds in reward-related terms. Crucially, the results were similar for the observation condition, indicating that seeing others' affective touch interactions also engages these areas. In addition, carriers' mean pleasantness ratings were lower than controls'. To determine the neural correlates of rating patterns, we extracted the value for the negative quadratic coefficient for each subject, reflecting the fit to a typical CT-related, inverted U-shaped curve across speeds (see Löken et al, 2009; Perini et al, Front Hum Neurosci,

2015).We used these values as covariates of interest during CT targeted touch (3 cm/s). Whereas the carriers' ratings covaried with parietal somatosensory and sensorimotor areas (primary somatosensory cortex, angular gyrus), the controls engaged areas associated with affect, reward, and social relevance (orbitofrontal cortex, ventral anterior insula, and superior temporal sulcus). These findings suggest that despite overall similarities in affective touch processing, evaluation of affective touch in R221W carriers is limited to somatosensory networks, while controls shift from somatosensory to more specific reward- and evaluation-related processing during caress stimulation.

Disclosures: I. Morrison: None. **I. Perini:** None.

Poster

240. Somatosensory Functional Organization

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 240.30/N23

Topic: D.09. Tactile/Somatosensory Systems

Support: Leverhulme Trust

Title: C-tactile afferent stimulating touch carries a positive affective value

Authors: *S. C. WALKER[1], R. PAWLING[1], P. D. TROTTER[1], P. R. CANNON[2], F. P. MCGLONE[1];
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Abstract: The rewarding sensation of touch in affiliative interactions is hypothesised to be underpinned by a specialised system of nerve fibres called C-tactile afferents (CTs), which respond optimally to slowly moving, gentle touch, typical of a caress. However to date, there is little empirical evidence to support the theory that CTs encode socially relevant and rewarding tactile information. Here, we employed psychophysiological and behavioural measures to examine whether CT activation carries an innate reward value. Firstly we used electromyography (EMG) to measure the facial responses of two key emotion-related muscle sites (zygomaticus major & corrugator supercilii), while participants evaluated the pleasantness of experimenter administered stroking touch, delivered using a soft brush, at CT optimal velocity (3cm/sec) and at a faster, CT non-optimal speed (30cm/sec). Subsequently, in an evaluative conditioning paradigm, participants rated the approachability of emotionally neutral faces. During conditioning, a subset of each participant's most neutrally rated faces was paired with tactile stimulation of their forearm, delivered by a stroking robot. For half the faces stroking was CT-optimal (3cm/sec), for the other half, stroking was faster (30cm/sec). Heart rate and skin conductance were recorded throughout. In the EMG study, a significant location x speed

interaction was found, with increased activation at the zygomaticus major seen to 3cm/sec stroking on the forearm, a response indicative of positive affect. In the conditioning study, a significant touch-type x time interaction was found. Whilst rated equally approachable at first, post-conditioning, faces paired with CT-optimal touch showed a significant increase in approachability, those paired with the faster control touch did not. Physiologically, we observed significantly greater heart rate deceleration and lower skin conductance responses to CT-optimal touch versus control. These results offer the first empirical evidence that tactile stimulation, which optimally activates CTs, carries an affective value that can be imbued to socially relevant stimuli. Furthermore, our findings support the theory that CT activation can reduce arousal levels, perhaps underpinning their role in social support and nurture.

Disclosures: S.C. Walker: None. **R. Pawling:** None. **P.D. Trotter:** None. **P.R. Cannon:** None. **F.P. McGlone:** None.

Poster

241. Spinal Cord Injury and Plasticity

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 241.01/N24

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH 5R01NS064004-07

Craig H Neilsen Foundation #261214

Title: Combining theta burst motor cortex stimulation with spinal cathodal DC stimulation to promote motor functional recovery after corticospinal tract injury

Authors: *W. SONG, D. RYAN, A. AMER, J. MARTIN;
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Abstract: Corticospinal tract (CST) damage produces weakness or paralysis. An important strategy for promoting the function of spared CST connections is to harness activity-dependent plasticity. Previously we found motor functional improvement after 6 hours of M1 stimulation each day for 10 days in pyramid-injured rats. To further facilitate activity dependent plasticity, in this study we combine forelimb M1 activation, with co-activation of its spinal target, to strengthen CST functional connections and to promote motor recovery in pyramid-injured rats. We hypothesized that combined stimulation would augment CST connections and this would increase axon terminal extension within the spinal cord and, in turn, promote motor functions after injury. To activate M1, we developed an electrical analog of intermittent theta burst stimulation (iTBS), using epidural stimulation to activate cortical motor output, and concurrently cervical spinal circuits were co-activated by cathodal trans-spinal direct current stimulation (c-

tsDCS), which independently facilitated motor output. We found that after unilateral pyramid transection, rats showed a significant contralateral forelimb impairment while performing skilled ladder walking. After testing, rats were assigned to either a stimulation group or a control group without stimulation. The stimulation group was treated with combined stimulation (iTBS/c-tsDCS) 25 min a day for 10 days. There was a significant reduction in motor threshold during the treatment period. Importantly, motor function was significantly improved, beginning 1 week after cessation of stimulation, with reduced errors in the ladder task. In contrast, the non-stimulated control group maintained significantly higher error scores. One month post injury, the CST was traced in the stimulated and control groups. There was a significantly higher density of axon outgrowth on the impaired side of the spinal cord (ipsilateral to M1 stimulation) in the stimulated group than the control group. Our results suggest that combining "top down" M1 stimulation with "bottom up" spinal stimulation activates the entire corticospinal system to induce durable CST structural changes within the spinal cord. And this, in turn, promotes motor function after pyramidal injury. The combined stimulation may be sufficiently robust to provide treatment after a severe spinal cord injury.

Disclosures: **W. Song:** None. **D. Ryan:** None. **A. Amer:** None. **J. Martin:** None.

Poster

241. Spinal Cord Injury and Plasticity

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 241.02/N25

Topic: D.10. Spinal Cord Injury and Plasticity

Title: Electrophysiological assessment of the cervical spinal cord networks of intact rats based on profiles of spinal evoked potential amplitudes and delays in multiple forelimb muscles

Authors: *L. MOORE[1], H. ZHONG[2], R. R. ROY[3], V. EDGERTON[3], D. C. LU[4];
[2]Dept. of Integrative Biol. and Physiol., [3]Dept. of Integrative Biol. and Physiology, Brain Res. Inst., [4]Dept. of Neurosurgery, Brain Res. Inst., [1]UCLA, Los Angeles, CA

Abstract: Abstract: Epidural spinal cord stimulation (electrical enabling motor control, eEmc) is an emerging therapy for the treatment of paralysis resulting from spinal cord injury. When applied to the lumbosacral spinal cord, eEmc enhances voluntary limb control in paraplegic patients (Harkema et. al., 2011). To begin to determine whether this therapy could also be used to enhance arm function, we studied the electrophysiological properties of the cervical spinal cord networks converging to multiple forelimb motor pools in intact adult male Long Evans rats (n = 13). Selected arm/forearm muscles were implanted bilaterally with intramuscular EMG electrodes. Epidural electrodes were implanted medially at spinal cord levels C4, C6, and C8. Bipolar stimulation was applied at 1 Hz across pairs of epidural electrodes in six different combinations and evoked potentials were recorded *in vivo* from the muscles implanted with

EMG electrodes. Data from the homologous muscles bilaterally were grouped for analysis and the evoked responses were evaluated based on their amplitudes and delays. Stimulation from C6+C4- and C8+C4- were least effective in evoking responses in the forelimbs. The C6 to C8 region showed the unique property of effectively evoking forelimb responses regardless of the direction of stimulation. Our data indicate that eEmc at the C6 to C8 segments produces the largest responses in multiple upper limb muscles. The data presented here combined with data obtained after a paralyzing injury may provide a guideline for the more effective sites to stimulate in order to neuromodulate the excitability of specific motor pools and muscles and, thus, to facilitate the success rate in completing motor tasks post-injury.

Disclosures: L. Moore: None. **H. Zhong:** None. **R.R. Roy:** None. **V. Edgerton:** None. **D.C. Lu:** None.

Poster

241. Spinal Cord Injury and Plasticity

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 241.03/N26

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH R01HD041487

Title: Changes in step height following locomotor training are not related to change in reflex excitability or voluntary muscle strength

Authors: *E. C. FIELD-FOTE[1,2], K. J. MANELLA[3], L. MELBOURN[4];
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Abstract: Background and Purpose: In individuals with motor-incomplete spinal cord injury (iSCI), inadequate step height during walking contributes to decreased walking efficiency and increased risk of falls. In the absence of changes in strength, an increase in step height is a possible indication of increased corticospinal control after locomotor training. To date, no study has examined the effect of different body weight supported locomotor training (BWSLT) approaches on step height during overground walking in persons with iSCI. The purpose of this study was to determine if locomotor training influences step height during walking, how different approaches influence changes in step height, and whether these changes are related to dorsiflexor or hip flexor strength, or to spinal reflex excitability. **Participants:** Twenty-eight subjects with chronic (> 1 year) SCI who participated in a larger study to compare locomotor training approaches. **Methods:** Subjects were randomized to 1 of 4 BWSLT groups: treadmill-based training with manual assistance (TM), treadmill-based training with electrical stimulation

(TS), overground training with electrical stimulation (OG), and treadmill-based training with a locomotor robot (LR). Data were captured before and after 12 weeks of training. Step height during overground walking was analyzed based on 3D motion capture. Biomechanical and electrophysiologic measures of lower extremity reflex excitability included pendulum test, H-reflex excitability and post-activation depression, reciprocal inhibition, and flexor reflex excitability. **Results**: Across all locomotor training groups there was a significant increase in step height during self-selected overground walking speed ($p=0.031$); between-group differences in step height were not significant. Changes in reflex excitability did not correspond to changes in step height, however, in the OG group there was greater modulation of H-reflex low frequency depression, reciprocal inhibition, and flexor reflex excitability. There was no significant relationship between change in dorsiflexor or hip flexor motor scores and change in step height. **Conclusions and Clinical Relevance:** Motor control related to ability to clear the foot during walking is improved with locomotor training, and appears to be unrelated to changes in voluntary control of the ankle dorsiflexors or hip flexors. These changes also appear not to be reliant on changes in lower limb reflex excitability. These results support the concepts of task-specific training for improved quality of movement.

Disclosures: E.C. Field-Fote: None. **K.J. Manella:** None. **L. Melbourn:** None.

Poster

241. Spinal Cord Injury and Plasticity

Location: Hall A

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Program#/Poster#: 241.04/N27

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH Grant R01NS064964

VR Grant 21076

VR Grant 11554

Title: Changes in activity of spinal postural networks at different time points after spinalization

Authors: *T. DELIAGINA, P. V. ZELENIN, V. F. LYALKA, L.-J. HSU, G. N. ORLOVSKY; Karolinska Inst., Stockholm, Sweden

Abstract: Spinalization, i.e., a complete transection of the spinal cord results in loss of postural functions, which do not recover over time. Instead, spastic, incorrectly-phased motor responses to postural sensory signals gradually develop, and later, bursting activity in muscles appears, suggesting plastic changes in the spinal postural networks. The aim of the present study was to reveal these plastic changes, i.e., to characterize the activity of spinal postural networks at different time points after spinalization. For this purpose, rabbits in 3, 7, 14, and 30 days after

spinalization (at T12) were taken in acute experiments. After decerebration, the head and vertebral column were rigidly fixed, whereas the hindlimbs were positioned on a platform. Lateral tilts of the platform caused sensory inputs from limbs. In preparations with intact spinal cord, these inputs evoked postural limb reflexes, i.e., activation of extensors in the loaded and flexing limb and decrease in extensor activity in the opposite (unloaded and extending) limb. Activity of different motoneuron pools was assessed by recording EMGs of hindlimb muscles. Putative spinal interneurons were recorded extracellularly in L5. The data were compared with those obtained in our earlier studies on rabbits with intact spinal cord (Control) and in rabbits after acute spinalization. As in Control, at each time point after spinalization, neurons whose activity correlated with platform tilts were found, suggesting that they receive tilt-related sensory input from the limbs. It was found that during the first month after spinalization, the proportion of neurons activated with ipsi-limb flexion and its extension, as well as the source of their modulation did not change (as compared to the acute state). In contrast, their activity and depth of modulation (which exhibited a significant decrease just after acute spinalization as compared to control) reached the control value in 3 days after spinalization. At this moment, motor responses to platform tilts were practically absent. This result suggests that there are two processes of plastic changes in the postural networks, which are triggered by spinalization - a slow process of recovery of the motoneuronal excitability (taking months), and a rapid process of restoration of the normal activity level in spinal interneurons (taking days). In addition, considerable changes in the receptive fields of neurons were observed. During one month after spinalization, there was a dramatic increase in the relative number of neurons activated from skin receptors (60% vs 7% and 4% in control and after acute spinalization, respectively).

Disclosures: **T. Deliagina:** None. **P.V. Zelenin:** None. **V.F. Lyalka:** None. **L. Hsu:** None. **G.N. Orlovsky:** None.

Poster

241. Spinal Cord Injury and Plasticity

Location: Hall A

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Program#/Poster#: 241.05/N28

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH Grant 5R01NS064004-07

Craig H Neilsen Foundation Grant 261214

Title: Using silicon probe electrode recording arrays to examine changes in spinal cord neuronal activity produced by spinal cord direct current stimulation

Authors: *J. H. MARTIN[1], W. SONG[2];
[1]Physiol, Pharmacol & Neurosci, [2]Physiology, Pharmacol. and Neurosci., The City Col. of the City Univ. of NY, New York, NY

Abstract: Transspinal direct current stimulation (tsDCS) has been shown to modulate cortical motor output, potentially to promote motor function after injury, including immediate expansion of the motor cortex (M1) motor map (Song et al, 2015). The spinal cord contains myriad neuron types and processes in different locations, with different sensory and motor functions. It is not known if spinal cord neuronal activity in the various regions is differentially modulated by applied DC currents. Here we used a two-dimensional silicon probe electrode array in the cervical spinal cord to record local field potentials (LFPs) simultaneously from 32 sites to M1 or peripheral stimulation. We determined changes produced by applied cathodal and anodal DC currents. LFPs were recorded in anesthetized rats to phasic M1 (motor evoked spinal potentials, MESP) or cutaneous stimulation (sensory evoked spinal potential, SESP). DC was passed between a stainless steel screw implanted in the C7 vertebra and a skin patch electrode. We constructed latency and amplitude maps of the first negative peak that arises from the initial (presumably monosynaptic) depolarization of the neurons around the recording area. MESPs showed polarity dependent changes during tsDCS. Difference maps of latency and amplitude showed distinct patterns depending on whether the recordings were located dorsomedially, corresponding approximately to the medial dorsal horn, or ventrolaterally, in the lateral intermediate zone and motor pools. Dorsomedial neurons were activated first and c-tsDCS shortened latencies and increased amplitudes, whereas anodal tsDCS (a-tsDCS) had the opposite, and smaller, effect. C-tsDCS strongly increased the power of the evoked oscillatory activities, after wavelet decomposition, from low (alpha/beta) to high (gamma) frequency bands in the ventromedial spinal cord, whereas a-tsDCS showed the opposite effect. SESP latency was shortest dorsomedially and amplitude, largest ventrolaterally. tsDCS changed latency minimally. C-tsDCS increased responses ventrolaterally whereas a-tsDCS decreased responses. C-tsDCS decreased evoked oscillatory activity power dorsomedially and increased ventrolaterally, whereas a-tsDCS showed a weaker opposite effect. Our results show that tsDCS immediately and differentially modulates spinal network excitability depending on location and DC polarity, including the ventral motor regions of the gray matter. Our study provides further evidence for using tsDCS as a “bottom up” rehabilitation method after motor systems injury.

Disclosures: J.H. Martin: None. **W. Song:** None.

Poster

241. Spinal Cord Injury and Plasticity

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: Canadian Institutes of Health Research

Title: Effect of interlimb coupling on spinal reflexes during cycling

Authors: *R. ZHOU[1,2], J. ASSH[2], L. ALVARADO[3], S. CHONG[3], V. MUSHAHWAR[2,1]; [1]Ctr. for Neurosci., [2]Div. of Physical Med. & Rehabil., [3]Univ. of Alberta, Edmonton, AB, Canada

Abstract: The spinal networks in the cervical and lumbar cord are actively coupled during locomotion, and the involvement of the arms shapes leg activity. The nature of coupling and modulatory interactions between the arm and leg centres in the spinal cord during locomotion still remains largely unknown, especially after spinal cord injury (SCI). The goal of this study was to investigate the spinal arm-leg intersegmental connectivity during cycling through the modulation of spinal reflexes. Fifteen people with intact nervous system and 4 with chronic (>1 year) incomplete SCI (iSCI) participated in the study. The injuries were between C3 and C7. All iSCI subjects received 60 hours of coupled arm-and-leg active cycling training over 3 months. Intact participants underwent 2 experimental sessions. In the 1st, the role of leg cycling on modulating the H-reflex amplitude in the flexor carpi radialis (FCR) muscle was studied. Stimuli for evoking 50% maximal H-reflex were delivered in a randomized order, with the leg positioned in each of 4 quadrants within the cycling revolution to assess phasic modulation. The legs either actively cycled or were passively placed in one of the quadrants to investigate the influence of dynamic leg movement on the upper limb spinal reflex. The stimuli were delivered to the FCR when the muscle was relaxed or contracted at 10% maximal isometric voluntary contraction (MIVC). In the 2nd session, a similar procedure examined the effect of arm cycling on the Soleus (SOL) H-reflex amplitude. The peak-to-peak amplitude of H-reflex in FCR or SOL was compared across all conditions (stationary vs. cycling; phase-dependency; muscle at rest or 10%MVIC) using a 3-way ANOVA. The iSCI participants only completed the 2nd session with the SOL muscle at rest; the testing was conducted pre- and post-training. Results from the intact participants showed significant reduction in the amplitude of both FCR and SOL H-reflexes during dynamic cycling of the opposite limbs ($p \leq 0.026$) but no significant effect at various phases. In participants with iSCI, there was no significant reduction in the SOL H-reflex during dynamic arm cycling either pre- or post-training; however, the 3-month coupled arm-and-leg training tended to decrease H-reflex amplitude ($p=0.078$), indicating a reduction in spasticity. The results demonstrate that rhythmic movements significantly decrease spinal reflexes of the arms and legs through intraspinal connectivity in the intact nervous system. Although this intersegmental modulation was disrupted in people with chronic iSCI, the leg spinal reflexes may still be substantially reduced after a longitudinal training intervention.

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Poster

241. Spinal Cord Injury and Plasticity

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: National Science Foundation Grant 1402984

Shriners Research Foundation Grant 85900

Title: A tilting task for brain-machine interface control of balance after spinal cord injury

Authors: *M. MEYERS, N. BRIDGES, J. GARCIA, K. A. MOXON;
Sch. of Biomed. Engineering, Sci. and Hlth. Systems, Drexel Univ., Philadelphia, PA

Abstract: Postural control is a critical function of the lower body that is impaired after spinal cord injury (SCI). Brain-machine interfaces could be used to by-pass the injury, taking control signals from supraspinal structures and using them to control functional electrical stimulation below the level of the lesion. However, little is known about supraspinal control of posture and the impact of spinal cord injury on those neural control signals. Since the rat is a good model of human spinal cord injury, there is a need to develop a task that can be used to study postural control before and after SCI in the rat. Previous studies used a task in which a rat maintains balance on a platform that rotates or tilts in the frontal plane. After severe SCI animals will require body-weight support (BSW) in order to continue working in this task. To characterize the impact of BWS, the center of pressure (CoP) when healthy animals were tilted with and without BWS were compared. Female Long-Evans rats were trained to stand stationary on a tilting platform, and their behavior was monitored by three single-point load cells which measured the normal forces between the paws and the platform. The platform moved in a trapezoidal trajectory, with stationary periods at neutral and tilted positions. The CoP of the ground reaction force distribution was computed from the load cell signals in both the anterior-posterior dimension and the left-right dimension. The experiment was repeated with and without the animals connected to the BWS device, and three parameters of the CoP trajectory were compared between the two conditions: the mean CoP during the stationary period at zero degrees, the net displacement of the CoP during an early period of motion, and the mean CoP during the stationary period at the tilted position. Two different weight-support devices were used: one consisting of a Velcro strip connecting the animal to a horizontal bar, able to pivot up and down and supported by a spring (Overhead BWS), and the other consisting of a sling supporting the animal from below (Sling BWS). When the Overhead BWS was used, significant differences were found between the weight supported and control conditions in all three CoP parameters, suggesting that the animals relied on the BWS to maintain balance. When the Sling BWS was used, the difference in CoP parameters between conditions was attenuated. These results suggest that animals rely less on the Sling than the Overhead BWS. Therefore, studies investigating postural control systems after SCI should use a Sling BWS. This task and the Sling BWS were also used in a study of trunk muscle EMG and for a closed-loop BMI for posture.

Disclosures: **M. Meyers:** None. **N. Bridges:** None. **J. Garcia:** None. **K.A. Moxon:** None.

Poster

241. Spinal Cord Injury and Plasticity

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Program#/Poster#: 241.08/N31

Topic: D.10. Spinal Cord Injury and Plasticity

Support: 1I01 RX000243

Title: Interlimb coordination: Effect of training strategy and locomotor task post-SCI

Authors: ***K. A. CHEFFER**[1,2,3,4], W. A. O'STEEN[1,3,4], K. L. WHYLAND[1,3,4], S. K. WINTER[1,3,4], N. PELISCH[1,3], D. R. HOWLAND[1,2,3,4,5];
[1]Kentucky Spinal Cord Injury Res. Ctr., Louisville, KY; [2]Anatom. Sci. and Neurobio., [3]Neurolog. Surgery, Univ. of Louisville, Louisville, KY; [4]Robley Rex VA Med. Ctr., Louisville, KY; [5]Kosair Charities Ctr. for Pediatric Neurorecovery, Louisville, KY

Abstract: Proper coordination of fore-hindlimb movements is important for efficient and stable quadrupedal locomotion. This coordination is mediated by long propriospinal tracts connecting cervical and lumbosacral levels of the spinal cord in addition to input from supraspinal regions. Also important is right-left hindlimb coordination which can be mediated by segmental commissural neurons and modified by descending pathways. A lateral thoracic hemisection partially disrupts descending pathways to the lumbosacral cord, but does not directly modify segmental connections. The purpose of this study is to characterize recovery and determine how training impacts spatiotemporal coordination features during different voluntary gait challenges. Adult, spayed, female cats with low-thoracic, lateral hemisection injuries are divided into three groups: 1) Untrained; 2) Basic trained with a focus on reciprocal stepping in overground and/or treadmill environments; and 3) Skill trained which combines use of basic and adaptive tasks. Adaptive tasks included a 2” wide narrow beam and custom horizontal ladder. All animals are followed for 16-20 weeks post-injury. Semi-quantitative and quantitative analyses are used and include footfall and support pattern diagrams, phase diagrams, and E1-F interval analysis. Preliminary results suggest that recovery is impacted by training with respect to onset of general performance recovery. Trained groups show accelerated recovery in their ability to cross the ladder and narrow beam compared to untrained animals. Trained groups also incorporated the ipsilesional hindlimb earlier and to a greater degree. Pre-injury, regardless of task, fore-hind and right-left limb coordination is characterized by a 1:1 ratio and consistent spatiotemporal features and patterns at moderate gait speeds. At acute post-injury time points, the ratio and coordination are disrupted across tasks. Spatiotemporal features show the earliest and greatest recovery during basic overground walking. The more challenging tasks show greater disruptions and persistence of alterations. Strategies used post-injury show not only distinct differences compared to pre-

injury, but also across tasks. These new features of spatiotemporal coordination which emerge after injury include changes in the limb stepping ratios between the fore- and hind-limbs as well as the right and left hindlimbs. Analyses are being run to assess for correlations between spatiotemporal features, training approaches, and numbers of supraspinal and long propriospinal neurons with descending axons crossing the lesion site.

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Poster

241. Spinal Cord Injury and Plasticity

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Program#/Poster#: 241.09/N32

Topic: D.10. Spinal Cord Injury and Plasticity

Support: NSF Grant 1402984

Shriners Research Foundation Grant 85900

Title: Cortical control of balance using an interactive brain-machine interface

Authors: *N. BRIDGES[1], R. MADINENI[2], A. SHARAN[2], J. GARCIA[1], M. MEYERS[1], K. A. MOXON[1];
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Abstract: After severe spinal cord injury (SCI) at the mid-thoracic level, postural control is impaired, contributing to paraplegia. Studies examining cortical reorganization after SCI suggest that these networks learn novel control strategies that contribute to functional recovery. Little is known, however about the plasticity in the adult cortex involved with learning new control strategies for maintaining posture. Brain-machine-interfaces (BMIs) serve as a unique tool for studying such processes because they provide the animal a means to relate neurophysiological modulations with observed outcomes through an investigator-determined nervous system framework (i.e. a decoder). To this end, we developed a novel closed-loop BMI balance task driven by neuronal activity in response to postural perturbations. 16-channel microwire arrays (Microprobe, Gaithersburg, MD) were bilaterally implanted chronically within the hindlimb sensorimotor cortex of Long Evans rats. Rats were subjected to rotational tilts in the lateral plane. Tilts of varying initial jerk (acceleration change) were applied at random intervals. Neuronal activity was continuously recorded and the magnitude of the initial jerk was decoded. If the magnitude of the initial jerk was correctly decoded, the animal was rewarded by having the platform return to the neutral position. If the initial jerk was incorrectly classified, the platform was moved to a 25 degree tilt and held for 2.5 seconds before returning to the neutral condition.

Rats were able to successfully use the BMI to control the platform and neuronal tuning properties changed over time. These findings suggest that our task is uniquely suited to study feedback-based learning processes in postural systems.

Disclosures: **N. Bridges:** None. **R. Madineni:** None. **A. Sharan:** None. **J. Garcia:** None. **M. Meyers:** None. **K.A. Moxon:** None.

Poster

241. Spinal Cord Injury and Plasticity

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: NIH R01HD053854

Title: Changes in corticomotor excitability and hand use in persons with tetraplegia following stimulation-augmented task-specific versus resistance training

Authors: ***J. GOMES-OSMAN**[1], J. TIBBETT[2], K. BRISSON[2], K. ROACH[2], E. C. FIELD-FOTE[3];
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Abstract: Purpose/Hypothesis: Small improvements in hand function can make a meaningful difference in the lives of persons with tetraplegia. Somatosensory stimulation may increase voluntary drive through spared corticospinal pathways after spinal cord injury (SCI). We hypothesized that peripheral nerve somatosensory stimulation plus functional task practice training (PNSS+FTP) would be associated with greater improvements compared to PNSS alone, or conventional resistance training (CRT; universal gym). We also hypothesized that the changes in hand function would be reflected in changes in cortical excitability. Participants: Forty-nine individuals with chronic (> 1 year) tetraplegia due to SCI with neurological of level C7 or above. Materials/Methods: In this single-blind, randomized clinical trial, subjects were randomized to 1 of 3 groups PNSS+FTP, PNSS alone, or CRT; they participated 5 days/week for 4 weeks). Motor evoked potentials (MEPs) acquired with transcranial magnetic stimulation (TMS) were used to assess cortical excitability. Pinch and grip forces were measured via dynamometer. Eleven items from the Chedoke Arm and Hand Activity Inventory (scored based on 7 levels of assistance) were used to assess change in hand function associated with the interventions. Results: Baseline values of MEPmax and area under the recruitment curve were significantly lower for the PNSS+FTP group compared to CRT or PNSS. Post training change in these measures was also lowest the PNSS+FTP group. Conversely PNSS+FTP was associated with the greatest change in pinch (weaker hand=1.5±2.8 lbs, stronger hand=1.9±3.7 lbs) and grip force

(stronger hand=2.5±7.4 lbs). On average, PNSS+FTP improved 28.6% of Chedoke items that primarily involved pinch, compared to 20% and 15% for the PNSS and CRT groups. In contrast, CRT improved 31.4% of Chedoke items that primarily involved grip, compared to 24.5% and 22.9% for PNSS+FTP and PNSS. Conclusions and Clinical Relevance: Despite significantly lower baseline levels of cortical excitability in the PNSS+FTP group, this training was associated with the largest increases in pinch, grip strength, and functional pinch performance. CRT was associated with the largest increases in functional grip performance. PNSS is a clinically accessible approach to augmenting the effects of FTP, and this combined intervention appears to be valuable for improving hand function.

Disclosures: J. Gomes-Osman: None. **J. Tibbett:** None. **K. Brisson:** None. **K. Roach:** None. **E.C. Field-Fote:** None.

Poster

241. Spinal Cord Injury and Plasticity

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Topic: D.10. Spinal Cord Injury and Plasticity

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Craig H Neilsen Foundation #261214

Title: Cholinergic inputs on motor neurons are modulated by manipulating the corticospinal projection

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Abstract: Corticospinal (CS) axons are one major pathway that conveys motor commands from motor cortex to spinal cord. CS inputs are integrated with sensory inputs and modulated by interneurons before reaching motor neurons. Premotor cholinergic interneurons receive CS inputs and directly innervate motor neurons, and thus may be particularly important in this process. In this study, by manipulating the corticospinal projection, we found a close association between changes in CS axons and that of cholinergic terminals on motor neurons. We hypothesize that cholinergic inputs to motor neurons are modulated by corticospinal projection and, in turn, influence motor output. We compared changes in cholinergic terminals on motor neurons, putative “C-boutons,” in 4 groups of animals in which CS axons were manipulated in different ways. 1) Pyramidal tract lesion (PTx), which complete disrupts contralateral CS projections from one hemisphere; 2) Unilateral M1 inactivation with Muscimol (10mM) infusion (Inact), which suppresses the excitability of CS axons; 3) Cervical dorsal rootlet rhizotomy (DRX) which completely deprives sensory inputs and induces competitive sprouting of CS

axons; and 4) Proprioceptive afferent stimulation (ES) which induces sprouting of PA and CS axon retraction. We anterogradely trace CST with BDA from motor cortex, and visualized cholinergic interneurons by immunohistochemical staining with ChAT antibody. In the medial intermediate zone, cholinergic interneurons receive direct CS projection, whereas proprioceptive projection is very rare, indicating that corticospinal axons are one of the primary inputs to premotor cholinergic interneurons. We showed significant loss of contralateral CS axons and/or projections in PTx, Inact and ES groups of animals, and increased CS projections in DRx animals compared with that of control. C bouton density was analyzed and found to decrease in response to PTx, M1 inactivation, or electrical stimulation of PA. In contrast, it significantly increased after DRx. The change in cholinergic terminals paralles that of the corticopsinal projection in each group, thus suggesting that the projection from corticospinal axons positively regulate cholinergic inputs onto motor neurons. Due to the lack of direct CS projections onto motor neurons in rodents, the modulation of cholinergic inputs to motor neurons induced by CS axonal change could be the key mechanisms that affects motor function, especial after PT lesion. Cholinergic interneurons thus are a potential target for restoring impaired motor function after injury.

Disclosures: Y. Jiang: None. **J.H. Martin:** None.

Poster

241. Spinal Cord Injury and Plasticity

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: F32NS080393 to MAL

HD32571 to TRN

Title: Mapping intermuscular force dependent reflex pathways selectively using intramuscular stimulation in the decerebrate cat

Authors: *M. A. LYLE, A. CLOUTIER, T. R. NICHOLS;
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Abstract: Force feedback from Golgi tendon organs (GTOs) are well known to have widespread intermuscular connections that are mediated by interneurons that share inputs from muscle spindles, among others. Because current methods to study the strength and distribution of GTO circuits use nerve stimulation or muscle stretch, both of which also activate muscle spindle afferents, the selective role of GTOs remains uncertain. Here, we tested the hypothesis that intramuscular stimulation could be used to examine GTO circuits selectively. The hypothesis was evaluated by measuring the effects of intramuscular stimulation evoked contraction with that

of muscle stretch as donor inputs on the motor output of recipient muscles. Recipient muscle motor output was the force magnitude in response to 2 mm ramp-hold-release stretches. Each trial included 20 to 40 stretch repetitions with alternate stretches paired with a donor muscle input. Stimulation evoked contraction and muscle stretch as donor inputs were evaluated in separate trials. Muscle pairs evaluated included those already known in the cat to exchange muscle spindle afferents only (medial & lateral gastrocnemius), GTO afferents only (flexor hallicus longus & gastrocnemius), and muscle pairs that share both afferents (gastrocnemius & soleus). It was found that muscle stretch, but not intramuscular stimulation, provided excitatory inputs to the recipient muscles when the muscle pair shared only muscle spindle afferents. Both muscle stretch and intramuscular stimulation inhibited recipient muscles similarly when the muscle pair shared GTO afferents only. In muscle pairs evaluated that shared both muscle spindle and GTO afferents, intramuscular stimulation evoked inhibitory effects only. These preliminary data collectively support the hypothesis that direct muscle stimulation evoked contraction can be used to selectively map intermuscular interactions attributed to GTO circuits. Beyond the ability to selectively activate GTOs, stimulation evoked muscle contraction, the well-known physiological stimuli for GTOs, is versatile and noninvasive in contrast to current methods. We propose this approach could be used to substantially advance our understanding of the functional role of intermuscular force feedback.

Disclosures: **M.A. Lyle:** None. **A. Cloutier:** None. **T.R. Nichols:** None.

Poster

241. Spinal Cord Injury and Plasticity

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: 1I01 RX000243

Title: Kinematics analysis using 3D sagittal and novel multiple plane models for the study of gait recovery in quadrupeds with neurological disorders

Authors: ***N. PELISCH**[1,2], K. A. CHEFFER[1,2,3,5], N. BROWN[1,4,5], K. L. WHYLAND[1,2,5], S. K. WINTER[1,2,5], W. A. O'STEEN[1,2,5], G. BERTOCCI[1,4], D. R. HOWLAND[1,2,3,5,6];
[1]Kentucky Spinal Cord Injury Res. Ctr., Louisville, KY; [2]Neurolog. Surgery, [3]Anatom. Sci. and Neurobio., [4]Bioengineering, Univ. of Louisville, Louisville, KY; [5]Robley Rex VA Med. Ctr., Louisville, KY; [6]Kosair Charities Ctr. for Pediatric Neurorecovery, Louisville, KY

Abstract: Three dimensional (3D) kinematic gait analysis is an important noninvasive technique to assess and quantify normal gait features as well as changes following neural injury in quadrupeds. Challenges and effects due to choices made during data capture, manipulation, and

analyses are compared using data from SPF, purpose-bred, intact cats and those with thoracic spinal injuries. Cats are conditioned to cross a range of runways from a flat, wide surface to those requiring paw placement precision and significantly challenge balance. A 10-camera Vicon Motion Analysis system, and custom code, is used for all analyses. Typical techniques track pelvic and hindlimb markers in the sagittal plane. Typical marker positions include: iliac crest, greater trochanter, stifle (knee), lateral malleolus, and base of the fifth metatarsal. Due to skin movement during gait, a tracked stifle marker does not accurately identify the joint position. We compare three approaches to generate a projected stifle using different inputs: 1. Lengths of proximal and distal hindlimb segments; 2. Distal hindlimb segment length + a vector marker on the distal fibula 2-3 cms proximal to the hock (ankle joint); and 3. Lengths of proximal and distal hindlimb segments + vector marker. For many reasons, markers may not be tracked at various points. Effects regarding the minimum number of cameras required for initiating and tracking a marker as well as gap filling options are presented. Although sagittal plane capture is the most common approach in animal laboratories, it does not directly quantify key out-of-plane motions typical of recovered and/or adapted gaits. To more effectively capture clinically relevant kinematic changes, we are developing and evaluating a pelvis and bilateral hindlimb model using joint coordinate systems. Kinematic outcomes using this multiple plane approach include flexion-extension (also represented in the sagittal plane model), as well as internal-external rotation, and abduction-adduction of the hind limb joints. In addition to evaluating stifle projection techniques, virtual marker generation accuracy (as compared to actual marker placement) is tested as a method to replace lost and/or obstructed markers. Furthermore, the importance of a static marker capture, limb position during capture and impact on accuracy of marker generation during different step cycle subphases are evaluated. Valuable insights regarding movement and recovery can be achieved using many approaches, but comparisons across kinematic models and data capture, tracking, and analysis methodologies indicate when data may be compromised and results should be interpreted cautiously.

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Poster

241. Spinal Cord Injury and Plasticity

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: NSERC

FQRNT

Title: Lack of adaptation during prolonged split-belt locomotion in the intact and chronic spinal-transected adult cat

Authors: V. KUCZYNSKI[1], Y. THIBAUDIER[1], M.-F. HURTEAU[1], C. DAMBREVILLE[1], A. TELONIO[1], *A. FRIGON[2];
[1]Pharmacology-Physiology, [2]Physiol. and Biophysics, Univ. de Sherbrooke, Sherbrooke, QC, Canada

Abstract: In human adults, the pattern of coordination between the legs adjusts during prolonged assymetrical locomotion on a split-belt treadmill where the speed of the left and right sides is independently controlled. Specifically, some interlimb parameters, such as double support periods and step lengths return towards symmetry during prolonged split-belt locomotion and show after-effects upon returning to tied-belt (equal left-right speeds) locomotion (Reisman et al. 2005, J Neurophysiol 94:2403-15). The adaptation (return towards symmetry and after-effects) to split-belt locomotion is considered to be one of the first stages of motor learning. However, the neural structures involved in locomotor adaptation are largely unknown. Due to the central role of the spinal cord in producing the basic pattern of locomotion, we hypothesized that locomotor adaptation could be achieved, at least in part, by intrinsic spinal mechanisms. To test this hypothesis, 4 intact and 4 spinal-transected (spinalized) adult cats performed 2 min of tied-belt locomotion (Tied1), followed by 10 min of split-belt locomotion and a return to tied-belt locomotion for 2 min (Tied2). The fast-slow speed ratios used ranged from 1.5 to 2.0. During experiments, video recordings were made from the left and right sides. Stance durations, double support periods and step lengths were measured bilaterally. Stance durations for the left and right hindlimbs were approximately equal on the left and right sides (i.e. symmetric) during Tied1. During split-belt locomotion, stance duration on the slow side was longer than on the fast side and this did not change over the course of 10 min for both intact and spinalized cats. Left-right symmetry returned immediately during Tied2. Double support periods and step lengths showed a similar adjustment pattern, with left-right symmetry during Tied1, slow-fast asymmetry that did not change during 10 min of split-belt locomotion and a return to left-right symmetry immediately during Tied2 in both intact and spinalized cats. Therefore, intact and spinalized cats do not appear to display locomotor adaptation during prolonged asymmetrical stepping, at least with the fast-slow speed ratios used. The lack of return towards left-right symmetry might be due to the more stable nature of quadrupedal locomotion in the cat and/or to differences in the neural structures involved in adjusting stepping in asymmetrical conditions when compared to human adults that walk in an upright bipedal position.

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Poster

241. Spinal Cord Injury and Plasticity

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Topic: D.10. Spinal Cord Injury and Plasticity

Title: Examination of the mechanisms that contribute to the induction and maintenance of a peripheral memory

Authors: *M. M. STRAIN[1], J. D. TURTLE[1], K. C. HOY[2], J. W. GRAU[1];
[1]Dept. of Psychology, Texas A&M, College Station, TX; [2]Dept. of Neurosci., MetroHealth Med. Ctr., Cleveland, OH

Abstract: Prior work has shown that spinal neurons can support some simple forms of learning. In particular, instrumental learning can be shown in spinally transected rats by administering a leg shock (to the tibialis anterior [TA] muscle) whenever the hindleg is extended. Rats trained in this manner exhibit a progressive increase in flexion duration. This training produces both a central modification (that fosters future learning) and a peripheral change (that maintains the shocked leg in a flexed position for over 30 min). The latter effect has been related to alterations at the neuromuscular junction (NMJ) (i.e., training with contingent shock increases both acetylcholine (ACh) and NMDA receptor (NMDAR) labeling). Further, once the behavioral modification (the memory) is induced, it is maintained minus input from the spinal cord. Additionally, we have shown that microinjection of a NMDAR (MK-801) or ACh (curare) antagonist into the TA eliminates the behavioral modification. The current study further explores the mechanisms that contribute to the induction and maintenance of this peripheral memory. It begins by examining whether training induces a peripheral modification by measuring the strength of the muscular response using electromyography (EMG). Results found that spinally transected rats given 30 min of training with response-contingent (controllable) shock exhibited a stronger EMG relative to subjects that received non-contingent (uncontrollable) shock. Further, microinjecting MK-801 into the TA eliminated this effect. We hypothesize that efferent input from the spinal cord, in conjunction with TA stimulation, could induce the peripheral memory. To explore this possibility, spinally transected rats had their sciatic nerve cut and had stimulation applied to the peroneal nerve, either paired or explicitly unpaired with TA stimulation. Preliminary results show that pairing nerve stimulation with TA muscle activation causes a lasting (30 min) increase in flexion vigor. On-going experiments are examining how this effect is related to stimulus number, duration, and temporal order.

Disclosures: **M.M. Strain:** None. **J.D. Turtle:** None. **K.C. Hoy:** None. **J.W. Grau:** None.

Poster

241. Spinal Cord Injury and Plasticity

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Topic: D.10. Spinal Cord Injury and Plasticity

Support: CIHR FRN 79413

Title: Intra-spinal network for motor learning revealed by dI3 interneuron silencing in complete spinalized mice

Authors: ***N. STIFANI**[1], A. SAMPALLI[1], I. WRIGHT[1], T. V. BUI[3], T. AKAY[1], R. M. BROWNSTONE[2];
[1]Med. Neurosciences, [2]Med. Neurosciences and Neurosurg., Dalhousie Univ., Halifax, NS, Canada; [3]Dept. of Biol., Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Progressive recovery of locomotor function following complete spinal transection has been described in cats and mice. In humans, treadmill training provides rhythmic sensory inputs to spared spinal networks leading to significant improvement in gait. These observations suggest an evolutionarily conserved spinal network that undergoes rearrangement to restore fundamental locomotor function. Yet, to date mechanisms responsible for this functional recovery are unknown. dI3 interneurons (INs) are a population of glutamatergic dorsal spinal INs genetically characterized by the expression of LIM homeodomain protein Isl1. dI3 INs have been implicated in spinal processing of sensory afferents and have been shown to be essential for grasping function. In order to reveal spinal networks responsible for locomotor recovery, we studied mice in which the output of dI3 INs has been genetically silenced (dI3OFF mice). dI3OFF mice exhibit only minor locomotor deficits compared to their control littermates. After complete spinal transection at lower thoracic level, all mice exhibited initial hindlimb paralysis. Treadmill locomotor training induced a progressive functional recovery in control animals but had limited effects in dI3OFF mice. Our results demonstrate that dI3 INs are not crucial for locomotion under normal conditions, yet they are critical for locomotor recovery after complete spinal transection. Our results reveal the nature of intra-spinal circuits involved in locomotor recovery. These circuits have the potential to restore locomotion after spinal cord injury.

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Poster

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Craig H Neilsen Foundation #261214

Title: Examining the molecular mechanisms underlying activity-dependent corticospinal axon growth

Authors: ***N. ZAREEN**[1], S. DODSON[2], K. ARMADA[2], R. MISIR[2], J. H. MARTIN[2]; [1]Physiology, Pharmacol. and Neurosci., The City Col. of the City Univ. of New Yor, New York, NY; [2]Physiology, Pharmacol. and Neurosci., The City Col. of the City Univ. of NY, New York, NY

Abstract: The corticospinal tract (CST), which originates from the primary motor cortex (M1) and projects to spinal motor circuits, is the main pathway for evoking skilled voluntary movements. Spinal cord injury can interrupt this pathway, resulting in significant weakness or paralysis. We have shown in healthy adult rats, that CST neurons respond to increased neuronal activity produced by chronic M1 electrical stimulation by sprouting axons into the spinal gray matter, forming novel synapses with existing motor circuits and enhancing evoked muscle response. In animals with unilateral pyramidotomy, we showed that stimulation of the intact M1 results in complete restoration of skilled motor function. Thus, chronic stimulation is an effective repair strategy following CST injury. Our aim was to understand the molecular mechanisms underlying this activity-dependent sprouting. We focused on the role of the mTOR pathway. We used epidural electrodes to stimulate M1 for 6 hrs/day (330 Hz, 45 ms trains/2 sec) in adult Sprague-Dawley rats, using our published protocol (Brus-Ramer et al, 2007; Carmel et al, 2010) and anterogradely traced CST projections to monitor CST sprouting stereologically. M1 stimulation upregulated the expression of the neuron activity marker, cFos, in cortical neurons. Western blots and immunohistochemical data show that following both 1 day and 10 days of M1 stimulation, the levels of an mTOR activity marker, phospho-S6 (pS6) protein, are upregulated in neurons, especially in layer V, of the stimulated M1 but not in that of the sham animals. Elevated pS6 levels associated with significantly more segmental axons crossing the midline, more sprouting into the spinal gray matter, and a greater number of putative CST synapses. This suggests the mTOR pathway could be involved in CST axon sprouting following M1 stimulation. To test if mTOR is necessary for activity-dependent CST sprouting we blocked mTOR with rapamycin. pS6 levels in cortical neurons failed to increase in stimulated, rapamycin-treated animals. Notably, we found fewer segmental axons crossing the midline, less gray matter sprouting, and fewer synapses compared to the vehicle treated group, showing mTOR activation is crucial to CST remodeling following M1 stimulation. Activity-dependent CST remodeling is a proven strategy for recovering from CST injury and here we provide insight into its underlying molecular mechanism. The mTOR pathway integrates various extracellular signals to regulate protein synthesis and has been shown to associate with CST axon regeneration. We now show that the mTOR pathway is also responsible for activity-dependent CST axon sprouting in healthy adults.

Disclosures: **N. Zareen:** None. **S. Dodson:** None. **K. Armada:** None. **R. Misir:** None. **J.H. Martin:** None.

Poster

241. Spinal Cord Injury and Plasticity

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Topic: D.10. Spinal Cord Injury and Plasticity

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Title: Timing errors during lower limb cycling under various conditions

Authors: *A. VALY[1], J. LACZKO[2,1];
[1]Pazmany Peter Catholic Univ., Budapest, Hungary; [2]Information Technol. and Biorobotics, Univ. of Pécs, Pécs, Hungary

Abstract: Timing errors in movements of 15 able bodied individuals (age 24 ±4) were studied during lower limb cycling against various resistances at specific speeds. Maintaining cycling speed during training or rehabilitation exercises is often desirable but always a difficult task for participants performing lower limb cycling. A number of physical and neurological factors contribute to what speed a subject performs each complete revolution. The goal of this research is to present a method which significantly imporves speed control during cycling when feedback on speed is available. Voluntary able bodied paticipants cycled on an ergometer (SciFit ISO7000R, Germany) at two cadences and three resistance conditions (at 45 rpm: 2,55 Nm, 9,55 Nm, 22,30 Nm, at 60 rpm: 2,55 Nm, 15,92 Nm, 34,24 Nm), resulting in six exercise sessions each with the duration of 20 seconds. The participants received visual feedback on their speed via the ergometer touchscreen. We measured lower limb movement kinematics using a Zebris CMS-HS system (Isny, Germany), with 7 ultrasonic kinematic markers to calculate crank and joint angles. For the whole duration of the exercises we also calculated the time instants at which the crank would have returned to the top dead center (TDC, designated 0 degrees) if the cycling speed was constant. From our measurements we extracted the actual times at which the crank was at the TDC and calculated the time difference between ideal and measured time instances at which the crank would return to the TDC. This error increased through consecutive complete revolutions during each session for each individual. Increased timing errors were associated to successive cycles. There was no significant difference between the timing errors observed in different resistance conditions (t test, p=0.05). Comparing the participants, we found that at low resistance the difference between timing error of the fastest and slowest participant was 1240 ms at 45 rpm and 810 ms at 60 rpm. At medium resistance this dropped to 580 ms at 45 rpm and 690 ms at 60 rpm. Going to high resistance timing errors further dropped but at a lower rate, to 460 ms at 45 rpm and 640 ms at 60 rpm. High resistance may result at an earlier fatigue. From the aspect of physical training and medical rehabilitation our results suggest, that employing a medium crank resistance when cycling on an ergometer enables subjects to better keep the desired cycling cadence producing smaller timing errors.

Disclosures: **A. Valy:** None. **J. Laczko:** None.

Poster

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Shriners Research Foundation Grant 85900

Title: The role of rat trunk muscles in postural control

Authors: *J. GARCIA, N. BRIDGES, M. MEYERS, K. A. MOXON;
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Abstract: Understanding the role of trunk muscles during postural control is important for restoring function after injury. Severe spinal cord injury results in loss of supraspinal control of lower body musculature leading to loss of postural control. Previous studies suggested that enhanced supraspinal control of trunk muscles could compensate for this loss. To understand how these muscles are activated during postural control a task in which a rat maintains balance on a platform that rotates or tilts in the frontal plane was used. Female Long-Evans rats were tilted in the frontal plane with varying initial jerk. The target angle of the tilt was set to either 15 or 25 degrees. Animals were trained to stand on the tilting platform and then chronically implanted with electromyography (EMG) electrodes into the rostral and caudal (relative to T8) longissimus unilaterally and spinotrapezius bilaterally. For each trial, after reaching the target angle the platform remained in this position for one second before returning to the neutral position. There was a 2 second inter trial interval where the platform remained in the neutral position. The position of the platform was monitored using an accelerometer and timestamps indicating the beginning and end of platform movement were recorded. After a 3-5 day recovery period, rats were again placed on the platform. EMG was acquired at 5 kHz, filtered (10-500 Hz, 4th order Butterworth) and rectified. Bursts were determined using a threshold equal to baseline activity plus 3 standard deviations. Probability of a burst and average number of bursts per unit time were examined in four windows: NEUTRAL position, START of tilt, HOLD at max tilt and RETURN to neutral position. Overall, the probability of a muscle burst was dependent on the degree of tilt. Differences in activation of spinotrapezius muscles compared to longissimus muscles was dependent on the direction of tilt (left versus right) and the magnitude of tilt. Understanding the role of these muscles in normal animals will lend insight into the changes that occur after spinal cord injury and help to develop strategies to restore posture.

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Poster

241. Spinal Cord Injury and Plasticity

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Support: NIH R00-111215

Title: Spinal pathways for the coordination of swallow and cough

Authors: *T. PITTS[1], S. KING[2], D. R. HOWLAND[2];
[1]Kentucky Spinal Cord Res. Ctr., [2]Univ. of Louisville, Louisville, KY

Abstract: The respiratory spinal network is activated by both cough and swallow. However current computational models do not account for local changes in excitability with the spinal cord. We compared in silico simulations to *in vivo* data from anesthetized cats. Coughs were elicited by mechanical stimulation of the intra-thoracic trachea. Electromyograms (EMG) of the parasternal (PS) and transversus abdominis (TA) muscles identified cough . Swallow was elicited by injection of water into the pharynx and EMGs of the thyroarytenoid (ThAr), PS, and geniohyoid (GH) identified swallow. Simulations predicted PS EMG amplitude of "post-swallow" cough would be greater than "pre-swallow" cough and this effect was observed *in vivo*. However, important temporal features that govern cough and swallow coordination when both behaviors are co-expressed were not observed in silico, such as, during repetitive coughing, the expiratory phase (E2) of cough is prolonged when swallow is present. We conclude the current model is predictive; the in silico simulations reproduce several important features of *in vivo* airway protection; however some temporal relationships between cough and swallow are not currently accounted for by the model. Supported by NIH R00-111215

Disclosures: T. Pitts: None. **S. King:** None. **D.R. Howland:** None.

Poster

242. Motor Control: Novel Techniques

Location: Hall A

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Program#/Poster#: 242.01/N44

Topic: D.13. Motor Neurons and Muscle

Support: The Ronald Dr. Deffenbaugh Family Foundation

NIH/NINDS R37 NS030853

Title: A three-dimensional map of the hindlimb motor representation in the lumbar spinal cord in sprague dawley rats

Authors: *J. A. BORRELL[1,6], S. B. FROST[2,3], J. PETERSON[4], R. J. NUDO[5,3];
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Abstract: The purpose of this study was to derive a three-dimensional topographical map of functional motor outputs from the lumbar spinal cord to hindlimb skeletal muscles as defined by intraspinal microstimulation (ISMS). Experiments were carried out in healthy, adult, male, Sprague Dawley rats using commercial neurophysiological recording equipment. Under ketamine/xylazine anesthesia, fine wire electromyographic (EMG) electrodes were implanted into 4 muscles in both hindlimbs: the lateral gastrocnemius (LA), tibialis anterior (TA), vastus lateralis (VL), and biceps femoris (BF) (total 8 muscles). After a laminectomy of the T13-L1 vertebrae and removal of the dura matter, a four-shanked microelectrode array (NeuroNexus Technologies) was used for ISMS. Shanks were 200 µm apart, and each shank had four 40 µm diameter stimulation sites that were 200 µm apart. Each stimulation site had an impedance in the range of ~30-40 kΩ at 1 kHz. The electrode array was inserted into the spinal cord at the lumbar level (under vertebrae T13-L1) along a three-dimensional (200 µm by 200 µm by 200 µm) stimulation grid. Stimulation sites ranged from ~140-2200 µm below the dorsal surface of the spinal cord, from ~400-800 µm lateral to the midline on both sides of the spinal cord, and from ~0.6-10 mm from the caudal end of the T12 vertebra. Three pulse biphasic trains with 200 µs duration square-wave per pulse at 300 Hz per train and each train separated by 1 sec were used to determine evoked movements and EMG activity. Movement threshold was determined by the lowest stimulation current that evoked a visible and consistent joint displacement. Stimulus-Triggered Averaging (StTA) was used on rectified EMG data to determine response latency. The ISMS responses typically consisted of leg, hip, knee, ankle, and toe movements. There was predominant hip movement in rostral T13. In caudal T13, there was a shift towards medial leg rotation. Moving caudally to L1, there was a mixture of ankle movement, digit movement, and medial leg rotation with no clear arrangement in either hindlimb. Further caudally in L1, there was predominant knee movement in both hindlimbs. StTAs of EMG activity showed a latency of ~4 ms. The derived motor map gives insight into the layout of the motor neuron pools within the hindlimb area of the normal spinal cord.

Disclosures: **J.A. Borrell:** None. **S.B. Frost:** None. **J. Peterson:** None. **R.J. Nudo:** None.

Poster

242. Motor Control: Novel Techniques

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Program#/Poster#: 242.02/N45

Topic: D.13. Motor Neurons and Muscle

Support: NS37400

Title: Increase in fALFF-measured spontaneous neuronal activities in sensorimotor cortices after muscle fatigue

Authors: *Z. JIANG[1,3], G. H. YUE[2,3,4];
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Abstract: Background: Prolonged muscle exertion results in muscle fatigue that may cause reduced performance and productivity, and increased chances of injury. Although peripheral mechanisms underlying voluntary muscle fatigue are relatively well known, central contributions to the fatigue are much less understood. The current study investigated brain signal oscillations during resting state before and immediately after a muscle fatigue task (MFT). Resting state functional MRI (rsfMRI) data of the sensorimotor cortices were analyzed by computing fractional amplitude of low-frequency fluctuation (fALFF). Recent rsfMRI studies have demonstrated that fALFF of BOLD signals are associated with spontaneous neuronal activities especially those involved over long distance communication in the brain. We hypothesized that sensorimotor network in the brain after MFT would experience abnormal fALFF compared to before MFT. Aim: Identify muscle fatigue-related dynamic signal adaptations during resting state in sensorimotor cortices by using rsfMRI signal-based fALFF, a novel frequency-domain signal analysis tool. Method: Ten subjects performed a 20-minute intermittent (3.5s ON/6.5s OFF, 120 trials total) handgrip task using the right hand at 50% maximal voluntary contraction (MVC) force level that induced significant fatigue. The rsfMRI slices covering the primary motor (M1) and somatosensory (S1) cortices were collected before and after the task using a 3T Siemens Trio scanner and an echo planar imaging sequence. All rsfMRI data were first motion corrected followed by spikes and linear trends removal using AFNI. Images were co-registered to high resolution structural scans collected in the same session. Region of interests were obtained from parcellation results using Freesurfer. The fALFF analysis was carried out using scripts released by 1000 Functional Connectomes Project (http://fcon_1000.project.nitric.org). Minor modifications were made to set up the batch running for our own data. Result: We observed marked shifting to higher fALFF in all analyzed brain regions: bilateral S1 and bilateral M1. Wilcoxon signed rank test confirmed significant fALFF increases in all regions (all $Ps<0.001$). This finding suggests augmented exchange of information among the M1 and S1 regions over a relative long distance (crossing hemispheres) after muscle fatigue. Conclusion: The increase of fALFF represents likely a physiological recovery process related to restoration of normal resting-state signal oscillations in the sensorimotor cortices after a temporary perturbation from the normal state by stressful muscle fatigue.

Disclosures: **Z. Jiang:** A. Employment/Salary (full or part-time):; Department of Biomedical Engineering, New Jersey Institute of Technology, Newark,NJ USA. **G.H. Yue:** None.

Poster

242. Motor Control: Novel Techniques

Location: Hall A

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Program#/Poster#: 242.03/N46

Topic: D.13. Motor Neurons and Muscle

Title: Unbiased estimate of the spinal cord neuronal population involved in primate motor control

Authors: *N. WILSON, P. ROSE, S. SCOTT;
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Abstract: The spinal cord plays an essential role in the sensory and motor functions that give rise to behaviours ranging from simple reflexes to complex goal directed movements. The ratio between motoneurons and interneurons in the spinal cord has been commonly computed to understand the relative computational capabilities of spinal motor networks (Walloe, 2011). Currently, there are few accurate estimates of the number of motoneurons and interneurons within the non-human primate spinal cord and this ratio is not well defined. Neurons within the spinal cord grey matter of non-human primates were quantified to provide a more robust anatomical framework for modelling upper limb motor control. The spinal cords of rhesus monkeys (Macaca mulatta) were examined from segments C2 to T1 using stereological methods to estimate the total number of neurons in specific areas of the grey matter. These areas included both those containing motor neurons (Lamina IX) as well as those containing interneurons associated with motor pathways (Laminae V - VIII). Total number of neurons was calculated to be 216,600 ± 5000 in laminae V and VI, 140,100 ± 4100 in VII and VIII and 52,500 ± 3300 within lamina IX. A ratio of 1:25 in the rhesus monkey was calculated using motoneuron and interneuron estimates. This ratio is higher than that of other mammalian and non-mammalian vertebrates, indicating that a more robust spinal network may underlie the ability of primates to generate complex upper limb movements.

Disclosures: **N. Wilson:** None. **P. Rose:** None. **S. Scott:** None.

Poster

242. Motor Control: Novel Techniques

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 242.04/N47

Topic: D.13. Motor Neurons and Muscle

Title: Feasibility of using causality changes to monitor fatigue during forearm repetitive movement in typically developing children

Authors: ***Y.-N. WU**[1], Y. CAO[2], C. STARK[1], K. CHAN[1], S.-C. YEN[3];
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Abstract: Fatigue contributes to declined motor performance in people with and without neurological disorders. When the high repetition of movement is required to engage neural plasticity during neurorehabilitation or skill learning, excess practice can cause fatigue that might be detrimental (causing longer period of rest that interrupts the neurorehabilitaiton routine). A reliable way to monitor fatigue developing and then guide practice is valuable yet less available. Fatigue is task and population dependent. Weakened muscle contraction force might not be a sensitive indicator to reflect fatiguing during sub-maximum contractions, which are often involved in nuerorehabilitation. Meanwhile electromyography (EMG) amplitude and spectrum analysis of maximum voluntary contraction has been extensively used to study muscle fatigue, but it lacks the dynamic information. On the other hand fMRI study demonstrating neural network disconnection due to muscle fatigue implies coupling changes occur when fatigue develops. To seek a potential tool for real-time fatigue monitoring, we aimed to apply EEG with Granger causality algorithm to examine the causal relation between motor function-related cortical areas during repetitive forearm movement. Eight typically developing children (9.7±1.9 y/o, 4 boys and 4 girls) were recruited in the study. 32-channels EEG with 10-10 system for electrode positioning was used to capture brain activities. Surface EMG electrodes were placed on the forearm muscles of both upper extremities of the child. The child was then seated in front of the robot that has sensors to recognize forearm motion. Through this robot, the child played ten (or less) 3-minute video games by repetitively rotate the left forearm. EEG, EMG, kinetic data and a modified perceived exertion scale were recorded for each game the child played. The task stopped before reaching 10th game if a child expressed really hard to perform (exertion scale= 7) at the end of the game just played. Signals of selected five electrodes overlying five distinct motor function-related cortical areas including C3, C4, Cz, Fz and Pz were used to examine any causality at each game using bi-vitiate Granger causality. The preliminary results show a trend of decreased causality strength, particularly between C4 and Pz while comparing the first 3-minute data and the data when a child first expressed starting to feel hard to perform the task. The decreased causality strength might be due to strengthened coupling of many brain areas activation, such as M1, PM, S1 and bilateral PFC reported in fMRI studies. Our results added another piece of evidence of using EEG as a tool to assist rehabilitation practice.

Disclosures: **Y. Wu:** None. **Y. Cao:** None. **C. Stark:** None. **K. Chan:** None. **S. Yen:** None.

Poster

242. Motor Control: Novel Techniques

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Topic: D.13. Motor Neurons and Muscle

Support: NIH (R21AG045766)

Title: Motor cortical input-output characteristics of the lower extremity in young and old adults

Authors: *H. HASSANLOUEI, C. W. SUNDBERG, J. SENEFELD, A. KUPLIC, S. K. HUNTER;
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Abstract: Changes in corticospinal excitability may be partially responsible for the age-related decline in neuromuscular function of the lower limb. Whether there are age-related differences in the input-output characteristics in lower limb motor cortical areas is not known. The purpose was to compare the input-output curves of motor evoked potentials (MEPs) elicited in motor cortical areas targeting the quadriceps muscles of young and old adults. MEPs of the vastus lateralis (VL) were recorded from 8 recreationally active young (19-30 yr, 3 women; 55 ± 25 MET.hr/week) and 11 old adults (61-79 yr, 5 women, 52 ± 40 MET.hr/week) with bipolar EMG electrodes by delivering single-pulse transcranial magnetic stimulation (TMS) over the motor cortex contralateral to the knee extensor muscles. The active motor threshold (AMT) was determined as the minimum stimulator intensity eliciting MEPs in the VL in at least 4 of 8 trials while subjects performed intermittent isometric contractions at 10% maximal voluntary contraction (MVC) (~3 s contraction, ~7 s relaxation). Ten stimuli were delivered (1 per 3 s contraction) at each stimulator intensity ranging from 90-165% of the AMT in 5% increments until: 1) a minimal increase in MEP amplitude was observed with increased stimulator intensity, or 2) the maximum stimulator output was reached. Electrical stimulation of the femoral nerve was used to elicit the maximal compound muscle action potential of the VL (Mmax). All VL MEP amplitudes were expressed as a percentage of Mmax and plotted against the stimulator output (%AMT) to generate an input-output curve. Input-output curves were fit with a 3-parameter Boltzman sigmoidal function to obtain the estimated maximal MEP amplitude (MEPmax), the stimulus intensity required to elicit a response equal to half MEPmax (S50), and the peak slope of the sigmoidal curve. The Mmax was less in old adults (7.7 ± 3.5 mV) compared with young adults (13.1 ± 3.5 mV, $P<0.05$). AMT was similar between the young and old adults (49.6 ± 8 vs 49.2 ± 8 % stimulator output). When the MEP amplitudes were expressed as a percentage of Mmax, there were no differences between young and old adults in MEPmax (30.2 ± 20.6 vs 35.3 ± 17.2 %), S50 (118 ± 10 vs 121 ± 14 %) and the peak slope (0.095 ± 05 vs 0.080 ± 02, $P>0.05$). Thus, among active older adults, aging does not appear to interfere with the motor cortical input-output characteristics of the lower limb during low-level volitional activity.

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Poster

242. Motor Control: Novel Techniques

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Topic: D.13. Motor Neurons and Muscle

Support: R01NS080954

Title: Peripheral motor neuron inhibition using novel inhibitory opsins

Authors: *C. GORINI, C. RAMAKRISHNAN, K. DEISSEROTH, S. DELP;
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Abstract: Peripheral motor neuron disorders, such as spasticity and cerebral palsy, result in excessive muscle tone and hyperreflexia. Modulation of motor neurons responsible for these dysfunctions is often difficult; however, optogenetic tools provide a method by which this hypertonia may be relieved. Viral methods for sufficient inhibitory opsin expression have proved difficult, as different virus types can produce dramatically different levels of opsin expression and trafficking. Here, we focus on achieving peripheral motor neuron inhibition using an AAV6 viral vector in conjunction with novel inhibitory, light-activated opsins in both wild type and nude mice. Two to five weeks after injection of the viral opsin construct into the medial and lateral gastrocnemius of four week old mice, we observe a reversible decrease in electrically generated Achilles tendon force upon light illumination of the sciatic nerve. This decrease is accompanied by a corresponding reduction in EMG activity in the medial gastrocnemius. Additionally, histology indicates robust retrograde transduction of opsin in the nerve and spinal cord following intramuscular injection of the gastrocnemius. Future directions include testing a variety of inhibitory opsins and viral routes, as well as classifying opsin trafficking malfunctions and any immune response preventing transfection of motor neurons.

Disclosures: C. Gorini: None. **C. Ramakrishnan:** None. **K. Deisseroth:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Circuit Therapeutics. **S. Delp:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Circuit Therapeutics.

Poster

242. Motor Control: Novel Techniques

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Topic: D.13. Motor Neurons and Muscle

Support: NIH Grant 5T32EB004314-15

VA CDA2: B9043W

Title: Muscle coordination patterns underlying dynamic hand function

Authors: *N. M. COLE[1,2], A. B. AJIBOYE[1,2];
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Abstract: A long standing theory of motor control is the neuromotor system simplifies control of multi-degree-of-freedom (DOF) movements by recruiting coordinated patterns of muscle activation (muscle synergies), which are combined to produce motor output. While there exists debate about whether muscle synergies are a physiological phenomenon, a muscle synergy based framework may simplify control of functional electrical stimulation (FES) arm and hand neuroprostheses. While many studies have extracted synergies associated with naturalistic arm reaching and static hand postures, the current study aimed to investigate muscle activation synergy patterns underlying dynamic hand tasks involving everyday object manipulations. In able-bodied persons, we recorded electromyogram (EMG) signals from twelve intrinsic and extrinsic hand muscles that were representative of muscles typically implanted for current FES hand systems. Fine wire electrodes, along with ultrasound image guidance ensured selective recording of the tightly bundled muscles. Hand kinematics (18 finger and wrist joint angles) were recorded with a CyberGlove. Each subject performed 10 - 15 trials of 13 tasks that replicate activities of daily living and include different postures, gross and fine movements, and various force levels (e.g. opening a jar, turning a doorknob, or writing). Synergies were extracted from the EMG recordings using a cross-validated NNMF decomposition algorithm. Synergy models are determined for each subject and compared across subjects to identify common patterns during the dynamic hand tasks involving object manipulation. We found that the 13 tasks and 12 muscles could be reduced to 6-8 static muscle synergy patterns accounting for 77 - 95% of the variance in the recorded data. Identified muscle synergy patterns could be visualized as whole hand opening (finger, thumb, and wrist extensors), hand closing (finger, thumb, and wrist flexion), and opening of the thumb and index finger (extensor indicus, distal thumb extension, and thumb abduction). Overall, this study establishes a set of generalizable muscle synergy patterns, with several of the identified patterns found to be similar across subjects. This work lays the foundation for evaluating if the identified synergies can be used for closed-loop control of an implanted FES hand system.

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Poster

242. Motor Control: Novel Techniques

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Program#/Poster#: 242.08/O3

Topic: D.13. Motor Neurons and Muscle

Support: 5R01NS047357

2P01NS057228

Title: Rabies virus retrograde tracing of monosynaptic premotor spinal interneuron networks extended to mature animals

Authors: L. GOMEZ-PEREZ, R. W. GRIFFITH, *F. J. ALVAREZ;
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Abstract: Locomotion matures during postnatal development. In human infants this is evidenced by the reversal of reflex signs and a progressive decline in co-contraction, suggesting spinal circuit modifications. However, possible changes in the connectivity of premotor interneurons (INs) are unknown because lack of methods to map their connections throughout development. Recently, a modified rabies virus (RV) vector was used to identify premotor networks in neonatal animals, but this technique reportedly does not work in mature animals. The method relies on infecting motoneurons (MNs) with a RV lacking the glycoprotein (Gly) gene necessary for infection replaced by a fluorescent protein. Transduction of MNs with AAVs carrying the Gly gene allows passage of mCherry or EGFP RV to INs connected to these MNs. RV does not move beyond this first monosynaptic step because INs lack the Gly gene, resulting in labeling the assembly of INs monosynaptically connected to the infected motor pools. Co-injection of both vectors in specific muscles has been used to dual infect motor pools. We confirmed that co-injections in the lateral gastocnemius muscle (LG) result in abundant IN labeling when done at P5, but not at P15. To characterize MN transduction by RV and AAV we systematically injected mCherry-RV and EGFP-AAV1 in the LG of animals of different ages (P5, P10, P15, P30, adult) and studied transduced MNs and INs at different postinjection times. RV infection is rapid but results in MN degeneration, with the period of MN stability becoming progressively shorter with age. RV-infected MNs are stable for two weeks after P5 infections, but only for a week at P15. Also, RV infection rates diminish at P30. Moreover, co-infection of muscle fibers with mCherry-RV and AAV1-Gly optimizes the number of RV-infected MNs, likely because muscle becomes a source of RV particles. In contrast, AAV1 MN transduction peaks two weeks after injection and the temporal mismatch between MN survival and AAV transduction increases with age causing the decrease in efficiency of the method. To overcome this problem we used mouse transgenics and sequential injections to express the Gly-AAV1 in muscles and/or MNs prior to RV infection and successfully extended the age range of the method. The preliminary results suggest that the complement of INs premotor to the LG pool does indeed change from P5 to P15. We are working to optimize RV infection past P30 and characterize the RV passage mechanism because when Gly is highly expressed in MNs labeling of high numbers of glial cells suggest abundant extrasynaptic transfer. In addition, RV virus enters both MNs and sensory afferents and both sources need to be considered.

Disclosures: **L. Gomez-Perez:** None. **R.W. Griffith:** None. **F.J. Alvarez:** None.

Poster

242. Motor Control: Novel Techniques

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Topic: D.13. Motor Neurons and Muscle

Support: GlaxoSmithKline

Title: A novel wireless microelectrode array implanted chronically provides reliable and selective stimulation

Authors: G. S. BENDALE[1], A. KANNEGANTI[1], F. DEKU[1], S. BREDESON[3], *J. L. SEIFERT[2,4], P. TROYK[3], S. COGAN[1], M. I. ROMERO-ORTEGA[1,4];
[1]Bioengineering, UT Dallas, Richardson, TX; [2]Bioengineering, UT Dallas, Little Elm, TX; [3]Biomed. Engin., Illinois Inst. of Technol., Chicago, IL; [4]UT Southwestern, Dallas, TX

Abstract: Peripheral neural interfaces play a vital role in providing motor control and sensory feedback following nerve damage due to injury or disease. These interfaces must be highly stable, chronically reliable, biocompatible and safe for long-term use. It is also important to meet patient needs in terms of comfort and ease of operation. Tethered electrodes have long wires and bulky external head-stage connectors that are potential points of failure and have been shown to be sources for inflammation. Wireless technology has vastly improved the field of biomedical devices and addresses many of the limitations of current interface designs. In this ongoing study, we have examined the stability and reliability of wireless floating microelectrode arrays (WFMAs) implanted in the sciatic nerve of adult female Lewis rats over a period of 6 months, by evaluating behavioral responses to *in vivo* stimulation. At various time points (days 1, 71, 141 and 172 post-implantation), stimulation thresholds were measured for each of the 16 electrodes on two different WFMAs, by correlation with hind limb motor response. The threshold current values (i.e., the minimum current required to elicit the motor response) did not change significantly over time ($p \geq 0.05$), thus supporting the reliability of the interface. We observed three distinct movements elicited by stimulation: dorsiflexion, plantar flexion and abduction. The types of movement elicited corresponded with the location and size of the electrodes, providing for stimulation specificity. The measured charge per phase for each electrode was below the tissue damage limit of 4nC/phase over the entirety of the time points evaluated, indicating the safety of the interface. In order to assess whether stimulation was causing pain, behavior was evaluated in an awake animal with repeated stimulation at the pre-determined threshold level. No signs of pain or discomfort were observed, i.e., biting, licking, vocalization, or chewing, thus indicating that the wireless stimulation does not activate pain fibers at the motor threshold level. The various sizes and heights of electrodes can be used to interface specific fascicles, thus

targeting unique muscle groups, which can be relevant in a wide range of clinical conditions. We thus propose that the specific distribution of electrodes along with the independence offered by the wireless interface will help achieve safe, reliable, targeted interfacing.

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Poster

242. Motor Control: Novel Techniques

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Program#/Poster#: 242.10/O5

Topic: D.13. Motor Neurons and Muscle

Title: A concurrent TMS/tACS approach to investigate the function of intrinsic brain oscillations in the motor system

Authors: *N. WENDEROTH, K. RUDDY, M. BÄCHINGER;
Neural Control of Movement Lab, ETH Zurich, Zurich, Switzerland

Abstract: The functional role of intrinsic oscillatory brain activity for motor control remains largely unknown. It has been suggested that alpha oscillations reflect functional inhibition of task irrelevant regions, as a means of directing information flow in the brain (Jensen & Mazaheri, 2010). Further, using electroencephalography (EEG) it has been reported that motor evoked potentials (MEPs) resulting from transcranial magnetic stimulation (TMS) are smaller when power in the alpha band is high and larger when it is low (Sauseng et al., 2009; Zarkowski et al., 2006). Even within the alpha cycle, it is possible that phase dependant modulation of excitability may occur, as has been demonstrated already for the visual system (eg. Busch et al., 2009). No such evidence exists for the motor system. It has been shown previously that intrinsic brain oscillations can be entrained by transcranial alternating current stimulation (tACS). This method is most efficient when the externally imposed frequency matches the individual's endogenous peak alpha frequency. Here we applied tACS with the intention to (1) synchronize alpha oscillations in the brain of the participant with the external, alternating current and to apply TMS in a phase locked manner; and (2) to increase the power of the alpha rhythm over primary motor cortex. TMS was applied before, during and at three timepoints after tACS stimulation (alpha frequency of tACS stimulation matched to individual alpha peak measured with EEG, 2 mA peak-to-peak intensity), to probe the excitability of corticospinal projections to the first dorsal interosseous (FDI) muscle. During stimulation, TMS was timed to coincide with peaks, troughs, rising and falling edges of the imposed tACS alpha cycle. Participants returned for two additional control conditions on separate days; one with tACS+sham TMS, and another with TMS+sham tACS. The order of sessions was counterbalanced across subjects. Preliminary results suggest no systematic decreases in MEP amplitude as a result of tACS stimulation, and

shed light on important methodological considerations for performing TMS recordings during tACS entrainment.

Disclosures: **N. Wenderoth:** None. **K. Ruddy:** None. **M. Bächinger:** None.

Poster

242. Motor Control: Novel Techniques

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Topic: D.13. Motor Neurons and Muscle

Title: Isolation and purification of actively translated rna from spinal motor neuron axon terminals

Authors: ***J. SHADRACH**[1], B. PIERCHALA[2];
[2]Biologic and Materials Sci., [1]Univ. of Michigan, Ann Arbor, MI

Abstract: Coordinated motor function is achieved by proper communication between spinal motor neurons (MNs) and muscle fibers at a specialized synapse called the neuromuscular junction (NMJ). While dysfunctions of the neuromuscular system that lead to diseases such as spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS) have complex disease etiologies, recent evidence suggests that deficient RNA processing and trafficking might form a common mechanism that underlies disease pathogenesis. To better understand the types of motor axon mRNA transcripts required for the normal maintenance of the NMJ, we have utilized the RiboTag transgenic mouse model developed by Sanz et al. 2009 to specifically isolate ribosomal complexes and their associated mRNA transcripts. Crossing RiboTag mice to a Cre line results in the expression of an HA-tagged RPL22 (RPL22-HA) protein in Cre expressing cells. As a core member of the ribosome particle, this RPL22-HA is incorporated into the ribosome complex such that the entire complex and any associated mRNA transcript can be immunoprecipitated with an HA-antibody. We expressed RPL22-HA specifically in MNs by crossing RiboTag mice with choline acetyltransferase (ChAT)-Cre mice. As expected, spinal MNs in these RiboTag;Chat-Cre mice demonstrate strong HA+ immunofluorescence. Additionally, HA-tagged ribosomes can be efficiently immunoprecipitated from both spinal cord tissue and skeletal muscles, indicating that RPL22-HA is not only being efficiently incorporated into ribosome particles within the MN cell body, but those complexes are also trafficked into distal axons. Finally, in preliminary studies, we have successfully isolated high integrity RNA transcripts (RIN>8) from immunoprecipitated ribosome complexes from both spinal cord and skeletal muscle tissues; however, especially for RNA isolated from the muscle we are currently working on ways to improve efficiency and further reduce the background noise. Ultimately, using RNAseq we will identify a pool of mRNA transcripts that are specially trafficked into motor axons *in vivo*. While this may shed light onto normal processes required for maintenance at the

NMJ, future application of this technique to models of nerve injury or neuromuscular degeneration will provide a unique insight into how the MN terminal responds to its environment.

Disclosures: **J. Shadrach:** None. **B. Pierchala:** None.

Poster

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Topic: D.13. Motor Neurons and Muscle

Support: NIH Grant AG031769

Title: Motor control differs for increasing and releasing force

Authors: ***S. PARK**, M. KWON, D. SOLIS, N. LODHA, E. CHRISTOU;
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Abstract: Control of the motor output depends on our ability to precisely increase and release force. However, the influence of aging on force increase and release remains unknown. The purpose of this study, therefore, was to determine whether force control differs while increasing and releasing force for young and older adults. Sixteen young adults (22.5 ± 4 years, 8 females) and sixteen older adults (75.7 ± 6.4 years, 8 females) increased and released force from 0 to 15% MVC during an ankle dorsiflexion isometric task. We recorded the force output and multiple motor unit activity from the tibialis anterior muscle and quantified the following outcomes: 1) variability of force using the SD of force; 2) mean discharge rate and variability of discharge rate of multiple motor units (MU); 3) power spectrum of the multiple MU from 0-4, 4-10, 10-35, and 35-60 Hz. Participants exhibited greater force variability while releasing force, independent of age ($p < 0.001$). Increased force variability during force release was strongly associated with decreased modulation of multiple MU from 35-60 Hz ($R2 = 0.82$). Changed modulation from 35-60 Hz was further correlated to the change in multiple MU discharge rate ($r = 0.66$) and modulation from 0-4 Hz ($r = -0.64$). In conclusion, these findings suggest that force control is impaired when releasing force due to an altered modulation of the motor units.

Disclosures: **S. Park:** None. **M. Kwon:** None. **D. Solis:** None. **N. Lodha:** None. **E. Christou:** None.

Poster

242. Motor Control: Novel Techniques

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 242.13/O8

Topic: D.13. Motor Neurons and Muscle

Title: Transcranial optogenetic mapping revealed longitudinal changes in motor maps of ipsi-lesional and contra-lesional cortex following mild traumatic brain injury

Authors: *T. NGUYEN, X. JIN;
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Abstract: Mild traumatic brain injury (mTBI) commonly results in short- or long-term cognitive, behavioral, and emotional impairments in patient, but the current clinical and laboratory evaluations of mTBI subjects cannot reveal specific structural and functional brain alterations, and the mechanism of mTBI is not poorly understood. To better understand functional changes of the cerebral cortex following mTBI, we applied a minimally-invasive optogenetic stimulation method to map motor cortex in transgenic mice that expressed light-sensitive channelrhodopsin-2. A closed-head mTBI model was created by using a controlled cortical impact device. Longitudinal optogenetic mappings of the forelimb areas of the ipsilateral and contralateral motor cortex were made by scanning a beam of blue laser on the skull at 2h, 6h, 12h, 1 day, 3 days, and 5 days post injury. Optogenetically evoked responses were recorded with electromyography (EMG) from the forelimb and electroencephalography (EEG) in the cortex. Optogenetic mapping revealed immediate suppression of EMG response and smaller motor maps of the ipsi-lesional cortex within 2 hours post-mTBI, which returned to the baseline level in 6 hours after injury. In contrast, there was an increase in motor map size of the contra-lesional motor cortex within the first 12 hours, which recovered at day 5 post-mTBI. Motor behavioral test using Rotarod showed a decreased mean riding time of the mTBI mice within the first 10 days after mTBI, suggesting a lasting effect of motor behavior. Our data suggest that optogenetic mapping of the motor cortex is a useful technique for longitudinal study of cortical functions following mTBI, and that mTBI induces a transient loss of cortical excitability on the ipsilateral motor cortex but a progressive hyperexcitability of the contralateral cortex after injury. These early electrophysiological changes may have important significance to subsequent posttraumatic neurological deficits and brain functional recovery.

Disclosures: **T. Nguyen:** None. **X. Jin:** None.

Poster

242. Motor Control: Novel Techniques

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 242.14/O9

Topic: D.13. Motor Neurons and Muscle

Title: Exploration of neuromuscular modeling approaches and gait adaptation across terrain types as a basis for prosthetic limb development

Authors: *T. R. CLITES[1], A. S. ARNOLD[2], H. M. HERR[1];
[1]Biomechatronics Group, Media Lab., MIT, Cambridge, MA; [2]Concord Field Station, Dept. of Organismic and Evolutionary Biol., Harvard Univ., Bedford, MA

Abstract: Current control paradigms for lower limb prostheses are unable to match innate gait adaptability, especially across terrains with varying mechanical properties. This deficiency leads to dramatic losses in comfort, efficiency, and patient acceptance of prosthetic devices. To create improved biomimetic controllers that interpret and react to varying ground surfaces, it is first essential to identify and characterize the mechanisms that underlie terrain adaptation. The purpose of this study was to develop and validate a neuromusculoskeletal model that predicts ankle moments, in a goat model, with sufficient accuracy to elucidate the neuromuscular dynamics that allow the animals to adapt to different terrains. The overarching aim of this work is to demonstrate a robust, neurally- and mechanically-integrated prosthesis in this animal model, and, ultimately, in human patients. Kinematic, kinetic, and electromyographic data were recorded in two goats walking on an instrumented walkway. The stiffness and damping properties of the walkway were varied to represent a range of natural conditions. Two different modeling approaches were used to develop a neuromusculoskeletal model of the ankle and surrounding muscles. In one approach, the bone geometry, joint kinematics, and muscle force-generating properties were measured in a cadaveric specimen and scaled to the goats of interest. In the other approach, an optimization algorithm was used to solve for the muscle-tendon properties that reproduce the joint moments from inverse dynamics while minimizing metabolic cost at the animals' chosen walking speed. The two modeling approaches were tested against published *in vivo* measurements of muscle fascicle lengths (obtained using sonomicrometry) and time-varying forces (obtained using an implanted tendon buckle) in goats of the same breed. The refined models allow inferences to be drawn about the neuromuscular dynamics that enable gait adaptation, and the comparisons with *in vivo* data provide new insights into the accuracy of neuromusculoskeletal models used in prosthetic controllers.

Disclosures: T.R. Clites: None. **A.S. Arnold:** None. **H.M. Herr:** None.

Poster

242. Motor Control: Novel Techniques

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Topic: D.13. Motor Neurons and Muscle

Support: NSF IOS-1025806

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W.M. Keck foundation

NAU TRIF

Title: A novel neuromuscular model for a robotic prosthesis based on velocity-dependent muscle activation

Authors: *U. TAHIR, J. L. PETAK, K. C. NISHIKAWA, J. TESTER;
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Abstract: Despite the success of the sliding filament theory, the goal of predicting muscle force output during movements remains elusive, suggesting that the accepted model of muscle contraction is incomplete. We developed a new radical model, the winding filament hypothesis (WFH) that includes a role for the giant protein titin that spans the half sarcomere. The elastic region of titin is composed three parts; the N2A region joins two springs with varying stiffness, the Ig domains (which collectively function as a compliant spring) and the PEVK region (which functions as a stiff spring). According to the WFH, titin is activated upon calcium influx, and the N2A region binds to the thin filament. The crossbridges, which not only translate, but also rotate the thin filament, wind the PEVK region of titin upon actin during muscle contraction, storing elastic energy. The mdm mutation in mice produces a deletion in the N2A region of the titin gene, resulting in different active and passive properties compared to wild-type mice. To predict changes in force during natural movements, we need to understand how the velocity-dependent behavior of muscle changes with activation. We investigated this using isovelocity stretch and shortening experiments in active and passive muscles from wild-type and mdm mice. Soleus muscles from mice were isolated and attached to a servomotor force lever. The muscles were stretched or shortened through a range of initial lengths, velocities and activation levels. Activation ranging from 0% to 100% was achieved by modulating stimulation voltage and frequency. Damping coefficients and the force-velocity relationship (FVR) scaled linearly in activated wild-type muscles. In passive muscles, the damping coefficient was smaller, suggesting that activation reduces viscosity. Passive isovelocity tests suggest that a mechanism in addition to crossbridges is needed to explain the FVR in the absence of activation. In contrast to wild-type muscles, mutant muscles demonstrated differences between the passive and active FVR, suggesting that the mechanism for decreased viscosity in response to activation is absent. Based on studies such as these, we developed control algorithms for a foot-ankle prosthesis; the iWalk BiOM. We implemented our WFH-based controller and tested subjects under a variety of conditions including level walking, stair ascent and uneven terrain. Our results indicate that the WFH-based control algorithm is capable of produces ankle torques during level walking, stair ambulation and uneven terrain that are similar to torques produced by intact individuals using a simple activation pattern and no change in parameters.

Disclosures: **U. Tahir:** None. **J.L. Petak:** None. **K.C. Nishikawa:** None. **J. Tester:** None.

Poster

242. Motor Control: Novel Techniques

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 242.16/O11

Topic: D.13. Motor Neurons and Muscle

Support: California Physical Therapy Fund

Title: Methodological study to identify trunk and hip muscle representation in motor cortex

Authors: *A. M. ALBISHI[1], J. ARMOUR SMITH[2], B. FISHER[2];
[2]Div. of Biokinesiology and Physical Therapy, [1]USC, Los Angeles, CA

Abstract: Background: Trunk and hip muscles contribute significantly to postural control, balance and locomotion. With aging, changes in activities of these muscles are evident. While modifications in motor behavior are linked to altered muscle representational areas in motor cortex, the relationship between motor cortex organization of the trunk and hip muscles and changes in motor behavior in older adults has yet to be established. Transcranial Magnetic Stimulation (TMS) has been used to identify muscle representation in the motor cortex. Mapping the trunk and hip muscles is challenging due to the small representations of these muscles in the medial motor cortex. Purpose: Establish a methodology to quantify the spatial representation of trunk and hip musculature in young and older adults. Method: Six young females participated in the study. Motor evoked potentials (MEPs) were quantified in the external oblique (EO), lumber longissimus (LES) and gluteus medius (GEMD) using a double cone coil and surface electromyography. After motor thresholding, mapping of a 6 by 4cm grid over the pre-central gyrus was conducted using neuronavigation during a submaximal active contraction (20% of maximum voluntary isometric contraction) for all three muscles. Average peak-to-peak amplitude of MEPs was calculated for each map location and utilized to determine the center of gravity (COG) for each muscle. Results: MEPs were elicited consistently in all three muscles. The average COG for GMED was medial to that of LES and EO. COG for all three muscles was localized within primary motor cortex (approximate x, y, z coordinates; GMED 12, -17, 63mm , LES 15, -17, 63mm, EO 14, -17, 63mm in Tailarach space). However, in some individuals the caudal supplementary motor area also contributed significantly to the motor maps. Conclusion: Initial data demonstrate that this methodology is feasible and can identify distinct trunk and hip representations in the motor cortex.

Disclosures: A.M. Albishi: None. **J. Armour Smith:** None. **B. Fisher:** None.

Poster

242. Motor Control: Novel Techniques

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 242.17/O12

Topic: D.13. Motor Neurons and Muscle

Support: Cleveland Clinic - RPC 6700

Title: Neural mechanisms of central fatigue during a prolonged motor task in patients with cancer related fatigue

Authors: *D. ALLEXANDRE[1], A. HOXHA[2], G. YUE[2];
[1]Human Performance and Engin. Lab., Kessler Fndn. Res. Ctr., West Orange, NJ; [2]Kessler Fndn., West Orange, NJ

Abstract: Background: We previously found a greater after-to-before fatigue task twitch force (TF) ratio in CRF than in controls (62%), suggesting that the muscle was less fatigued at the time of perceived exhaustion for cancer patients, pointing to greater contribution of central fatigue to ask failure compared to control subjects. Objective: To investigate neural markers of central and peripheral contributions to motor task failure in patients with cancer-related fatigue (CRF) by studying changes in cortical muscle coherence (CMC) and in EEG sources activity associated with the task. Methods: 14 patients with CRF who were off chemo and radiation therapies and 10 healthy controls with similar age and body mass index were enrolled. All participants completed Brief Fatigue Inventory (BFI) and performed a fatigue task consisted of intermittent elbow-flexion contractions at submaximal (40% maximal) intensity till self-perceived exhaustion. Each contraction lasted 4 seconds with a 2-second rest between trials. To assess the mechanism of neuromuscular fatigue, twitch force (TF) (elicited by supramaximal electrical stimulation) of biceps brachii (BB) muscle at rest before and immediately after the fatigue task, electromyography (EMG) of the BB and brachioradialis muscles, and scalp electroencephalogram (EEG) activities were measured throughout the trials. Results: BFI scores were higher ($P<0.001$) in CRF than controls, indicating greater feeling of fatigue in CRF patients than controls. The after-to-before fatigue task TF ratio in CRF (81%) is higher ($P<0.05$) than that of controls (62%), suggesting that the muscle was less fatigued at the time of perceived exhaustion in CRF patients than controls. Conclusions: Task failure is more of central origin in cancer patients. Ongoing analysis of EEG data will help shade lights on the neurophysiological mechanisms of central fatigue.

Disclosures: D. Allexandre: None. **A. Hoxha:** None. **G. Yue:** None.

Poster

242. Motor Control: Novel Techniques

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 242.18/O13

Topic: D.13. Motor Neurons and Muscle

Support: GPN UIC University Fellowship

Foundation for Physical Therapy

UIC CCTS

Title: Effect of aerobic exercise intensity dose on corticomotor excitability, intracortical inhibition and intracortical facilitation

Authors: *S. KEIL[1], D. CORCOS[2], S. MADHAVAN[1], M. RAFFERTY[1];
[1]Univ. of Illinois Chicago, Chicago, IL; [2]Northwestern Univ., Chicago, IL

Abstract: Background: Single bouts of aerobic cycling have been shown to have an immediate effect on corticomotor excitability, intracortical inhibition, and intracortical facilitation measured with transcranial magnetic stimulation (TMS). The purpose of this study was to determine whether the response was sensitive to aerobic intensity dose. Prior experience with high intensity exercise was examined as a possible predictor of variability in dose response. Methods: Twenty-two participants completed 20 minutes walking at a brisk pace on an incline at 65% and 80% of their age-predicted maximum heart rate on two, non-consecutive days. They were tested with single and paired pulse TMS before and after exercise. Results: Across all participants, there was a dose response where MEP amplitude increased following moderate intensity exercise, but did not increase following high intensity exercise ($p=0.05$). There was also a trend toward lengthened CSP duration following moderate intensity exercise and no change or shortened CSP duration following high intensity exercise ($p=0.09$). The subset of 11 participants who were identified as low-moderate intensity exercisers had a significant increase in resting motor threshold following both exercise doses compared to the high intensity exercisers ($p=0.02$). Discussion: Immediate changes in corticomotor excitability and intracortical inhibition tends to occur following moderate intensity, but not high intensity exercise. Some changes in TMS measures may be driven by inter-individual differences in frequency of high intensity exercise experience. Future research should include increased numbers of participants due to the variability observed in TMS measures.

Disclosures: S. Keil: None. **D. Corcos:** None. **S. Madhavan:** None. **M. Rafferty:** None.

Poster

242. Motor Control: Novel Techniques

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 242.19/O14

Topic: C.04. Neurodegenerative Disorders and Movement Disorders

Title: The Pasta gnawing test: A simple and stress-free test for motor behavior

Authors: *R. RABL, M. FARCHER, D. AMSCHL, B. HUTTER-PAIER;
QPS Austria Gmbh, Grambach, Austria

Abstract: Measuring motor behavior in rodent disease models is often performed in long lasting tests with time consuming evaluations (paper pencil) under high stress conditions (e.g. Rota Rod, Challenging Beam Walk). Beyond that, results of these tests are influenced by body constitution (esp. bodyweight) and the emotional status of the animals. In 2011, Kane et al. noticed, that when performing a Pasta handling test for evaluating the lesion rate of 6-OHDA, unilateral lesioned rats, not only the number of adjustments with each paw was altered, but also the gnawing noise was different. To check whether this finding could also be observed in motor impaired transgenic mice, we evaluated the gnawing noise of three mouse models of three different indications containing motor deficits and compared it with the performance in the Rota Rod Test. Mouse models of Parkinson`s disease (TNWT#61), ALS (TAR6/6) and Niemann Pick`s disease (NPC1) were tested in the Pasta gnawing test and Rota Rod test over time and the gnawing speed and the number of gnaws within one gnawing interval, as well as the latency to fall of the Rod (Rota Rod) were evaluated. Our results revealed that the Pasta gnawing test, evaluating a natural and unforced behavior, did display motor impairment in transgenic murine disease models similar to commonly used motor tests like the Rota Rod, in which the animals are forced to move. Furthermore, we found out, that this measurement was independent from by the body weight, other than for the Rota Rod performance. The Pasta gnawing test is a reliable, easy to perform and stress-free test to evaluate motor performance in mice.

Disclosures: **R. Rabl:** None. **M. Farcher:** None. **D. Amschl:** None. **B. Hutter-Paier:** None.

Poster

243. Posture: Muscle Activity, Exercise and Biomechanics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 243.01/O15

Topic: D.16. Posture and Gait

Support: NIDRR Grant # H133E070013.

Title: Online vs offline learning of challenging balance tasks post-stroke

Authors: R. LOPEZ-ROSADO[1], *D. A. BROWN[2];
[1]Physical Therapy and Human Movement Sci., Northwestern University, Feinberg Sch. of Med., Chicago, IL; [2]Dept Physical Therapy, Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Purpose/Hypothesis: The aim of this study was to investigate online (within session) and offline (between sessions) task performance of stroke survivors in a balance training

program for both their paretic and non-paretic lower extremities, using three different approaches (clinician guarded, high repetitions; robot-guarded, high repetitions; and robot-guarded, low repetitions with increased height difficulty). We hypothesized that participants who received the robot-guarded, training program while challenged by increasingly difficult heights would demonstrate greater online and offline gains in both extremities. Number of Subjects: 36 stroke survivors, randomly assigned to one of 3 groups: 1) clinician guarded, high repetitions (N=12); 2) robot-guarded, high repetitions (N=12); and robot-guarded, low repetitions with increased height difficulty (N=12). Materials/Methods: The KineAssist® Balance and Gait Training Robotic System was used to guard participants. Part of a larger scope balance training study, although all participants practiced 9 x 5 minute repetitive practice tasks per week, with both the paretic and nonparetic leg dominating the task, we analyzed six of the tasks over a six week period (step up on step, step up on foam, forward reach, long step, hurdle, sit to stand). Task height level of difficulty, number of repetitions and exercise load (# of reps x step height) for each task was recorded. Results: Improved online performance occurred during the sessions where participants were progressed to more difficult task levels within each five- minute practice period (robot-guarded, low repetitions with increased height difficulty). With respect to offline learning over the course of six weeks, on average all participants tested at higher task difficulty levels in all groups for both the non paretic and paretic lower limbs (offline learning gains). Exercise load was generally higher for the clinician-guarded regimens, even though the progression in learning was lesser for this group. Conclusions: Robot-guarded training provides a suitable environment for stroke survivors to practice very challenging, and often risky, challenging balance tasks. When the task is made increasingly difficult so that loss of balance occurs, the robot-guarded condition allows for continued online learning. Clinical Relevance: This study provides evidence that learning is improved when individuals post- stroke are challenged to succeed at balance tasks at high difficulty levels beyond their initial ability.

Disclosures: R. Lopez-Rosado: None. **D.A. Brown:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); HDT Robotics. F. Consulting Fees (e.g., advisory boards); HDT Robotics.

Poster

243. Posture: Muscle Activity, Exercise and Biomechanics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 243.02/O16

Topic: D.16. Posture and Gait

Support: FINATEC

UnB

CAPES

CNPq

Title: Postural adaptive strategies of young basketball players in different conditions: possible impact of a proprioceptive training protocol

Authors: *R. A. MEZZARANE, T. C. L. BEZERRA, L. C. GOMES, D. C. SILVA, L. HAGSTROM, T. G. RUSSOMANNO;
Univ. of Brasilia (unb), Brasília, Brazil

Abstract: A number of approaches can be used to evaluate the risk or minimize the occurrence of injury due to sports practice. The analysis of time and frequency-domain parameters in challenging postural conditions can be useful to detect changes in motor control strategies after different interventions (such as proprioceptive training). Significant differences in bilateral modulation of the soleus (SO) muscle activity between elderly and young participants have been recently reported during quiet upright stance. Coherence analysis of the rectified electromyogram (EMG) showed that the main effects were confined to two distinct frequency regions, 0-4 Hz and 8-12 Hz. Here, we investigated postural oscillations and the co-modulation of the SO muscle of young basketball players (12-17y) in different conditions. Twelve participants remained in quiet upright stance over a force platform during 70 seconds in 4 conditions, eyes open, eyes closed, standing on a high-density foam pad with either eyes open or closed. All subjects gave written informed consent and the procedures were approved by a local human ethics committee. The EMG was simultaneously recorded from the SO muscle of both legs at a frequency of 2 kHz. Magnitude squared coherence was estimated for each subject using Welch's averaged modified periodogram method in order to identify common oscillations between both EMG signals. The FFTs of 12 segments were averaged. A Hanning window with 10000 points was specified (without overlapping) resulting in a spectral resolution of 0,2 Hz. Both the root mean square (RMS) and the mean velocity (MV) of the center of pressure were calculated. The coherence estimates of each subject were converted into z-scores and the averaged regions of interest were compared trough a two-way (type of support x vision) repeated measures ANOVA. The same statistical procedure was applied to both MV and RMS. The significance level was set at $p < 0.05$. Lack of visual input strongly affected MV over the foam (significant interaction; $p<0.05$). Significant difference in the z-score was found only for the factor "support" in the range of 0-4Hz ($p<0.05$) consistent with an increased bilateral modulation of motor unit discharges from both legs. This might be ascribed to the disrupted proprioceptive input induced by the foam pad that affects the balance control performed by the central nervous system. We expect that some of these parameters change after the same group of participants undergoing a proprioceptive training period of eight weeks. Eventual changes in these posturographic parameters could indicate adaptive alterations resulted from neuronal plasticity induced by the training ptrotocol.

Disclosures: R.A. Mezzarane: None. **T.C.L. Bezerra:** None. **L.C. Gomes:** None. **D.C. Silva:** None. **L. Hagstrom:** None. **T.G. Russomanno:** None.

Poster

243. Posture: Muscle Activity, Exercise and Biomechanics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 243.03/O17

Topic: D.16. Posture and Gait

Title: Role of body asymmetry in control of vertical posture

Authors: *B. CHEN, Y.-J. LEE, A. ARUIN;
Univ. of Illinois At Chicago, Chicago, IL

Abstract: Humans frequently assume asymmetrical postures while walking or standing in a crowded space with an object in one hand (a cellphone or a cup of coffee) and experience a body perturbation. Understanding the role of such a body asymmetry in the presence of the body perturbation is important for the optimization of the postural control. 10 young healthy individuals stood on the force platform and held an object in the right (target) hand. They were exposed to external perturbations applied to their shoulders while standing with either normal or narrow base of support (BOS). Bilateral eletromyographic activity (EMG) of dorsal and ventral trunk and lower extremities muscles and center of pressure (COP) displacements were recorded during the phases typical for the anticipatory and compensatory postural adjustments. Integrals of EMG and indexes of co-contraction (C) and reciprocal (R) activation of muscles were calculated and analyzed. Reciprocal activation of muscles on the target side and co-activation of muscles on the contralateral side was observed when standing asymmetrically and being symmetrically perturbed. Decreased magnitudes of co-contraction and reciprocal muscle activation in APA phase were seen while standing with the narrow BOS. Peak COP displacement was increased when participants stood with the narrow BOS. The findings highlight the importance of investigating the role of body asymmetry in control of vertical posture and provide a foundation for future studies focusing on improvement of postural control in individuals with body asymmetry due to unilateral impairment.

Disclosures: **B. Chen:** None. **Y. Lee:** None. **A. Aruin:** None.

Poster

243. Posture: Muscle Activity, Exercise and Biomechanics

Location: Hall A

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Topic: D.16. Posture and Gait

Support: NHMRC Fellowship APP1002190

NHMRC Program Grant ID631717

Title: Balance strategies of professional ballet dancers with a history of low back pain are more similar to non-dancers than dancers without low back pain: Analysis of balance with non-linear measures

Authors: *P. W. HODGES, J. GILDEA, W. VAN DEN HOORN;
Univ. Queensland, Brisbane, Australia

Abstract: Balance is critical in ballet. Although dancers are presumed to have superior balance ability to non-dancers, available data are conflicting. One possible reason for the variable results of previous studies of ballet dancers is the failure to consider history of low back pain (LBP) in this group. LBP is highly prevalent in dancers and although LBP is associated with compromised balance in non-dancers, its impact on the balance of dancers is unclear. Further, interpretation of balance in dancers is complicated by the assumption that better balance ability is characterized by less movement, and by the analysis of data with linear measures that provide little detail of balance mechanisms. This study aimed to compare balance between professional ballet dancers with and without LBP and non-dancers, when standing with feet parallel and in the dance-specific turned out "first" position. Centre-of-pressure (CoP) trajectory in the anteroposterior and mediolateral directions was analyzed using linear and non-linear measures in 22 dancers (15 with LBP, 7 without LBP) and 15 pain-free, age-matched non-dancers. Diffusion analysis of CoP trajectories demonstrated that dancers without LBP had greater movement away from an equilibrium position and moved further before correction in the short-term component of balance control (critical point distance; non-LBP vs. non-dancers: $P<0.02$) and displayed greater movement towards an equilibrium position in the long-term component of balance control than non-dancers (parallel feet, anteroposterior direction). This observation of greater motion was supported by some linear measures. Dancers with LBP demonstrated a similar strategy to non-dancers characterized by reduced critical point distance (LBP vs. non-LBP: $P<0.02$, LBP vs. non-dancers: $P=1$) and greater long-term diffusion rate (LBP vs. non-LBP: $P<0.01$, LBP vs. non-dancers: $P=1$). These data show that to control balance, dancers without LBP generally used more movement whereas dancers with LBP used less movement. The balance strategy of dancers with LBP was more similar to non-dancers than to their LBP-free counterparts. The results imply that less movement does not define optimal balance in dancers. Furthermore, impaired balance in dancers with a history of LBP may impact performance quality.

Disclosures: P.W. Hodges: None. **J. Gildea:** None. **W. van den Hoorn:** None.

Poster

243. Posture: Muscle Activity, Exercise and Biomechanics

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Topic: D.16. Posture and Gait

Support: CAPES Scholarship

PIBIC/CNPq Scholarship

FAPESP Grant # 2012/19943-0

Title: Ballet dancers take more advantage of additional sensory information to reduce their postural sway

Authors: *R. B. GARBUS[1], R. C. MARINHO[2], S. M. S. F. FREITAS[2];
[1]Univ. Cidade De São Paulo, Santos, Brazil; [2]Univ. Cidade De São Paulo, São Paulo, Brazil

Abstract: In daily activities, ballet dancers perform several movements that need an adequate balance or postural stability. Additional somatosensory information provided by the light touch of the index fingertip on a fixed surface is able to reduce the postural sway of individuals during quiet standing. However, it is still unknown in the literature whether ballet dancers are able to take more advantage of the light touch to improve their postural stability during upright stance. Nine ballet dancers and nine non-dancers, all women, right-handed stood, as quiet as possible, on an AMTI force plate during 35 seconds. Participants performed three trials for each visual condition (open and closed eyes) and each touch condition (with and without the right index fingertip touching a fixed bar composed by a force sensor ATI Nano 17). For the touch condition, participants were instructed to maintain the elbow in extension while touched a rigid surface using their right index finger and applied force less than 1 N. If the force greater than 1 N was applied, the computer emitted a beep sound to inform the participants to reduce it. The time series of forces and moments of the force plate as well as the force of the touch bar were recorded at 100 Hz and later used to compute the center of pressure (COP) at anterior-posterior and medial-lateral directions. The area, mean sway amplitude and velocity of COP at both directions and the mean and standard deviation of the force applied on the bar during the trials with touch were assessed. The values of COP area, mean sway amplitude and velocity increased when participants stood with closed eyes compared to trials with open eyes. Participants of both groups reduced the COP area, mean sway amplitude and velocity when they touched the bar regardless of the visual conditions. None of this observed effects differed between groups. The applied force was less than 1 N for both groups; however, ballet dancers applied less force compared to the non-dancers. Overall, the results suggest that women who practice ballet are able to take more advantage of the additional somatosensory information provided by the light touch to reduce the postural sway, in spite of the fact that they applied less force on the external surface.

Disclosures: **R.B. Garbus:** None. **R.C. Marinho:** None. **S.M.S.F. Freitas:** None.

Poster

243. Posture: Muscle Activity, Exercise and Biomechanics

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Program#/Poster#: 243.06/O20

Topic: D.16. Posture and Gait

Support: Scientific Research (B)23300238

Title: Effects of lower leg muscle and balance training on periodic floor oscillation task with fixing the knee, hip and trunk in the elderly

Authors: *K. FUJIWARA[1], N. KIYOTA[2], M. IREI[3], C. YAGUCHI[4], H. TOYAMA[1]; [1]Kanazawa Gakuin Univ., Kanazawa, Japan; [2]Japan Hlth. Care Col., Eniwa, Japan; [3]Osaka Hlth. Sci. Univ., Osaka, Japan; [4]Hokkaido Bunkyo Univ., Eniwa, Japan

Abstract: Effects of lower leg muscle and balance training on periodic floor oscillation task with fixing the knee, hip and trunk were investigated in the elderly. Thirty-six elderly subjects were divided into three groups: only lower leg muscle training without balancing (MT); lower leg muscle training with balancing (BT); and untrained control groups (CON). Each training group performed 100 trials per day using a balance board over 4 weeks. Before and after the training period, the subjects maintained a standing posture with the knee, hip and trunk immobilized by the brace while the platform oscillated in the anterioposterior direction at 0.5 Hz with 2.5-cm amplitude. For 5 trials of 1-minute oscillation, the center of foot pressure in the anterioposterior direction (CoPap), Electromyogram (EMG) of the tibialis anterior (TA), gastrocnemius (GcM), and soleus (Sol) and event-related brain potential (ERP) from a Cz electrode were measured. In addition, dorsi- and plantar flexor strength at the ankle and muscle thickness of TA, GcM and Sol were measured with sitting at 90° knee and hip flexion. In MT and BT, significant training effects were found as follows: increases in the both flexor strength, and all muscle thickness. Before the training, mean speed of CoPap fluctuation did not change with the trial repetition in all groups. Only in BT, spectrum amplitudes of CoPap at 0.5 Hz significantly decreased through the training. This effect was recognized in the initial trial, and then it became prominent with the trial repetition, especially in balance against backward disturbance. An individual difference in the peak timing of GcM and Sol activation decreased in BT after the training, whose activation peaks were observed around the anterior reversal. On the other hand, TA activation peak was recognized around the posterior reversal. Negative ERP peaks were found around the anterior reversal more than the other phase. Before the training, there was a significant correlation between these ERP and EMG peak times in all groups. After the training, the highest correlation was found in BT. Peak amplitude of the negative ERP was tend to slightly increased through the training in MT and BT. These results suggest that in the elderly, anticipatory postural control restricted mainly to the ankle would not be improved by only muscle training of the lower leg. However, lower leg muscle training with balancing, including the neural control, improved the postural control restricted to the ankle.

Disclosures: **K. Fujiwara:** None. **N. Kiyota:** None. **M. Irei:** None. **C. Yaguchi:** None. **H. Toyama:** None.

Poster

243. Posture: Muscle Activity, Exercise and Biomechanics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 243.07/O21

Topic: D.16. Posture and Gait

Support: Canadian Institute of Health Research Postdoctoral Fellowship

Title: Response of the plantarflexor muscles to maintaining balance on an unstable platform

Authors: *C. L. POLLOCK[1], D. BACHMANN[2], J. M. WAKELING[1];
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Abstract: There is a moderate relationship between the excitation of the plantarflexor muscles of the ankle and the displacement of the centre of pressure (COP) during quiet standing. This relationship has been suggested to demonstrate the contribution of the ankle plantarflexors to anterior-posterior postural sway, and it is quantified using cross-correlation analysis of the muscle excitation (EMG) to the position of the COP. It is less well understood how this relationship changes when the balance task is made increasingly less stable. The purpose of this study was to examine the relationship of ankle plantarflexor EMG with the anterior-posterior centre of pressure (AP-COP) under conditions of increased challenge to standing balance. Methods: Participants stood with both feet on a single force platform with their arms crossed across their chests to decrease upper extremity involvement in balance. Three conditions were tested: standing with eyes open, and standing on an unstable base of support with eyes open and with eyes closed. The novel unstable base of support condition was achieved by mounting the force platform on custom made rockers that decreased the stability in the anterior posterior direction and required continuous postural adjustment to remain stable. Participants were instructed to remain standing tall with bias to ankle strategy to maintain balance on the board, practice was provided prior to data collection. Each condition was collected for one minute. Parameters measured included; COP displacement, surface EMG of the tibialis anterior, soleus (SOL), medial (MG) and lateral (LG) gastrocnemius muscles. Kinematics were measured for the ankle, knee and hip joints. The EMG intensities were cross-correlated with the AP-COP displacement, and the time-shifts and maximum correlation values determined. Results: Each plantarflexor muscle demonstrated a moderate relationship with AP-COP during quiet standing. The SOL muscle tended to show stronger relationship with AP-COP under the increasingly challenging tasks of the unstable base ($p<0.1$). The LG muscle tended to demonstrate a decrease in strength of relationship between modulation of EMG and AP-COP during the unstable base conditions ($p<0.1$). The MG muscle did not demonstrate significant difference between the conditions. Conclusions: These data suggest a difference between the soleus muscle and the gastrocnemius muscles in response to increased challenge to standing balance and provides insight into the role of each of the plantarflexor muscles during conditions that challenge standing balance.

Disclosures: **C.L. Pollock:** None. **D. Bachmann:** None. **J.M. Wakeling:** None.

Poster

243. Posture: Muscle Activity, Exercise and Biomechanics

Location: Hall A

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Program#/Poster#: 243.08/O22

Topic: D.16. Posture and Gait

Support: NSERC

Title: Revealing the person-specific characteristics of the control of upright balance in younger adults

Authors: ***H. A. OMAÑA, JR**[1], B. W. N. BADIUK[1], V. G. DEPAUL[1,2], W. E. MCILROY[1]; [1]Applied Hlth. Sci., Univ. of Waterloo, Waterloo, ON, Canada; [2]Toronto Rehabil. Inst., Toronto, ON, Canada

Abstract: Currently, literature depicts young healthy adults as a homogeneous group with a comparable ability to control upright stability. There is a lack of attention to the meaning of measured between subject variability. Evidence of individual differences accounting for variation in other behaviors such as cognitive capacity, raises the possibility of important person-specific differences in the capacity to control upright stability. The study tested the hypothesis that, among a cohort of healthy young adults, there would be systematic between-person differences in measures of postural sway across a range of task challenges. Individuals would be characterized by consistent within-group performance rankings across tasks of varying difficulty. It is proposed this pattern would reflect an important trait contribution to the control of balance in young healthy adults. Thirteen healthy young adults (age 24.7 years; SD ± 3.6 years; 8 males) were recruited. Two main factors were manipulated: stance width (shoulder width, Romberg, tandem), and vision (eyes open, eyes closed). Five, one-minute trials were completed for each condition. Center of pressure variability, mean velocity, mean frequency and path length were measured from two force plates. Individual performance was ranked across each task condition and then averaged for all individuals in order for comparisons to be made. As hypothesized, increasing postural challenge increased the amplitude of postural sway, path length and velocity. Important to the present study, individuals relative postural performance (sway), compared to other subjects, remained similar across task challenge. There were significant differences across individuals with respect to average ranks. Individuals who had greater sway in the easier tasks tended to have greater sway in the more challenging tasks. In addition, this relative difference was similar across measures of sway (frequency and amplitude) suggesting this was not a difference in control strategies. The unique between-subject differences in relative performance across tasks provides support for the idea that the capacity to control upright stance varies even in healthy young adults. The determinants of this trait contribution to balance control would

provide an important complement to our existing understanding of state factors that influence postural sway. This study provides an initial step towards the understanding of between subject variability that may be useful for highlighting aspects of the central nervous system control of upright balance in a range of postural tasks and that may inform about age-related changes in some older adults.

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Poster

243. Posture: Muscle Activity, Exercise and Biomechanics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 243.09/O23

Topic: D.16. Posture and Gait

Support: R00 HD073240

Title: Key factors leading to falls in stroke survivors: insights from biomechanical analysis of laboratory-induced falls

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Abstract: Stroke survivors are at a significant risk of falling which can cause severe injury and death. Our limited understanding of the mechanisms resulting in the increased prevalence of falls in this group necessitates further study on falls. The purpose of this study was to identify the key kinematic and stability factors predict a fall in stroke survivors. Our secondary objective was to evaluate the specific strategies that stroke survivors utilize to avoid a fall. The long term goal of this study is to create training programs that target these key factors and train the most ideal strategies to reduce falls in stroke survivors. Seventeen subjects with unilateral stroke were evaluated during dynamic balance challenges designed to evoke a fall. Three posterior directed perturbations of varying difficulty were delivered to subjects while they stood on a treadmill. For safety, the intensity was delivered small to large while they had a harness on. Body kinematics (e.g. trunk flexion angle and angular velocity, step length, etc.) and stability measures (e.g. anteroposterior distance of center of mass (COM) from base of support (BOS), etc.) were evaluated at step initiation and heel strike. Further, clinical scores of function (e.g. Berg balance) were analyzed. We found that the ability to arrest body movement prior to heel strike, the length of the first compensatory step, and stability measures were the most critical response features to avoid a fall. Interestingly, these features have been previously identified as the most important features in fall avoidance in older adults (Owings et al., 2001). Therefore despite the significant

differences between older adults and stroke survivors (e.g. spasticity, delayed responses), stroke survivors fall for remarkably the same reasons as older adults. Three strategies utilized by stroke survivors were identified. A traditional compensatory step (36.4% of fallers used during all trials), pivot strategy(63.6% of fallers and 67% of non fallers used this strategy at least once) and a hopping strategy (18.2% of fallers and 66.7% of non fallers used it). We are the first to identify these strategies but more research about effectiveness of these strategies is needed. Clinical scores were not different between fallers and non-fallers further supporting the literature that these tests are not sensitive enough to predict fallers. Our most significant finding was that stroke survivors fall for similar reasons as older adults. As we have found previous success in modifying the critical factors that lead to falls in older adults, this result suggests our programs developed to decrease falls in older adults may translate for stroke survivors.

Disclosures: **M. Nevisipour:** None. **M. Grabiner:** None. **C. Honeycutt:** None.

Poster

243. Posture: Muscle Activity, Exercise and Biomechanics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 243.10/O24

Topic: D.16. Posture and Gait

Support: Siemensfonden 2014

Title: Ankle joint positioning sense following soccer induced fatigue

Authors: D. K. LARSEN, A. LORENTZEN, G. K. JEPPESEN, S. FRICKE, N. MRACHACZ-KERSTING, *U. G. KERSTING;
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Abstract: Causes of soccer ankle injuries are multifactorial, but muscular and neural fatigue are often identified as risk factors (Woods *et al*. 2004 *Br J Sports Med* 38: 36–41), specifically since most injuries occur during the latter half of the game. Currently no data exists on the amount of fatigue and its effect on joint position sense during the cause of a soccer match. The aim of this study was to quantify the effect of a soccer-specific fatigue protocol ($SAFT^{90}$, Small *et al*. 2010. J Sci Med Sport 13: 120-125), on central and peripheral fatigue and joint position sense in the ankle joint. Eight experienced male soccer players participated in two randomized testing sessions where they were asked to perform the $SAFT^{90}$ or to rest for the same duration of time. Presence of fatigue was defined as a reduction of the maximal voluntary contraction (MVC) force. Voluntary activation (VA), resting twitch (RT) and the H-reflex and V-wave measured during static and dynamic (running) tasks were used to evaluate the extent of central and/or peripheral fatigue. Finally, joint position sense (JPS) of the ankle at 15° and 30° plantar flexion was evaluated. All measurements were performed before (T_0) and after (T_{90}) the $SAFT^{90}$ or

resting protocol. The MVC force decreased by 22% from T_0 (1016±209N) to T_{90} (788±220N; p=0.007) and remained unchanged for the rest condition. The mean VA decreased by 3% and the RT force by 7% following the fatigue protocol while for the control session both parameters increased by 10 and 11% respectively. The static H-reflex decreased by 9% while the dynamic H-reflex increased by 15% following the $SAFT^{90}$. Similarly, the static V-wave decreased by 9%, while the dynamic V-wave increased by 13% following the $SAFT^{90}$. This trend could not be observed for the control session data. The absolute error in JPS increased by 22% for 15° and by 55% for 30° plantar flexion. In the control session, the absolute error decreased by 22% for 15° and increased by 50% for 30° plantar flexion. Preliminary results for this ongoing study reveal that the $SAFT^{90}$, a test deigned to mimic a real soccer game, was successful in inducing fatigue as evaluated by the decrease in the MVC force. While the typical measures of VA, RT force and static H-reflex and V-waves support previous studies on fatigue, this is the first study to demonstrate that H-reflexes and V-waves elicited during dynamic tasks are not decreased but in fact increased. This has important implications for our understanding of fatigue mechanisms during real-life tasks such as soccer games and may provide a platform for investigating further how fatigue during the latter half of the game may be linked to the high occurrence of ankle injuries in this popular sport.

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Poster

243. Posture: Muscle Activity, Exercise and Biomechanics

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Program#/Poster#: 243.11/O25

Topic: D.16. Posture and Gait

Support: JSPS KAKENHI 23700715

JSPS KAKENHI 15K01474

Title: Three phases of postural adjustments during obstacle avoidance in a real and virtual environment

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Abstract: This study was aimed to investigate postural control while lifting a leg to avoid a real or virtual obstacle. To this end, electrical activity of seven legs and trunk muscles was recorded and analyzed during the early postural adjustments (EPAs), anticipatory postural adjustments (APAs), and compensatory postural adjustments (CPAs). Ten healthy adults were asked to stand

upright and avoid colliding with an approaching block presented in a real or virtual environment by lifting their left leg while supporting their body with the right leg. Display conditions were (1) real environment and (2) virtual environment of computer-simulated animation presented in stereoscopic head mount display. The approaching blocks of three heights (10, 20, and 30 cm) were used. The surface electromyography (EMG) signals were recorded bilaterally from the tibialis anterior (TA), medial gastrocnemius (MG), rectus femoris (RF), biceps femoris (BF), rectus abdominis (RA), erector spinae (ES), and external oblique (EO). The EMG signals of the supporting side (right) were then integrated from -550 to -150 ms (EPAs), from -150 to +50 ms (APAs), and from +50 to +250 ms (CPAs) in relation to the moment of the initiation of the leg lifting (T0). The same time windows were used for the analysis of the integrated EMG signals of the lifting side (left). A two-way ANOVA (display × height) for the EPAs showed a significant main effect of display in MG of the supporting side (right), and post-hoc analyses indicated that the virtual condition evoked lower EMG activity than the real condition. For the APAs on the supporting side, the virtual condition also yielded significantly lower EMG activities than the real condition in TA, MG, RA, and ES. Furthermore, during the CPAs, TA, MG, and RF showed significantly lower activities in the virtual condition as compared to the real condition. Decreased EMG activity in the muscles on the lifting side (left) were observed in the virtual condition in RF during the early phase, in RF and ES during the anticipatory phase, and in TA and BF during the compensatory phase. Significant main effects of the block height on the EMG activities were found only in the compensatory phase (EO, RA, ES of the supporting side and RF, BF, and EO of the lifting side). The results indicate that postural control is modulated depending on the display conditions: such a modulation starts during EPAs although it becomes more obvious during APAs and CPAs. However, the effect of the obstacle height was seen mainly in the compensatory phase. The findings suggest that dealing with virtual objects affects the control of posture.

Disclosures: H. Ida: None. **S. Mohapatra:** None. **A.S. Aruin:** None.

Poster

243. Posture: Muscle Activity, Exercise and Biomechanics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 243.12/O26

Topic: D.16. Posture and Gait

Title: Developmental changes from childhood to adolescence in activation patterns of postural muscles during bilateral arm flexion

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Kanazawa, Japan; [3]Dept. of Physical Therapy, Fac. of Human Sci., Hokkaido Bunkyo Univ., Eniwa, Japan

Abstract: We investigated the developmental changes from childhood to adolescence in the activation patterns of postural muscles during bilateral arm flexion. A total of 244 subjects participated in this study (number of subjects (n): 3-4 years: n = 48; 5-6 years: n = 69; 7-8 years: n = 25; 9-10 years: n = 27; 11-12 years: n = 36; 13-14 years: n = 39). Subjects stood on the force platform with closed stance. In response to a visual stimulus (LED signal) presented at 1-3 s after a warning signal, the subjects initiated bilateral arm flexion as quickly as possible and then stopped their arms voluntarily at a horizontal position. After 5 practice trials, 10 test trials were performed with a 30 s-rest between the trials. Electromyogram activity of the following muscles were recorded from surface electrodes: the anterior deltoid (AD), rectus abdominis, erector spinae (ES), rectus femoris, biceps femoris (BF), tibialis anterior (TA), and soleus. The reaction time of the activation onset of AD (D0) in response to the LED signal was measured. Activation rate calculated as the percentage of trials with the burst activation among 10 trials. Time difference between D0 and the activation onset in the postural muscles were calculated as the start time of postural muscles. The activation rate of posterior muscles in the trunk and thigh (ES and BF) was more than 90% in every age group. However, even in 11-12 years, the rate of anterior muscles (RA, RF and TA) was more than about 40% and these rates at 13-14 years were significantly decreased than at 11-12 years. A significant effect of age was observed for the start time of ES and BF, which showed high activation rate. The start time of ES and BF was shortened with age. The start time of ES was significantly earlier than D0 after 5-6 years. The start time of BF was significantly shortened at 7-8 years than at 3-4 and 5-6 years. However, even in 13-14 years, the activation of BF was not preceded to that of AD, with simultaneous activation. These results suggest that for the trunk muscle, the adult-like postural control would be organized by 5-6 years old, but for the thigh muscle, even the early adolescence would be still in the process of development of postural control in spite of the remarkable change in the activation timing at 7-8 years old.

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Poster

243. Posture: Muscle Activity, Exercise and Biomechanics

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 243.13/O27

Topic: D.17. Voluntary Movements

Title: Muscle Synergies in the Neck

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Abstract: Muscle synergies are the coordinated activity of different muscle groups that can be combined to produce a wide variety of movements. Some researchers suggest they reduce the complexity faced by the central nervous system (CNS) when controlling multi-muscle systems (Trech & Jarc, 2009; Bizzi & Cheung, 2013). Muscle synergies have not been evaluated in the neck despite it being a complex multi-muscle system that could benefit from them. Here we investigate if neck muscle activity during gaze shifts to visual targets can be decomposed into a small number of muscle synergies. Subjects sat facing a board fitted with 17 light emitting diodes (LEDs) arranged with one LED at the centre, and two concentric circles at visual angles of 17° and 35° with 8 equally spaced LEDs each. Their torso was constrained and the central LED was placed at eye level in their neutral posture. Subjects were told to reorient their gaze as quickly as possible with a peripheral LED when it illuminated and hold until it turned off, and then return to centre. Subjects performed 3 trials for each LED (48 trials in total). Muscle activity was recorded using wire electrodes inserted under ultrasound guidance into the sternohyoid, sternocleidomastoid, splenius capitis, occipital capitis inferior, rectus capitis posterior major, and semispinalis capitis. Head motion in space and eye motion relative to the head were tracked using helmet mounted markers and a motion tracking system plus a glasses frame mounted gaze tracking system. Electromyography (EMG) signals were rectified, low-pass filtered (20Hz), integrated over non-overlapping 10ms windows, and averaged over 3 repetitions. We used an optimization algorithm to generate sets of muscle synergies (d'Avella et al., 2006) that were combined to best match the experimental EMG traces by scaling the amplitude and shifting the time of each synergy. Timing and amplitude across the muscles within each synergy was fixed. Four synergies were needed to fit the experimental EMG data well (R^2>0.80). The effect of some but not all of the synergies on head motion could be implied from the biomechanics of the muscles involved. For example, one synergy likely generates head extension due to the strong involvement of extensor muscles (semispinalis capitis and rectus capitis posterior major). This work shows that the motor signals to the neck muscles during gaze reorientation involving head motion can be well described by a small number of synergies. This work could be used to simplify muscle control in head and neck models. Further work is needed to determine if these neck muscle synergies simply describe EMG patterns, or if they are indeed used by our CNS to control the neck.

Disclosures: J.B. Fice: None. **P.A. Forbes:** None. **G.P. Siegmund:** A. Employment/Salary (full or part-time):; MEA Forensic. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MEA Forensic. **J. Blouin:** None.

Poster

244. Cortical Planning and Execution: Primary Motor Cortex

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 244.01/O28

Topic: D.17. Voluntary Movements

Title: Developing a method that is suitable to unveil the relevance of monosynaptic corticospinal projections for motor function

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Abstract: Human motor behaviour is remarkably complex and adaptable. The motor repertoire of humans, especially with respect to fine skilled and dexterous movements, outreaches that of other mammals. An important reason for this supremacy has been argued to be the existence of monosynaptic corticospinal projections (called CM projections in the following), directly connecting corticomotoneurons in the cortex with spinal motoneurons (Lemon, 2008). These projections are less developed in old-world primates, absent in the cat, and its developmental status was proposed to be related to the level of dexterity the respective species can achieve. However, such conclusions remain elusive, at least for humans, as there exists no method with which the state of CM projections can be assessed and related to behavioural function. In the present study, we introduce a method that can tonically modulate the excitability of CM projections and thus allows testing how this modulation affects the execution of movements. We used H-reflex conditioning by transcranial magnetic stimulation of the primary motor cortex to selectively assess the excitability of monosynaptic corticospinal projections (Leukel et al., 2012) in 8 healthy subjects. The excitability was quantified and visually displayed as biofeedback to the subjects. In two randomly executed sessions the subjects had to either increase or decrease this excitability analogue to a method previously described for changing spinal reflexes (Thompson et al., 2013). Subjects were rewarded when able to modulate the excitability of CM projections in the requested direction (increase or decrease). As main result, all subjects were able to change the excitability in a single session of 150 trials. The change in excitability was selective, meaning that no other corticospinal projection we tested was affected. We conclude that our method is useful to unveil the relevance of CM projections for human motor function and therefore answer an important open question in motor control. References Lemon RN (2008) AnnuRevNeurosci 31. Leukel C et al. (2012) Eur J Neurosci 35. Thompson AK et al. (2013) J Neurosci 33.

Disclosures: **C. Leukel:** None. **A. Gollhofer:** None. **W. Taube:** None.

Poster

244. Cortical Planning and Execution: Primary Motor Cortex

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Topic: D.17. Voluntary Movements

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NSF GRFP

Title: Somatosensory information drives motor cortex activity during feedback, but not feedforward, adaptations of locomotion

Authors: *E. E. STOUT[1], I. N. BELOOZEROVA[1], N. DOUNSKAIA[2];
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Abstract: Previous studies have suggested that somatosensory information plays little role in driving motor cortical neuron activity during locomotion, as temporary loss of somatosensory inflow leads to little change in neuronal discharge patterns during locomotion. To elucidate the role of somatosensory information in the motor cortex during locomotion, we studied the activity of motor cortical neurons with different somatosensory receptive field characteristics in the cat during multiple complex locomotion tasks. These tasks involved perturbations to locomotion that were overcome using feedforward or feedback-driven motor adaptations. We found that when using feedforward motor adaptations, changes to motor cortical neuronal activity were consistent regardless of somatosensory receptive field characteristics. When using feedback motor adaptations, neurons receiving somatosensory information from distal portions of the forelimb (the elbow and wrist) were significantly more likely to exhibit activity changes than other groups. Kinetics analysis of the swing phase of locomotion revealed that the proximal and distal joints played different roles during both feedforward and feedback-driven motor adaptations. Muscle torque at the shoulder generated interaction torque that was the primary cause of motion of the elbow and wrist in each particular condition. Muscle torque at the distal joints was used to make small adjustments in motions of these joints. Although this structure of joint control was used for both types of adaptation, feedforward adaptations in joint control were initiated at the very beginning of the swing phase while feedback-driven adaptations were produces during the last quarter of the motion. The results from both the analysis of motorcortical neuron activity and kinetic analysis support each other and suggest that somatosensory information drives motor cortical activity during locomotion during feedback but not feedforward locomotion adaptations, and that this information is used to guide whole-body movement adaptations.

Disclosures: E.E. Stout: None. **I.N. Beloozerova:** None. **N. Dounskaia:** None.

Poster

244. Cortical Planning and Execution: Primary Motor Cortex

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BrainScaleS (EU Grant 269912)

Helmholtz Portfolio SMBH

Title: Variability of motor cortical spiking activity depends on the behavioral context

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Abstract: Exploring the nature and origin of neuronal variability is essential for our understanding of information processing in cortical networks. We hypothesize that the variability of spiking activity varies as a function of the behavioral context. We thus analyzed spiking variability in monkey motor cortex during both an instructed delay and movement execution (for the experiment see Riehle et al. 2013) using three different measures. (i) The coefficient of variation (CV) measures the (ir)regularity of spike sequences and is defined as the dispersion of inter-spike-intervals (ISIs). (ii) The Fano factor (FF), computed as the variance of spike counts divided by their mean, provides an estimate of the spike count variability across trials. (iii) The serial rank-order correlation (SRC) between neighboring ISIs is a measure of the deviation from a renewal process (Farkhooi et al. 2009) which is classically predicted as $FF=CV^2$. Because the CV largely overestimates irregularity for changes in firing rate, we use its local measure CV2, introduced by Holt et al. (1996). We analyzed the spiking activity of 3295 motor cortical neurons, recorded during 34 sessions using chronically implanted Utah arrays in two monkeys. We investigated the effect of behavioral context on variability measures by comparing data in selected 500ms-windows during wait (instructed delay) and movement (execution). CV2 and firing rate are significantly lower and FF is significantly higher during wait than during movement. Furthermore, the relation between $CV2^2$ and FF depends considerably on the behavioral context. There is a strong tendency that the renewal prediction ($FF=CV2^2$) is fulfilled during movement, but not during wait, where $FF>>CV2^2$. A higher percentage of neurons has a significant SRC during wait than during movement. Farkhooi et al. (2009) proposed that positive SRCs lead to an increased FF. During wait almost exclusively positive SRCs are encountered, whereas only during movement negative SRCs occur and the amount of negative and positive SRCs is almost balanced. This balance may explain that the null hypothesis for renewal processes is almost satisfied during movement, but not during wait. Our data suggest that during wait, ongoing brain processes dominate, thereby resulting in spike trains that are highly variable across trials, visualized by the increasing FF. During movement, task-related activity increases at

the expense of ongoing processes, and FF decreases. In other words, ongoing processes in cortical networks provide an additional source of variability (Arieli et al. 1996), being not task-related and absent during movement execution.

Disclosures: **A. Riehle:** None. **T. Brochier:** None. **S. Grün:** None.

Poster

244. Cortical Planning and Execution: Primary Motor Cortex

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 244.04/O31

Topic: D.17. Voluntary Movements

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NIH F32NS077816

Title: Neural circuit dynamics in motor cortex linking sensory input to goal-directed motor output

Authors: ***E. ZAGHA**, X. GE, D. A. MCCORMICK;
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Abstract: Goal-directed behavior requires making selective and controlled responses to diverse external stimuli. To study neural mechanisms of behavioral control, we implemented a somatosensory detection task in mice. From pharmacological block of motor cortex, we find that this region is essential for strict behavioral control during the task. To understand the neural dynamics underlying this function, we recorded task-related neural activity in motor cortex. Following target onset, we observed one population of neurons that rapidly enhanced activity and a second intermixed population that was simultaneously suppressed. This divergence of neural activity occurred for both correct and incorrect motor responses. Correlation analyses and circuit modeling suggest that enhanced and suppressed neurons are not operating independently, but rather form competing neural ensembles that interact to convert a transient, sensory stimulus to a sustained motor command. During task performance motor cortex neurons were maintained in the activated state, with spiking initiated by large and transient membrane potential depolarizations. Our results reveal the cellular, circuit and subthreshold dynamics of motor cortex neurons and neural ensembles as they link sensory inputs to goal-directed motor outputs.

Disclosures: **E. Zagha:** None. **X. Ge:** None. **D.A. McCormick:** None.

Poster

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NIH EUREKA

DARPA REPAIR

Title: Motor cortical neurons reflect the active goal-dependent feedback control policy

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Abstract: In both humans and non-human primates, corrective motor responses following mechanical limb perturbations are modulated by contextual factors, such as higher-order task goals and environmental constraints (Scott et al., 2015). Following mechanical perturbations, motor cortical neurons exhibit rapid, context-modulated changes in firing rate, suggesting motor cortex operates as a feedback controller whose control policy is modulated by task context. Here, we tested the hypothesis that primate primary motor (M1) cortical activity might reflect not only the details of the movement presently being produced, but also the particular feedback control policy being engaged, which could then shape the context-dependent response to errors and perturbations. We trained a rhesus macaque to move a haptic feedback device (delta.3, Force Dimension) in the vertical plane, which controlled the position of a visual cursor on a screen, in order to produce brisk, accurate reaches towards visual targets while avoiding virtual obstacles. On 40-75% of trials, we delivered an unpredictably directed, step-force perturbation (3 N) early in the movement. We designed a set of target and obstacle arrangements to vary the control policy employed by the monkey during movement. In the "high-gain" arrangement, the monkey was required to resist lateral perturbations to avoid flanking obstacles, whereas in the "low-gain" arrangement, the monkey needed to respond with less corrective force and re-aim for a more-laterally positioned target. As the initial target was identical in both arrangements, hand kinematics on high- and low-gain non-perturbed trials were nearly identical. However, on perturbed trials, high-gain reaches diverged from low-gain reaches back towards the central target with a latencies of 106-185 ms (Hedges' $g > 0.25$, $p < 0.05$), consistent with goal-dependent modulation of a transcortical feedback loop. We found that most M1 units responded rapidly after perturbation onset (364/405, Kruskal-Wallis test, $p < 0.05$ over 25 ms). However, we also found that in some units, firing rates for high-gain vs. low-gain trials were significantly different before a perturbation was delivered (202/405) and during non-perturbed trials

(314/405). These results suggest that motor cortex (along with other motor feedback regions) might engage different dynamics when different feedback control policies are engaged, enabling the context-specific dynamics to shape neural responses to perturbation and the resulting corrective movements. This suggests that motor cortical dynamics support adaptive feedback control in addition to motor command generation for voluntary movement.

Disclosures: D.J. O'Shea: None. **E.M. Trautmann:** None. **S. Lin:** None. **K.V. Shenoy:** None.

Poster

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Title: Cortico-cortical coupling during bimanual forces in intact humans

Authors: *J. LONG[1,2], T. TAZOE[1,2], D. SOTEROPOULOS[3], M. A. PEREZ[1,2];
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Abstract: Primary motor cortices (M1s) are involved in the generation of bimanual isometric forces. However, cortical processes involved in M1s interactions during bimanual force generation remain poorly understood. Using coherence analysis between electroencephalographic (EEG) signals from M1s, and the ipsilateral cortical silent period (iSP; a measure of transcallosal inhibition) in an intrinsic finger muscle we examined interactions between M1s during unilateral and bilateral isometric index finger abduction at 10, 40 and 70% of maximal voluntary contraction (MVC) in 16 healthy subjects. We demonstrate that interhemispheric EEG-EEG coherence between M1s in the alpha (8-13 Hz) but not in the beta (13-30 Hz) frequency band decreased during bilateral compared to unilateral contractions at 40% and 70% but not during 10% of MVC. The iSP area increased during bilateral compared to unilateral contractions at 40% and 70% of MVC. Notably, changes in the iSP area and coherence

in the alpha frequency band were negatively correlated at 40% and 70% of MVC, suggesting higher transcallosal inhibition was associated with reduced cortico-cortical coherence. To further examine the relationship between the iSP and coherence we stimulated the ulnar nerve at different frequencies (8 and 30 Hz, at a resting motor threshold intensity for the FDI muscle). We found that peripheral nerve stimulation at 8 and 30 Hz increased coherence in the alpha and beta frequency band, respectively; whereas the iSP area decreased during bilateral 70% of MVC to a larger extent with 8 but not 30 Hz or sham stimulation. These findings suggest that pathways mediating transcallosal inhibition and alpha oscillations between M1s interact during strong bilateral force generation in intact humans.

Disclosures: J. Long: None. **T. Tazoe:** None. **D. Soteropoulos:** None. **M. A. Perez:** None.

Poster

244. Cortical Planning and Execution: Primary Motor Cortex

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Craig H. Neilsen Foundation Grant 261299

Title: Crossed corticospinal facilitation between arm and trunk muscles in humans

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Abstract: A voluntary contraction of muscles with one arm increases corticospinal excitability in the contralateral resting arm, a phenomenon known as crossed facilitation. Although many motor tasks engage simultaneous activation of the arm and trunk, interactions between corticospinal projections targeting these segments remain poorly understood. Using noninvasive cortical and cervicomedullary stimulation we examined in healthy humans motor evoked

potentials (MEPs) and the activity in intracortical circuits (short-interval intracortical inhibition, SICI) in the resting erector spinae (ES) muscle when the contralateral arm remained at rest or performed 20% of isometric maximal voluntary contraction (MVC) with the first dorsal interosseous (FDI), abductor pollicis brevis (APB), biceps (BB) and triceps brachii (TB). We demonstrate that the size of cortically evoked MEPs in the ES was increased during voluntary contraction of the FDI and BB but not the APB and TB. Notably, SICI measured in the ES was also decreased during voluntary contraction of the FDI and BB but not the APB and TB. In contrast, cervicomedullary MEPs in the ES remained unchanged across conditions. Our findings reveal selective interactions between hand and arm muscles and the trunk - with pronounced crossed facilitation between the index finger and biceps and the trunk related, at least in part, to changes at the cortical level. We argue that these interactions might reflect the different role of these muscles during functionally relevant arm/trunk movements.

Disclosures: S. Chiou: None. **P.H. Strutton:** None. **M.A. Perez:** None.

Poster

244. Cortical Planning and Execution: Primary Motor Cortex

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Topic: D.17. Voluntary Movements

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Title: Keeping up with a virtual monkey: Representation of self-generated actions vis-a-vis observed actions of a competitor by monkey cortical ensembles during an arm reaching task

Authors: *A. RAMAKRISHNAN[1,2], Z. LI[4], S. SHOKUR[5], M. A. LEBEDEV[1,2], M. A. L. NICOLELIS[1,2,3,5];
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Abstract: A ‘shared representational system’ has been postulated to represent both self-generated and observed actions (Prinz 1997). Yet, it is unclear how this system would work when self-generated actions occur concurrently with the action performed by a different individual, for example, a partner or a competitor. To examine such conditions neurophysiologically, we trained rhesus macaques on a reaching task that required them to compete with a virtual monkey. Monkeys operated a hand-held joystick to reach visual targets with an arm image. They simultaneously viewed a virtual monkey, face-to-face. The virtual monkey reached to the same targets (60% of the trials), or to distracting targets (20%), or did not move (20%). The virtual monkey was relevant and attracted attention because the monkeys

received twice as much juice if they acquired the target sooner than their virtual competitor. In a control condition, called 'passive observation', the monkeys were rewarded for observing the virtual monkey move, but did not move themselves. Many neurons in the primary motor cortex (> 63%) were directionally tuned to the monkeys' own movements, and 30% of the neurons were tuned to both self-generated movements and the movements of the virtual monkey. The strongest tuning to the virtual monkey was observed during passive observations. Moreover, the virtual monkey's actions were represented differently depending on whether they were fast (high challenge) slow (low challenge). These results suggest that self-generated actions and the actions of others are represented in distributed fashion by overlapping populations of cortical neurons. The overlap in cortical representations of self versus non-self may facilitate learning in such interactive paradigms.

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Poster

244. Cortical Planning and Execution: Primary Motor Cortex

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Topic: D.17. Voluntary Movements

Support: NIH Grant R01 NS045853

Title: Spatiotemporal signature of force production in orofacial motor cortex

Authors: *A. A. TOBAA[1], M. D. BEST[2], F. I. ARCE-MCSHANE[1], N. G. HATSOPOULOS[1]; [1]Dept. of Organismal Biol. and Anat., [2]Committee on Computat. Neurosci., Univ. of Chicago, Chicago, IL

Abstract: The amplitude of beta-band oscillations (15-30 Hz) of the local field potential (LFP) in primary motor cortex (MI) attenuates before the onset of tongue movements. This reduction in amplitude coincides with an epoch of enhanced corticospinal excitability, and is thought to reflect the activation of MI neurons. At the same time, it has recently been shown that these neurons undergo changes in their responses during motor skill acquisition (Arce-McShane et al., 2014). Here, we examined how these changes at the single cell level affect the timing and reduction in amplitude of the beta LFP at movement onset. We interpret these properties in the context of motor skill acquisition. We used 96-channel Utah microelectrode arrays to record LFPs from the orofacial MI of two rhesus macaques while they engaged in a tongue force production task (see Arce-McShane et al., 2014 for details). We identified a characteristic spatiotemporal pattern of beta attenuation preceding force onset such that the attenuation timing varied linearly across the motor cortical surface. Although the orientation of this pattern was

generally consistent across days, the relative timing of beta attenuation between electrodes, as well as its overall absolute timing relative to force onset changed as a function of motor skill acquisition. These results suggest that motor skill acquisition refines the recruitment order of neuronal ensembles. References: Arce-McShane, Fritzie I., Nicholas G. Hatsopoulos, Jye-Chang Lee, Callum F. Ross, and Barry J. Sessle. "Modulation Dynamics in the Orofacial Sensorimotor Cortex during Motor Skill Acquisition." The Journal of Neuroscience 34, no. 17 (April 23, 2014): 5985-97. doi:10.1523/JNEUROSCI.4367-13.2014.

Disclosures: A.A. Tobaa: None. **M.D. Best:** None. **F.I. Arce-McShane:** None. **N.G. Hatsopoulos:** None.

Poster

244. Cortical Planning and Execution: Primary Motor Cortex

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Topic: D.17. Voluntary Movements

Title: Test-retest reliability of TMS measures of corticospinal excitability across the age spectrum

Authors: *K. COLLINS, N. KENNEDY, A. CLARK, V. POMEROY;
Univ. of East Anglia, Norwich, United Kingdom

Abstract: Background: As people age there are changes within the nervous system such as decreased brain volume (grey and white matter), decreased intra-cortical connections, and microstructure changes within the neural pathways. A key pathway for upper limb function is the corticospinal pathway; which has been frequently studied using transcranial magnetic stimulation (TMS). Research is inconclusive as to how the aging process may influence the corticospinal pathway, and thus its measurement using TMS, and the reliability of TMS measurement. The aim of this project is to investigate the test-retest reliability of TMS measures of corticospinal excitability in neurologically intact adults across the age spectrum. Methods: Fifty-one neurologically intact healthy adults over 18 years of age will be recruited to participate. Participants participated in 2 identical TMS sessions separated by 5-7 days. TMS measures include active and resting motor threshold, recruitment curve, silent period, MEP amplitude, and MEP latency of the bilateral biceps brachii, extensor carpi radialis, and abductor pollicis brevis. Reliability was assessed using the Intraclass Correlation Coefficient (ICC) and the Limits of Agreement (LOA). Results: This project is on-going, data has been collected on 49 participants whose mean age is 43.2± 16.4 years (20-74), 28 women, 21 men. The preliminary findings include the ICC of active and resting motor threshold for bilateral upper extremities. The ICC and 95 % confidence intervals for the biceps brachii is ICC= 0.67-0.77 (0.48-0.87), extensor carpi radialis ICC= 0.60-0.71 (0.38-0.82), and abductor pollicis brevis ICC= 0.53-0.67 (0.30-

0.80). The reliability of additional TMS measures including the silent period, recruitment curve, and the MEP latency will be investigated. Conclusions: These preliminary findings suggest that in this group of participants the biceps brachii and extensor carpi radialis exhibit the highest ICC values for motor threshold. This study will further investigate the possible influence of age, gender and other factors on the reliability of TMS measurement to further evaluate the use of TMS as an assessment tool for people of all ages.

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Poster

244. Cortical Planning and Execution: Primary Motor Cortex

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Topic: D.17. Voluntary Movements

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Title: Mapping horizontal connections in primate motor cortex using intracortical microstimulation

Authors: *Y. HAO[1,2], A. RIEHLE[1,3,4], T. BROCHIER[1];
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Abstract: Exploring the functional organization of motor cortex at the mesoscopic scale provides a unique way to understand how cortical networks control movement. Previous anatomical studies have demonstrated that distant cortical points are interconnected through long range axon collaterals of pyramidal cells (Gatter and Powel, 1978). However, the functional properties of these local connections and the way cortical signals propagate within this cortical network have not been systematically investigated. To address this issue, we used multielectrode Utah arrays chronically implanted in motor cortex of two rhesus monkeys and applied single pulse intracortical microstimulation (sICMS) at one electrode to analyze its effect, on the

neuronal activity recorded at all other electrodes. We quantified the spatio-temporal distribution of single unit (SU) and multiunit (MUA) evoked responses to determine the properties of horizontal propagation. At the recording sites, the typical responses were characterized by a brief excitatory peak followed by inhibition of longer duration. Significant excitatory responses to sICMS could be evoked up to 4 mm away from the stimulation site, but the strength of the response decreased exponentially and its latency increased linearly with the distance. Inhibitory effects had a more limited spatial spread than excitatory ones. We then applied trains of ICMS at each electrode to distinguish between arm, wrist and hand-related sites and questioned how this somatotopic organization influences the spatial propagation of sIMCS effects. We used 2D Gaussian filters to quantify the direction and strength of the propagation from each stimulation site. We observed that following sICMS at hand-related sites, the propagation had no spatial bias towards other hand-related sites. On the other hand, sICMS at arm-related sites induced a propagation characterized by a weak but consistent bias toward other arm-related sites. Overall, these results indicate that the spread of activity does not confine to intra-area domains but show consistent inter-area interactions, i.e., between hand and arm areas. However the fact that these interactions are not entirely symmetrical may characterize a critical functional property of motor cortex for the control of upper limb movements.

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Poster

244. Cortical Planning and Execution: Primary Motor Cortex

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Title: Supplementary motor area and voluntary movement initiation: neural population responses and the effects of microstimulation

Authors: *A. H. LARA[1], J. C. CUNNINGHAM[2], M. M. CHURCHLAND[1];
[1]Neurosci., [2]Statistics Dept., Columbia Univ., New York, NY

Abstract: The supplementary motor area (SMA) has long been implicated in voluntary movement initiation. Numerous studies in human and non-human primates have shown that SMA activity precedes movement initiation mainly when movement is self-generated, relative to when movement is initiated in response to an external cue. The SMA has extensive reciprocal connections with primary motor (M1) and dorsal premotor (PMd) cortex, and is thus well positioned to provide an input that could initiate movement. To explore the relative roles of the SMA and M1/PMd, we trained two rhesus monkeys to reach to visual targets under three initiation contexts: cue-initiated, self-initiated and quasi-automatic. In the cue-initiated context, monkeys were shown a target and after a brief delay were cued to reach to the target. In the self-initiated context, a continuously growing target signaled reward size; monkeys reached at times of their choosing based on a tradeoff between the desire for large reward and the growing desire to collect the current reward. In the quasi-automatic context, monkeys made low-latency reaches to intercept moving targets. We used intra-cortical microstimulation (ICMS) to causally probe the role of SMA in initiating movement. In the self-initiated context, ICMS caused early movement initiation by an average of ~300 ms. In contrast, for the cue-initiated context, ICMS tended to delay movement. For the quasi-automatic context, ICMS had essentially no effect. These results support the long-standing hypothesis that the SMA plays a specialized role in self-initiated movements, with the caveat that ICMS also had some clear effects in the cue-initiated context. We then compared the neural population response in SMA with that of M1/PMd using principle component analysis. Context strongly impacted SMA responses but not M1/PMd responses. For example, the SMA activity pattern just before movement onset was quite different for self-initiated vs. cue-initiated movements, consistent with previously reported effects at the single-neuron level. This effect was seen in M1/PMd or in the muscles of the arm. Yet despite robust effects of context, the SMA responded as strongly (on average) for quasi-automatic reaches as for self-initiated reaches. Indeed, the largest response component - 1st principal component - was virtually identical across all three contexts. Taken together, our results support the current hypothesis that the SMA is particularly involved in self-initiated movement. Yet the largest SMA response component was, surprisingly, identical across contexts. This suggests that ‘context-specific’ computations converge onto a ‘context-independent’ decision to move.

Disclosures: A.H. Lara: None. **J.C. Cunningham:** None. **M.M. Churchland:** None.

Poster

244. Cortical Planning and Execution: Primary Motor Cortex

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NIH Director's New Innovator Award Program

Title: Motor cortex but not muscles approximate a dynamical system during a novel cycling task

Authors: *A. A. RUSSO, S. M. PERKINS, B. M. LONDON, M. M. CHURCHLAND; Columbia Univ., NYC, NY

Abstract: Motor cortical response properties remain the subject of much debate. Based primarily on studies of reaching, it has been postulated that motor cortical activity might 1) code high-order commands, 2) code muscle activity, or 3) function as a dynamical system that generates patterns from which muscle activity can be built. While the commonly used reach tasks possess many virtues, reaches are a subset of all possible movements. To further explore and compare the above hypotheses one desires a task where (1) movements can be either brief or sustained, and (2) the three hypotheses predict different time-evolving patterns of activity. To this end, we designed a novel task in which monkeys navigated a virtual environment by grasping pedal with their hand and cycling for prescribed distances. Behaviorally the task is simple - the monkey can only move forward or backward on the virtual track. The task is slightly richer at the kinematic level: the hand traces a two-dimensional trajectory. We expect further increased dimensionality at the level of the muscles reflecting the forces required to accelerate, sustain, and decelerate the device. Additionally, there may exist extra features in the neural responses reflecting population dynamics. We recorded neural responses from motor and premotor cortex of two monkeys (114 and 106 neurons) and EMG recordings from the arm, shoulder, and chest (36 and 22 recordings). Muscle responses were indeed temporally complex relative to the simple kinematics. Yet, neural responses were at least as complex and contained a number of features rarely or never found in the muscle responses, including preparatory activity, doubling or tripling of peaks per cycle, and extra peaks at the beginning or end. To assess whether these extra features reflect the internal dynamics that could create outgoing muscle commands, we fit the neural population response with a linear dynamical system. Fits were surprisingly good ($R^2 = 0.73$ and 0.69) especially when compared to that for the muscle populations ($R^2 = 0.36$ and 0.21). Thus, for the neural population but not the muscle population, the future population state could be predicted from the current state, consistent with strong dynamics. We found that this difference was in large part due to how activity evolved between forward and backward conditions. In neural space, the activity during these conditions evolved orthogonally to one another and thus did not produce opposing flow fields. This was not true in muscle space. These results are compatible with the

hypothesis that motor cortex acts as a dynamical system whose goal is to produce complex muscle commands.

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Poster

244. Cortical Planning and Execution: Primary Motor Cortex

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Deutsche Forschungsgemeinschaft

Title: Membrane potential dynamics of single neurons in mouse forelimb motor cortex correlate with movement parameters during voluntary reaching

Authors: ***B. C. VOIGT**[1], L. ESTEBANEZ[1,2], J. F. A. POULET[1];
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Abstract: Motor cortex (M1) neurons are active during voluntary movement but little is known about the subthreshold activity in awake behaving animals. Here we made whole-cell recordings of layer 2/3 and layer 5 M1 neurons during a targeted reaching task. Head-fixed mice were trained to reach and touch a sensor to obtain a water reward. Injection of the GABA-A agonist Muscimol into M1 abolished reaching. On a slow time scale (seconds), in resting mice, most neurons show slow (3-6 Hz), large amplitude membrane potential fluctuations and low firing rates. During movement, many neurons undergo a change in state and slow oscillations are replaced by smaller amplitude, faster events with low variance. Moreover many neurons show subthreshold correlates with kinetic parameters of arm movement. On a faster time scale (milliseconds), a brief depolarization of the membrane potential was recorded at movement onset, which was internally generated, as it occurs without sensory input from the forelimb. Membrane potential modulations recorded at different phases of the reach were larger in layer 2/3 than layer 5 neurons. Current work focuses on disentangling sensory and motor components of M1 subthreshold activity during reaching.

Disclosures: **B.C. Voigt:** None. **L. Estebanez:** None. **J.F.A. Poulet:** None.

Poster

244. Cortical Planning and Execution: Primary Motor Cortex

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Support: NIH Grant R01 NS079664

Title: Spike recordings from the primary motor cortex correlate more strongly with individual muscles or original joint angles than with muscle synergies or joint angle PCs

Authors: *Z. LIU[1], A. G. ROUSE[2], M. H. SCHIEBER[2];
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Abstract: High-dimensional muscular or kinematic spaces can be reduced mathematically to low-dimensional synergies which capture most of the original variance. Such synergies can reduce the computational burden of controlling neuro-prosthetic devices. The extent to which such synergies are used in natural neural control, and if so, which parts of the CNS generate synergies, remains uncertain. If synergies originate from the primary motor cortex (M1), we hypothesize that the activity of M1 neurons should correlate more strongly with synergies than with the individual muscles or the original joint angles from which the synergies were extracted. We recorded electromyographic (EMG) activity from 14-15 muscles, arm and hand motion as 22 joint angles, and spiking activity from microelectrode arrays implanted in the M1, all while two monkeys (Macaca mulatta) reached and grasped one of four objects each in up to eight different locations. Muscle synergies were extracted from the original EMG recordings with non-negative matrix factorization. Six muscle synergies explained over 86% of the EMG variance in both animals. Kinematic synergies were extracted from the original joint angles with principal component analysis. The first 6 principal components (PCs) explained over 93% of the joint angle variance in both animals. For each single-unit or multiunit spike recording we cross-correlated firing rate with i) each individual muscle's rectified EMG, ii) each of the 6 muscle synergies, iii) each of the 22 original joint angles, and iv) each of the first 6 principal components, all over leads and lags up to ±400 ms. We then compared the maximal absolute value of the cross-correlation (MAXC) achieved with the activity of any muscle to the MAXC achieved with any muscle synergy. We also compared the MAXC achieved with any original joint angle to the MAXC achieved with any joint angle PC. Across the population of significantly correlated spike recordings from each monkey, MAXC values with individual muscles were greater than MAXC values with muscle synergies (monkey L, $p<1e-7$; monkey X, $p<1e-7$, Wilcoxon signed rank tests). Likewise, MAXC values with individual joint angles were significantly greater than MAXC values with kinematic PCs (monkey L, $p<1e-10$; monkey X, $p<1e-2$, Wilcoxon signed rank tests). Our results fail to support the notion that muscle or kinematic synergies originate from the primary motor cortex. Muscle or movement synergies

may be generated from other centers of the motor system, such as the pontomedullary reticular formation and/or the spinal gray matter, while M1 neurons sculpt synergies so as to individuate movements.

Disclosures: **Z. Liu:** None. **A.G. Rouse:** None. **M.H. Schieber:** None.

Poster

244. Cortical Planning and Execution: Primary Motor Cortex

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 244.16/O43

Topic: D.17. Voluntary Movements

Support: Imperial College London

Title: Comparison of motor cortical excitability of projections to trunk muscles during anticipatory postural adjustments in standing and lying

Authors: T. REED, M. HURRY, S.-Y. CHIOU, *P. H. STRUTTON;
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Abstract: Voluntary movement of the upper limbs is associated with increased activity in the trunk muscles in order to stabilise the body's centre of mass. This activity can occur concurrently with the activity seen in the prime mover muscles; this is too fast to be a result of somatosensory feedback from the induced body movement and is therefore known as an anticipatory postural adjustment (APA). There is evidence to suggest APAs are controlled, in part, by the motor cortex as corticospinal excitability to trunk muscles is increased at the time of upper limb movements. Whether this increase reflects the demands of the trunk muscles in order to maintain the body upright or the nature of the interactions between upper limb and trunk motor control remains unclear. Therefore, the aim of the present study was to examine corticospinal excitability of projections to trunk muscles using transcranial magnetic stimulation (TMS) during voluntary dynamic upper limb flexion in both standing and lying positions, where the postural demands differ. Seventeen healthy subjects performed rapid shoulder flexion in response to a visual cue, a catch trial was included where no cue was presented but TMS was delivered. Electromyographic (EMG) activity was recorded bilaterally from anterior deltoid (AD), erector spinae (ES) at T12 vertebral level and rectus abdominis (RA). TMS was applied to the hotspot for ES over the primary motor cortex at several time points prior to the expected increase in EMG activity of AD; this was calculated using a recognition reaction time task prior to experimentation and in both positions, these were not different between standing and lying. Motor evoked potentials (MEPs) in ES were larger at time points closer to the rise in AD EMG activity; on the contrary, this was not observed in RA. Additionally, the increase in excitability did not differ between the standing and lying positions. Background EMG activity in the trunk muscles did not change

across time points and was not different between standing and lying. The similar profiles of excitability observed in the two positions imply that APAs are coordinated as part of a hard-wired response, since the postural demands between the two positions are different.

Disclosures: **T. Reed:** None. **M. Hurry:** None. **S. Chiou:** None. **P.H. Strutton:** None.

Poster

244. Cortical Planning and Execution: Primary Motor Cortex

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 244.17/O44

Topic: D.17. Voluntary Movements

Support: Imperial College London

Title: Motor cortical excitability of projections to trunk muscles during anticipatory postural adjustments

Authors: *M. HURRY, T. REED, S.-Y. CHIOU, P. H. STRUTTON;
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Abstract: The increase in corticospinal excitability prior to voluntary movement has been well characterised in limb muscles directly involved in the task. Voluntary limb movements are also associated with increases in trunk muscle activity, some of which occur within a time window considered too fast to be induced by sensory feedback; these are termed anticipatory postural adjustments (APAs). Animal and human studies suggest there is cortical involvement in APAs, with cortical lesions impairing APAs and stimulation of the motor cortex inducing APAs. However, the excitability of corticospinal projections to the trunk muscles prior to voluntary limb movement has not been investigated and was the purpose of the present study. Seventeen healthy subjects performed rapid shoulder flexion in response to a visual cue whilst standing. Electromyographic (EMG) activity was recorded from anterior deltoid (AD), erector spinae (ES) at T12 vertebral level and its antagonist, rectus abdominis (RA). Transcranial magnetic stimulation was delivered to the hotspot for ES over the primary motor cortex at several time points prior to the expected increase in EMG activity of AD; this was calculated using a recognition reaction time task prior to experimentation. The sizes of motor evoked potentials (MEPs) in ES and RA were examined and compared across time points. MEPs in ES were larger at time points closer to the rise in AD EMG activity. MEPs in RA did not differ over the time course examined. Additionally, background EMG activity in the trunk muscles was minimal and did not change across time points. This study demonstrates the profile of corticospinal excitability of two trunk muscles prior to a voluntary limb movement. Further, it reveals task specificity in cortical control of the trunk and substantiates previous evidence for cortical involvement in APAs. These results highlight the interactions between upper limb and trunk

motor control and underline the importance of using functional task training in subjects with deficits of postural control, e.g. low back pain and stroke, which may be relevant to the development of rehabilitative strategies.

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Poster

244. Cortical Planning and Execution: Primary Motor Cortex

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Topic: D.17. Voluntary Movements

Support: HRC PhD Scholarship

Title: Paired-pulse transcranial magnetic stimulation suppresses ipsilateral motor evoked potentials

Authors: ***A. B. MCCAMBRIDGE**, J. W. STINEAR, W. D. BYBLOW;
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Abstract: Skilled movement with the upper-limb requires input from both contralateral and ipsilateral primary motor (M1) cortices. Exactly how the ipsilateral M1 contributes to upper-limb movement is still largely unknown. Paired pulse transcranial magnetic stimulation (TMS) can be used to assess intracortical inhibitory circuits in M1, termed short-interval intracortcial inhibition (SICI). SICI is typically performed by using a subthreshold conditioning stimulus, followed ~ 2 ms later by a suprathreshold test stimulus, to produce motor evoked potentials (MEPs) in muscles of the contralateral upper-limb. To our knowledge, SICI has only been measured from contralateral MEPs. This is the first study to investigate SICI of ipsilateral MEPs (iMEPs). Ipsilateral MEPs are assumed to reflect excitability of an uncrossed oligosynaptic pathway, and can be evoked in proximal upper-limb muscles using high intensity TMS and pre-activation of the muscle. We hypothesized that iMEPs would be suppressed by the conditioning stimulus, therefore demonstrating SICI of iMEPs. TMS was delivered to the dominant M1 to evoke conditioned (C) and non-conditioned (NC) iMEPs in proximal muscles of the non-dominant upper-limb of healthy subjects during weak bilateral contraction. The test stimulus intensity was the lowest stimulus intensity to evoke a reliable iMEP, and a range of conditioning stimulus intensities were tested at short inter-stimulus intervals. The ratio of the C to NC iMEP area at each conditioning stimulus intensity was calculated to assess the amount of inhibition present. Preliminary results show paired-pulse TMS over ipsilateral M1 can suppress iMEPs at short inter-stimulus intervals. Investigating SICI of iMEPs may be a useful tool to better understand the role of ipsilateral M1 and uncrossed motor pathways during upper-limb movement.

Disclosures: **A.B. McCambridge:** None. **J.W. Stinear:** None. **W.D. Byblow:** None.

Poster

244. Cortical Planning and Execution: Primary Motor Cortex

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 244.19/0O46

Topic: D.17. Voluntary Movements

Support: Howard Hughes Medical Institute

Title: Imaging dendritic computation during perceptual decision making and motor planning

Authors: *A. M. KERLIN[1], B. MOHAR[2], B. MACLENNAN[1], D. FLICKINGER[1], K. SVOBODA[1], N. JI[1];
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Abstract: The summation of synaptic inputs in the dendrites of cortical neurons is shaped by nonlinear interactions over multiple length scales. To study these interactions during behavior, we have constructed a two-photon imaging system that allows rapid (~10 Hz) imaging of up to 300 um of contiguous dendrite while resolving calcium transients in individual dendritic spines. Two galvanometers and a remote focusing mirror (Botcherby et al., 2008) steer 16 kHz lines produced by a resonant mirror arbitrarily in three dimensions. We use this system to image spine and dendritic calcium transients in pyramidal neurons of the anterior motor cortex of mice performing a tactile discrimination task (Guo and Li et al., 2014). Spines on the same dendritic branch have diverse selectivity during sensation, movement perpetration, and movement execution. Our findings imply convergence of sensory and motor information onto individual dendritic segments of frontal pyramidal neurons during decision making.

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Poster

244. Cortical Planning and Execution: Primary Motor Cortex

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Topic: D.17. Voluntary Movements

Support: The work is supported by grants from Canadian Institutes of Health Research(CIHR)

Title: Reversible inactivation mapping of cortical sites required for voluntary forelimb movements in VGAT-ChR2 transgenic mice

Authors: *R. KATREDDI[1], G. SILASI[1], J. D. BOYD[1], J. M. LEDUE[1], S. H. SCOTT[2], T. H. MURPHY[1];
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Abstract: Objectives: The aim of the study is to understand the sensory-motor interactions of the cortical regions that are required to execute a voluntary forelimb movement. Methods: Vesicular γ-aminobutyric acid transporter-channelrhodopsin2(VGAT-ChR2) transgenic mice with chronic cranial windows were water restricted and trained in head fixed stage to pull a robotic lever to get a water reward. In these mice, ChR2 is expressed in all GABAergic interneurons and hence blue laser targeting leads to local inhibition by activation of inhibitory neurons. A blue laser was triggered at contralateral and ipsilateral points of the cortex to cause reversible local inhibition while the mouse pulls the lever repeatedly over a 1 min sampling epoch. The total number of pulls done by the mouse at each cortical point was compared with the control point which was out of the cranial windows to probe their role in directed movements. Results: Different frequencies (10, 50, 100Hz) of the blue laser light at different laser power (1, 3, 6mW) were targeted to the regions of interest. Our preliminary results show a 6mW train of 5ms pulses delivered at 100Hz to the contralateral primary motor cortex(M1) reduced the number of rewarded lever pulls by 90±16.8% relative to a control site, while 50 and 10 Hz were less effective. This extent of inhibition was not seen while photo-activating other contralateral points such as M2, visual, retrosplenial cotex, parietal association area and even ipsilateral M1. Moreover, M1 inhibition was fully reversible as the mouse started pulling the lever within seconds once the laser was off the contralateral M1 site. Conclusions: As expected contralateral M1 is required for the execution of voluntary movement; however, M2 and putative hubs such as the retrosplenial cortex, parietal association area were not required to execute lever pulling.

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Poster

244. Cortical Planning and Execution: Primary Motor Cortex

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Topic: D.17. Voluntary Movements

Support: MRC Grant 6EKW

Title: Functional distinction of interneuron circuits in human motor cortex during motor behaviour

Authors: R. HANNAH, S. JERJIAN, S. CAVANAGH, M. SOMMER, *J. C. ROTHWELL; Inst. Neurol, London, United Kingdom

Abstract: We tested the hypothesis that different excitatory interneuron circuits in primary motor cortex (M1) participate in different types of motor behaviour. We adopted two different approaches. In the first we examined how the excitability of different circuits was affected at distinct stages of a motor task; in the second we assessed whether differentially changing excitability levels in these circuits produced different effects on task performance. A controllable pulse parameter TMS (cTMS; Rogue Resolutions Ltd.; Peterchev et al. 2014, J Neur Eng) device was used to activate distinct interneuron circuits in M1 by changing the coil orientation (posterior-anterior, PA; anterior-posterior, AP) and TMS pulse width (30 - 120 μs). Experiment 1 (n=15): motor evoked potentials (MEPs) were elicited in the first dorsal interosseous muscle using short AP- (AP30) and long PA-directed (PA120) pulses, at various stages during a left/right choice reaction task (CRT) in which a non-informative warning cue appeared 500ms prior to the imperative signal. Participants maintained slight voluntary muscle contraction (5-10% MVC) throughout the CRT. Experiment 2 (n=15): monophasic intermittent theta burst stimulation was applied over M1 (AP45 and PA75) or control site (vertex, V45) in order to precondition the excitability of the two circuits. We measured its effect on simple reaction time, CRT, grip strength and finger tapping rate for the following 30 minutes. MEPs evoked by short AP- (AP30, AP45) pulses had a longer latency than those evoked by long PA-directed (PA120, PA75) pulses, confirming that different excitatory interneurons were recruited by the different combinations of pulse width/orientation. In experiment 1, the excitability of AP-sensitive, but not PA-sensitive, interneurons was suppressed at the time of the imperative stimulus and in the non-selected hand near to the onset of movement in the responding hand. In experiment 2, PA-directed theta burst stimulation facilitated finger tapping rate, whilst there was no significant effect after AP-directed stimulation. Motor performance in the other tasks was unaffected. The present data showed that AP sensitive circuits in M1 are preferentially involved in co-ordinating the inhibition of premature responses and in deselection of the non-responding hand. Additionally, the dissociation of finger tapping rate following AP- and PA-directed theta burst suggests that the interneurons excited by a PA pulse are specifically engaged in the tapping task. Together, these data are suggestive of specific functional roles of AP- and PA-sensitive interneurons in motor preparation and execution.

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Poster

244. Cortical Planning and Execution: Primary Motor Cortex

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 244.22/P1

Topic: D.17. Voluntary Movements

Title: The role of primary motor cortex in the coordination of digit force and position for dexterous object manipulation

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Abstract: To perform object manipulation while reducing the risk for performance errors, i.e., drinking from a cup of coffee without spilling it, the central nervous system must coordinate two variables at the onset of manipulation: digit placement and forces. When subjects grasp an object using self-selected (unconstrained) locations on an object, it has been found that trial-to-trial variability in digit position is compensated by a covariation in digit forces so as to preserve task dynamics (Fu et al. 2010, 2011; Fu and Santello 2014). In the present study we investigated the role of primary motor cortex (M1) in the coordination of digit position and forces for dexterous manipulation in this more natural grasping task paradigm using transcranial magnetic stimulation. We hypothesized that a 'virtual lesion' over M1 would impair the modulation of force-to-position during a manipulation task. Subjects first learned to generate a torque to minimize the roll of an object with asymmetrical mass distribution at object lift-off. Continuous theta burst stimulation (cTBS) was then delivered offline to contralateral M1 or vertex (control site) to understand the effects on digit position-force coordination necessary to generate the learned torque. We found that a 'virtual lesion' over M1, but not vertex, induced a significant impairment in the digit placement on the object, namely a decrease in the vertical distance between thumb and index finger placement for the first post-cTBS trial ($p < 0.001$). Moreover, subjects did not modulate digit forces in reponse to this change in digit placement, i.e. a significant change in digit load forces to compensate for decreased vertical distance between the digits ($p = 0.102$). This transiently imparied subject's ability to modulate digit force to position necessary for generating the learned compensatory torque at object lift onset for the first post-cTBS trial ($p < 0.001$). Subjects in the M1 group re-established the ability to generate the learned torque within ~2 trials, but exhibited significantly greater variability in torque generation ($p = 0.005$) relative to the vertex condition for all trials after the 'virtual lesion' over M1. These results suggest that after learning to perform a manipulation task in an unconstrained context, M1 (1) stores sensorimotor memory about digit placement, and (2) plays a significant role for the modulation of digit forces in response to changes in digit placement. Subject's ability to quickly re-establish pre-virtual lesion grasp behavior suggests that brain regions in the grasp network besides contralateral M1 are important for digit force-to-position coordination.

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Poster

244. Cortical Planning and Execution: Primary Motor Cortex

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Topic: D.17. Voluntary Movements

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Title: Corticomotoneuronal cells represent muscle function in the motor cortex

Authors: *D. M. GRIFFIN[1,2,3], D. S. HOFFMAN[1,2,4], P. L. STRICK[1,2,3,4];
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Abstract: Muscles are multi-functional. They serve as agonists for some directions of movement and as synergists, fixators and antagonists for others. Movement dexterity depends on the central control over the precise timing and amplitude not only of agonist muscle activity, but also of muscles performing other functions. Here we examined the contribution of each "functionally tuned" corticomotoneuronal (CM) cell population to the different functions of individual target muscles. We trained a rhesus monkey for > 9 years to perform a wrist task capable of dissociating intrinsic (muscle-like) and extrinsic (movement-like) parameters of wrist movement (Kakei et al., '99). During task performance we recorded individual neurons in the primary motor cortex concurrently with 12 task related wrist and digit muscles. We used spike-triggered averaging (SpTA) of electromyographic (EMG) activity to identify CM cells and their target muscles. We examined 20 directionally-tuned CM cells and their target muscles during wrist movement in eight directions across three different wrist postures. We found that each CM cell displayed a distinct functional relationship with its target muscles. Some CM cells were selectively active when a specific target muscle was used as an agonist. Other CM cells were selectively active when the same muscle was used as a synergist, fixator or antagonist. Thus, separate populations of CM cells generate one of the muscle functions for a given target muscle.

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Poster

244. Cortical Planning and Execution: Primary Motor Cortex

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Topic: D.17. Voluntary Movements

Title: Neural processes during the preparation and the mental simulation of action

Authors: *C. RUFFINO[1,2], C. PAPAXANTHIS[1,2], I. GREENHOUSE[3,4], L. LABRUNA[3,4], R. B. IVRY[3,4], F. LEBON[1,2];
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Abstract: Motor imagery (MI) is a specific cognitive state, during which the corticomotor system is specifically involved and correlated to the content of the represented action. Interestingly, the neural simulation theory (Jeannerod, 2001) postulates that motor imagery engages similar neurocognitive mechanisms to those operating during movement execution and preparation. In a previous study, we analysed the modulation of corticospinal (CS) excitability, with the transcranial magnetic stimulation technique, during the preparation of imagined movements. We found a dynamic CS inhibition during this preparation, similar to those observed in the preparation of the actual movement (Duque et al., 2009), CS excitability being progressively reduced during the delay period. The purpose of the present study was to probe the neural processes during the transition between preparation and simulation. We used single and paired-pulse TMS to measure CS excitability and short-interval intracortical inhibition (SICI), respectively. Right-handed participants were instructed to prepare and to imagine a left or right finger movement, in response to different cues. RT was first measured for actual trials, as the duration between the imperative signal and the time when the electromyographic activity increased above baseline. TMS was triggered at different times during imagined trials: at rest, 50ms before imperative, and at ¼, ½, ¾ and 3/2 of individual actual RTs. Motor evoked potentials (MEPs) were recorded in the right first interosseous muscle. Similarly to our previous study, we observed a decrease of CS excitability during the preparation of the imagined movement (50ms before imperative) and an increase during MI (3/2 of RT) in comparison to baseline. The transition between inhibition and facilitation was progressive and dynamic. The decrease of CS excitability was maximum at half of the RT period, then CS excitability was progressively facilitated. Similarly, SICI increased until half of the RT period then it was reduced when participants imagined the movement. The increase of SICI during the preparation may be responsible for the decrease of CS excitability, and inversely. We could consider that the modulation of CS excitability during the transition between preparation and mental simulation would reflect the balance between inhibitory and excitatory processes, similarly to actual movements.

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Poster

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Topic: D.17. Voluntary Movements

Support: NIH Grant NS0085570

Title: Transcranial magnetic stimulation measures of intrinsic motor system excitability and task-based inhibition exhibit intra-subject stability across weeks

Authors: *I. GREENHOUSE, M. KING, R. B. IVRY;
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Abstract: Individual differences in resting corticospinal excitability are highly reliable. For example, the resting motor threshold (rMT) for single-pulse transcranial magnetic stimulation (TMS) and the motor evoked potential (MEP) recruitment curve are stable across days. However, it is unknown whether task-based measures of corticospinal excitability show similar reliability. To explore this question, we examined TMS signatures of preparatory inhibition across two test sessions. . At two visits (13.7 ± 2.5 days apart), 26 participants performed a choice RT, delayed response task. On each trial of the task a cue signaled the preparation of a lateral flexion of either the left or right index finger followed 900 ms later by an imperative stimulus. Catch trials were included to limit anticipatory responses. TMS intensity during the task was set to 115% of rMT, as determined on an individual basis. The TMS coil was positioned over right M1, with MEPs measured from the left first dorsal interosseous muscle. Single TMS pulses were applied during a baseline intertrial interval or 800 ms into the preparatory delay. . The rMT was highly reliable across visits ($R = .92$, $p < .001$), as were baseline MEP amplitudes ($R = .60$, $p < .005$). Replicating earlier work, MEPs were inhibited relative to baseline when the left hand was selected (Visit 1: $t = 5.1$, $p < .001$; Visit 2: $t = 6.3$, $p < .001$) or not selected (Visit 1: $t = 3.2$, $p < .005$; Visit 2: $t = 5.4$, $p < 0.001$) for the forthcoming response. Inhibition was also greater for the selected than the non-selected hand (Visit 1: $t = 3.0$, $p < .01$; Visit 2: $t = 2.0$, $p < .05$). The degree of inhibition in the selected hand was reliable across visits ($R = .53$, $p < 0.005$) and was marginally reliable in the non-selected hand ($R = .35$, $p = .08$). Importantly, baseline MEP amplitudes did not predict the amount of task-based inhibition (all p's > .16). . The results demonstrate that processes underlying preparatory inhibition of corticospinal excitability are stable across weeks within individuals (albeit marginally for the non-selected hand). The fact that these dynamic, task-based measures were not predicted by resting static measures of excitability suggests that preparatory inhibition reflects the recruitment of a distinct neural mechanism.

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Poster

244. Cortical Planning and Execution: Primary Motor Cortex

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Topic: D.17. Voluntary Movements

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Title: The relationship between neural and muscle synergies investigated with common decomposition methods

Authors: *R. L. HARDESTY, JR[1], W. J. TALKINGTON[1], E. OLESH[2], V. GRITSENKO[1]; [1]Neurosci., [2]West Virginia Univ., Morgantown, WV

Abstract: Limb dynamics, defined as the sum of non-linear forces that arise directly or indirectly from muscle contractions, requires complex control by the neural motor system. Some of these forces are caused by the effect of gravity on the moving limb, others are caused by mechanical coupling between limb segments, termed interaction torques. Healthy motor control involves the generation of coordinated muscle contractions, or muscle synergies, that are appropriately scaled to overcome or take advantage of limb dynamics for any given movement. We investigated whether common decomposition methods can provide accurate representations of neural synergies that underlie muscle synergies engaged during arm movements of humans. Subjects performed three reaching tasks that consisted of pointing to spherical targets in virtual reality. The locations of targets were chosen so that movement to them is accompanied by varying directions of interaction and gravitational torques. Neural synergies were assessed using single-pulse transcranial magnetic stimulation (TMS) of the primary motor cortex at varying time points during movement. Motion capture and electromyography of 12 muscles spanning the shoulder, elbow, and wrist were recorded synchronously. Data analysis was carried out in MATLAB (Mathworks). To normalize TMS responses to the background motoneuronal excitability, the integration of inputs by motoneurons and the contribution of spindle afferents were mathematically modeled. The relationships between TMS responses, background muscle activity, and kinematic and dynamic variables of limb motion were investigated using non-negative matrix factorization (NNMF) and principal component analysis (PCA). Results indicate that during movement the TMS responses are modulated together across several muscles in agreement with muscle synergies extracted with NNMF from background muscle activations. These synergies also showed relationships with kinematic or dynamic components of movement. These results support the use of NNMF to extract neural synergies and suggest that these synergies may represent specific variables of limb dynamics.

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Poster

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Title: Short interval intrahemispheric inhibition (SICI) in a shoulder girdle muscle in humans

Authors: *K. MUDGAL[1], C. ALEXANDER[2];
[1]Imperial Col. London, Staines-Upon-Thames, United Kingdom; [2]Physiotherapy, Imperial Col. Healthcare NHS Trust, London, United Kingdom

Abstract: In order to control the hand in space the muscles of the scapula are required to help position the arm. Indeed it is clear that without appropriate positioning and stability of the shoulder region, manipulation of the hand becomes difficult (Levin 1996). Consequently, control of the shoulder region muscles is relevant to upper limb function. Surprisingly, little is understood about the mechanisms enabling modulation of activity of these muscles, which is in contrast to the understanding of control of hand muscles. To investigate control of shoulder region muscles we have examined short interval intrahemispheric inhibition (SICI) in both upper and lower fibres of trapezius. With ethical approval and informed consent and using standard techniques, the surface EMG of the dominant side of upper trapezius (UTr), lower trapezius (LTr) as well as the first dorsal interosseous muscle of the hand (FDI) were recorded from 16 healthy subjects. A figure of eight coil from a magnetic stimulator was positioned over the motor cortex upon the hotspot for the muscle under investigation. All stimuli were conditioned at a test condition interval of 2.5ms. The aim was to deliver a test stimuli at an intensity to evoke MEPs in the upper and lower trapezii between 0.5mV and 1mV. The conditioning stimulus was varied by 10% increments from 50-90% of the resting motor threshold. Conditioning and test stimuli were randomly interleaved such that 10 stimuli at each conditioning intensity were delivered and 10 test stimuli were delivered alone. Any effect upon the test stimulus was measured as a percentage modulation of the amplitude of the test MEP. In addition, the experiment was repeated with the test muscle active at 5% of MVC using 10 of the subjects with the conditioning stimuli intensity ranging from 50-90% of active motor threshold. As has been previously described, SICI could be evoked at rest in the right FDI (inhibition 32% ±21%; p =0.00). SICI at rest was strongest at a conditioning stimulus of 70% (49% ± 38% UTr; 60% ± 29% LTr). Interestingly we were unable to evoke SICI when the test muscle was active at only 5% of MVC in either FDI or UTr. However, SICI could be evoked in LTr (p=0.008) with a conditioning intensity of 70% and 80% of active motor threshold (82% and 80% respectively). Reference List Levin MF (1996) Interjoint coordination during pointing movements is disrupted in spastic hemiparesis. Brain 119 (Pt 1):281-293

Disclosures: K. Mudgal: None. **C. Alexander:** A. Employment/Salary (full or part-time):; imperial college healthcare nhs trust. 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.; national institute of health reseach.

Poster

244. Cortical Planning and Execution: Primary Motor Cortex

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 244.28/P7

Topic: D.17. Voluntary Movements

Title: A prepared mind is a quite brain

Authors: K. MAJIMA, M[1], M. HASEGAWA, M[2], T. K. SATO, M[3], Y. KAMITANI, M[1], *T. SATO[2,4];
[1]ATR, Keihanna, Japan; [2]Ctr. For Integrative Neurosci., Tuebingen, Germany; [3]UCL Inst. of Ophthalmology, London, United Kingdom; [4]JST, PRESTO, Tuebingen, Germany

Abstract: Our movements are initiated more efficiently when prepared in advance. This facilitation effect has been considered to arise from brain states during a preparatory period before motor execution. However, the precise properties of the neural network that induces a prepared state are unknown. Here, we show that sparse and low activity in cortical circuits facilitates the reaction time for subsequent behavior in mice performing a delayed reaching task that we have developed. Unlike previous behavioral paradigms such as licking or whisking tasks, our reaching task has incorporated variable delay periods and thus enabled us to study the network state specifically when mice prepared for an intended movement. Using *in vivo* two photon imaging with a highly-sensitive calcium sensor (GCaMP6), we monitored simultaneously the activity of up to hundreds of neurons in the motor cortex and decoded it using machine-learning algorithms. The reaction times of forepaw movements were significantly shorter when the cortical network exhibited a characteristic pattern of spatially sparse and low activity. Our results indicate that shifting the motor cortex network to a state in which a majority of neurons are inactive facilitates effective initiation of motor behavior, and highlight the importance of sparse activity with low noise for efficient cortical processing.

Disclosures: K. Majima: None. **M. Hasegawa:** None. **T.K. Sato:** None. **Y. Kamitani:** None. **T. Sato:** None.

Poster

244. Cortical Planning and Execution: Primary Motor Cortex

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 244.29/P8

Topic: D.17. Voluntary Movements

Support: Spanish MINECO BFU2011-29089

Spanish MINECO BFU2011-29286

Junta de Andalucía Spain BIO-122

Junta de Andalucía Spain CVI-02487

Junta de Andalucía Spain P07-CVI02686

EU FP7 FET-Open HIVE project 222079

Title: Enhancing acquisition of classical eyeblink conditioning by non-invasive transcranial direct current stimulation (tDCS) in alert rabbits

Authors: *C. AMMANN, A. GRUART, J. M. DELGADO-GARCÍA, J. MÁRQUEZ-RUIZ; Univ. Pablo de Olavide, Seville, Spain

Abstract: Transcranial direct-current stimulation (tDCS), a non-invasive brain stimulation technique, has been successfully applied for modulation of cortical excitability. The aim of this study was to investigate whether the application of tDCS on primary motor cortex (M1) could modulate the acquisition of an associative learning. Additionally, to rule out that thermal effect underlies the observed changes, a second experiment was carried out. For the first objective six male rabbits were prepared for classical eyeblink conditioning and simultaneous tDCS on M1. tDCS was applied on M1 through a disk electrode (0.5 cm2) over the skull, with a saline-soaked sponge attached to the contralateral ear serving as a counterelectrode. We applied a delay paradigm using a 350 ms tone as CS followed 250 ms from its onset by a 100 ms air puff directed to the left cornea as US. The presence of conditioned responses (CRs) was determined by recording the EMG activity of the ipsilateral orbicularis oculi muscle. Anodal and cathodal tDCS (1 mA, ± 2 mA/cm2, ~ 30 min) were applied during conditioning day 3 (C3) and day 8 (C8), respectively. Sham stimulation implicated the same electrode placement and conditioning protocol, except for tDCS duration, consisting of a 30 s application. The results showed that the presence of anodal tDCS during C3 potentiated the acquisition of the classical eyeblink conditioning, whilst cathodal tDCS during C8 did not present a significant effect on the percentage of CRs, nevertheless this tDCS polarity reduced magnitude and increased latency of the CRs. To discard a potential thermal effect associated to tDCS, three male rabbits received anodal and cathodal tDCS on M1 during 60 or 120 min through the disk electrode over the skull. An epidural NTC thermistor was implanted on M1 for brain temperature measurement before, during and after tDCS. Different current densities of tDCS were applied (±0.029, ±0.29, and ±2.9 mA/cm2). No significant changes of the epidural temperature were detected for any of the different current densities neither with anodal nor with cathodal tDCS application. The present work highlights the potential use of anodal tDCS on M1 to enhance the acquisition of an associative motor learning and the modulating capability of cathodal tDCS on motor responses. On the other hand, the results established that no thermal effect was induced over the brain

cortex when density currents two orders of magnitude higher than those applied in humans were used. These findings confirm that the prolonged application of tDCS can be considered safe from a thermal point of view, suggesting that tDCS effects are not related to an induced variation of brain temperature.

Disclosures: C. Ammann: None. **A. Gruart:** None. **J.M. Delgado-García:** None. **J. Márquez-Ruiz:** None.

Poster

245. Sexual Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 245.01/P9

Topic: E.01. Neuroendocrine Processes

Support: NICHD Grant 5R00HD055446-04

Title: Kisspeptin cell-specific deletion of PTEN results in sex- and nucleus-specific trophic effects in the adult mouse brain

Authors: *A. L. NEGRON[1], U. BOEHM[2], M. ACOSTA-MARTÍNEZ[3];
[1]Stony Brook Univ., Stony Brook, NY; [2]Pharmacol. and Toxicology, Univ. of Saarland Sch. of Med., Homburg, Germany; [3]Physiol. and Biophysics, Stony Brook Univ. Med. Ctr., Stony Brook, NY

Abstract: In mammals, kisspeptin and its receptor Kiss1R, play an important role in the control of gonadotropin-releasing hormone and are necessary for normal reproductive function and pubertal development. There are two principal hypothalamic kisspeptin-expressing neurons in rodents: one in the anteroventral periventricular nucleus (AVPV) and a second in the arcuate (ARC) nucleus. Kisspeptin protein and mRNA expression in the AVPV is sexually dimorphic (greater in females than males), which partly explains the ability for females and not males to produce a luteinizing hormone (LH) surge. During specific periods of development, hormone-dependent and -independent pathways are responsible for shaping the sexually dimorphic expression of kisspeptin. However, the molecular mechanisms underlying the hormonal and non-hormonal organizational actions on kisspeptin expression and function remain unknown. Phosphatase and tensin homolog (PTEN) is a dual protein/lipid phosphatase that regulates neuronal cell survival, size, and proliferation. Using adult Kiss-Cre PTEN$^{flx/flx}$ mice (Kiss-PTEN KO) and DAB immunostaining, we found that male Kiss-PTEN KO mice had more kisspeptin-immunoreactive (ir) cells compared to WT littermates, whereas female KOs had less compared to WT. The intense kisspeptin fiber density in ARC and the increased peptide content in AVPV make accurate cell counting and cell area measurements difficult. Therefore, we crossed Kiss-PTEN KO mice with a R26-YFP reporter mouse (Kiss/YFP-PTEN KO). In the AVPV, male

Kiss/YFP-PTEN KO mice had a higher number of Kiss-ir cells/section compared to WT, confirming our previous findings using DAB staining. In contrast, neither significant genotype effects in AVPV Kiss-ir cell number was observed in females, nor any differences in the ARC Kiss-ir cell numbers of either sex. In addition, compared to Kiss/YFP-WT littermates, Kiss/YFP-PTEN KO females showed increased cell area of AVPV Kiss neurons. Because PTEN deletion can activate mammalian target of rapamycin (mTOR) signaling, which regulates cell size and protein synthesis, we co-labeled Kiss/YFP with pS6, a marker of mTOR activity. A significant increase in the percentage of co-labeled cells in both the AVPV and ARC of female KO mice compared to WT was observed. Our findings suggest that PTEN signaling in kisspeptin neurons participate in the hormone-dependent and independent organizational events leading to sex-specific hypothalamic kisspeptin expression. Kisspeptin neurons exhibit hypertrophy in postmenopausal women and in ovariectomized monkeys. Thus, decreased PTEN signaling may also lead to premature kisspeptin cellular senescence.

Disclosures: **A.L. Negron:** None. **U. Boehm:** None. **M. Acosta-Martínez:** None.

Poster

245. Sexual Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 245.02/P10

Topic: E.01. Neuroendocrine Processes

Support: DoD W81XWH-13-1-0377

Title: Effects of estrous cycle on fear conditioning and extinction, and on single prolonged stress induced extinction recall deficits

Authors: *C. V. CHEN, I. LIBERZON;
Univ. of Michigan, Ann Arbor, MI

Abstract: Post-traumatic stress disorder (PTSD) is a deleterious mental health condition with a lifetime prevalence of 6-9% in the US, affecting twice as many women as men. Since gonadal hormones play a crucial role in many sex differences, they may contribute to sex differences in susceptibility to PTSD. Using a PTSD rodent model - Single Prolonged Stress (SPS), our lab has shown that SPS in male rats leads to a deficit in retention of fear extinction, a postulated key deficit in PTSD. Here, we sought to determine how fluctuating hormone levels throughout the estrous cycle in female rats affect various aspects of fear associated learning like fear conditioning, fear extinction, and SPS induced extinction recall deficits. Adult female rats' vaginal impedance was measured daily for twelve days to determine their estrous stage. Four groups of animals were then exposed to SPS during proestrus, estrus, diestrus 1, or diestrus 2. As previously described, SPS consisted of restraint, forced swim and ether anesthesia, followed by a

7-day quiescent period. Control animals were left undisturbed during the 7-day quiescent period. Following the undisturbed time, all rats were tested for fear conditioning (FC), fear extinction (FE) and extinction recall (ER). Preliminary data indicate that, in female controls, hormone levels on the day of learning affect memory performance . When hormone levels are high during FC, animals show higher levels of initial freezing the following day. Conversely, rats with high hormone levels during FE show the stronger evidence of extinction during ER. However, SPS exposure interacted with the hormonal effects on fear and extinction memory. When SPS was performed during proestus, it subsequently enhanced conditioning, showing higher freezing during FE and ER, as compared to animals undergoing SPS under other estrous stages. When matched with controls on the same estrous stage, females in proestrus on the day of SPS showed SPS-characteristic ER deficits. Low hormonal levels during FC, FE and ER, did not interact with SPS effects and animals showed SPS-induced ER deficits compared to stage matched controls. Future studies will seek to clarify whether estrogen, progesterone or both are responsible for these effects. These results, however, indicate that cycling hormones may explain the differential susceptibility to PTSD in the female population.

Disclosures: C.V. Chen: None. **I. Liberzon:** None.

Poster

245. Sexual Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 245.03/P11

Topic: E.01. Neuroendocrine Processes

Support: NSF Grant IOS1050367

Title: Progesterone receptor immunoreactivity in the brains of neonatal PRA knockout (PRAKO) or PRBKO mice

Authors: *D. LALITSASIVIMOL[1], K. D. ACHARYA[2], S. NETTLES[2], M. J. TETEL[2], O. M. CONNEELY[3], C. K. WAGNER[1];
[1]Psychology, Univ. At Albany, Albany, NY; [2]Neurosci. Program, Wellesley Col., Wellesley, MA; [3]Mol & Cell Biol., Baylor Col. of Med., Houston, TX

Abstract: During critical developmental periods, progesterone receptor (PR) is expressed in neural structures that influence sexually dimorphic behaviors in adulthood, including the ventrolateral subdivision of the ventromedial nucleus of the hypothalamus (VMN), and the medial preoptic nucleus (MPN). There is a significant sex difference in the expression of PR in the MPN, in which perinatal males express higher levels of PR immunoreactivity (PR-ir) in the MPN compared to females, suggesting a developmental window during which the male brain is more sensitive to progesterone than the female brain. The two PR isoforms, the full length PRB

and the truncated PRA, can differentially regulate the expression of specific genes. In the present study, we investigated the differential expression of PRA and PRB in the VMN and MPN during development by comparing PR-ir in neonatal male and female PRA knockout (PRAKO) and PRBKO mice and their wildtype (WT) counterparts. In both the MPN and the VMN, there was a significant main effect of sex, a main effect of genotype, and a significant interaction between sex and genotype. Levels of PR-ir were higher in WT males than in WT females ($p<0.001$) consistent with previous results from the developing brain. However, this sex difference was abolished in both PRAKO and PRBKO mice, with PR-ir levels in knockout males significantly lower than WT males, but similar to WT females. Knockout females did not differ from WT females in either region. These findings suggest that the sex difference in PR-ir in the neonatal MPN and VMN can be attributed to the expression of both PR isoforms and implicate PR in the sexual differentiation of these regions and the behaviors they regulate in adulthood.

Disclosures: D. Lalitsasivimol: None. **K.D. Acharya:** None. **S. Nettles:** None. **M.J. Tetel:** None. **O.M. Conneely:** None. **C.K. Wagner:** None.

Poster

245. Sexual Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 245.04/P12

Topic: E.01. Neuroendocrine Processes

Support: RO1 MH52716-018

Title: Role of microglia in sexual differentiation of the amygdala

Authors: *J. W. VANRYZIN[1], M. M. MCCARTHY[2];
[2]Dept. of Pharmacol., [1]Univ. of Maryland, Baltimore, Baltimore, MD

Abstract: Microglia are the dominant resident immune cells of the brain and function in multiple ways outside their traditional capacity of responding to insult. During development microglia regulate tissue homeostasis, neuronal precursor populations, and synaptic circuitry. We recently implicated microglia as an integral component of sexual differentiation of the preoptic area and control of male copulatory behavior, suggesting these immune cells also function to organize sex-specific brain structure and function (Lenz et al. J Neurosci 33(7), 2013). The amygdala is also a sexually dimorphic brain region that regulates social behaviors known to differ in males and females. We reported a sex difference in the number of newly born cells in the developing rat medial amygdala (MeA) that is mediated by endocannabinoids, with females having higher numbers of newly born cells than males and a lower endocannabinoid tone. These differences in newly born cells correlated to behavioral changes, as newborn females treated with the CB1/2 agonist WIN55,212-2 exhibited masculinized juvenile social play behavior and reduced cell

genesis in the developing amygdala (Krebs-Kraft et al. PNAS 107(47), 2010). Further investigation suggests microglia may be central in establishing the observed sex difference in newly born cells, as males have more Iba1+ microglia exhibiting phagocytic morphology compared to females in the medial amygdala during the sensitive period for sexual differentiation. Treatment with minocycline, a tetracycline derivative and inhibitor of microglial activation, increased male BrdU+ cell counts to female levels, but did not alter the number of female BrdU+ cells in the MeA. We are now deciphering the relative contributions of microglia phagocytosis and trophic signaling in the regulation of newly born cells using methods that selectively deplete microglia or prevent phagocytosis. Ultimately, these studies will provide valuable insight into new facets of immune regulation of brain sexual differentiation and development. This work was supported by RO1 MH52716-018 to MMM.

Disclosures: J.W. Vanryzin: None. **M.M. McCarthy:** None.

Poster

245. Sexual Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 245.05/P13

Topic: E.01. Neuroendocrine Processes

Support: NINDS award 040726B1 to MMM

Title: Sex difference in microRNA-124 in neonatal hippocampus and impact on NKCC1

Authors: *K. E. KIGHT[1], M. M. MCCARTHY[2,3];
[1]Program in Mol. Med., [2]Dept. of Pharmacol., [3]Program in Neurosci., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Neurogenesis is regulated by numerous intrinsic factors, and varies among brain regions and across the lifespan. We have previously reported that developmental cell genesis in the hippocampal formation of the Sprague Dawley rat is greater in the dentate gyrus of males compared to females during the first week of life, but not the second (Bowers et al., Bio Sex Diff 2010). We have also found a sex difference in the amount of several microRNAs known to be functionally involved in neuronal cell genesis, differentiation, and maturation. Female rat pups have higher baseline expression of several mature microRNAs in the dentate gyrus during the first postnatal week, including miR124, which promotes cell-cycle exit and neuronal maturation. One of the predicted targets of miR124 is the SLC12A2 transcript, which encodes the bumetanide-sensitive Na-K-2Cl cotransporter, NKCC1. As the primary channel facilitating chloride ion influx in immature neuronal cells, NKCC1 is critical for conferring the depolarizing actions of GABA on neuronal precursors and immature neurons, and its expression is downregulated in maturing neurons. Here we report that males had higher levels of NKCC1

mRNA compared to females in the neonatal dentate gyrus. This positively correlates with the male-biased proliferation and is inversely related to the sex difference in miR124 expression. Antagonism of miR124 via i.c.v. infusion of locked-nucleic acid-modified oligonucleotides downregulated NKCC1 expression. The higher levels of miR124 in females and the inhibitory effect of miR124 on NKCC1 expression suggest a mechanism for the earlier developmental shift to hyperpolarizing GABA seen in the hippocampus of females during the first postnatal week (Nuñez and McCarthy, Dev Neurobiol 2007). These results also suggests that differential expression of microRNAs between males and females, particularly miR124, may direct the sex difference in early postnatal neurogenesis through downstream effects on depolarizing GABA.

Disclosures: K.E. Kight: None. **M.M. McCarthy:** None.

Poster

245. Sexual Differentiation

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Topic: E.01. Neuroendocrine Processes

Support: NIH Grant RO1MH52716-018

Title: CB1 and CB2 endocannabinoid receptors act in concert to modulate sex differences in the developing amygdala and social play behavior

Authors: *K. J. ARGUE[1], J. W. VANRYZIN[2], M. M. MCCARTHY[1];
[1]Pharmacol., [2]Program in Neurosci., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Males of most species display higher levels of juvenile social play behavior compared to females. In rats, this sex difference is negatively correlated with cell proliferation in the developing amygdala. Both sex differences in play behavior and cell proliferation are mediated by the endocannabinoid system (PNAS 107; 2010). Further investigation into involvement of the endocannabinoid receptor types revealed surprising evidence of cooperation between the two receptors. Neonatal activation of either CB1 or CB2 alone between postnatal days 0-3 was not sufficient to increase female play behavior, however dual-agonist treated newborn females displayed increased levels of social play when tested as juveniles during postnatal days 27-38 (ANOVA $p < 0.05$), and activation of either CB1 or CB2 was sufficient to decrease the number of BrdU+ cells in the female developing amygdala (ANOVA $p < 0.001$). Dual-agonist treatment had no effect on play in males. Antagonizing both receptors in females reduced play behavior (ANOVA $p < 0.05$). Both CB1 and CB2 are known to mediate cell proliferation, however traditionally they were thought to be expressed on different cell types. We used a flow cytometry approach to identify newly proliferating cells in the developing amygdala, and found that neonatal females contain more newly proliferating cells coexpressing CB1 and CB2 compared to

males (t-test $p < 0.05$). Together with our behavioral analyses, these data suggest that developmental sex differences in endocannabinoid receptor expression mediate effects on juvenile social play behavior. Currently we are performing further characterization of the newly born cells that coexpress CB1 and CB2, and assessing how sex differences in neuronal morphology in the juvenile amygdala correspond to endocannabinoid-mediated effects on social play behavior. This study was funded by RO1MH52716-018 to MMM.

Disclosures: **K.J. Argue:** None. **J.W. VanRyzin:** None. **M.M. McCarthy:** None.

Poster

245. Sexual Differentiation

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Project #UND0019798

Title: High-throughput identification of sexual and developmental dimorphisms within the hypothalamus and pituitary gland

Authors: *K. L. GRUCHALLA, T. RHEN;
Dept. of Biol., Univ. of North Dakota, Grand Forks, ND

Abstract: The hypothalamus is the main control center of the endocrine system in vertebrates, and the pituitary gland acts as an intermediary between the hypothalamus and peripheral endocrine organs. The hypothalamus is a sexually dimorphic region that requires precise organization during development for proper sex-specific function throughout life. Yet, few high-throughput analyses have been performed to identify sex differences in gene expression during early development. We therefore isolated total RNA from hypothalami and pituitary glands of male and female snapping turtles during embryogenesis and at hatch. We used the Illumina platform to sequence all expressed genes at each time point and in each sex. We combined reads from hypothalamus/pituitary, gonads, and intestine to assemble a complete transcriptome for the snapping turtle. We then used CLC Genomics Workbench to map hypothalamus/pituitary reads to the transcriptome, calculate gene expression values, and test for differences in gene expression between the sexes and across developmental stages. We identified differentially expressed genes

using a false discovery rate of $p<0.05$. We annotated differentially expressed genes by BLASTing snapping turtle sequences against proteomes from human, chicken, and Chinese softshell turtle. We are using BLAST2GO, DAVID, and GeneMANIA to assign Gene Ontology terms for further analyses. Relatively few genes are differentially expressed between the sexes at either time point. At mid-embryonic development, 180 genes are differentially expressed between males and females, and at hatching, 164 genes are differentially expressed between the sexes. Many more genes exhibit developmental changes in expression between embryonic and hatch time points, with 1533 and 3170 differentially expressed genes in males and females, respectively. This suggests major neurological changes occur during the second half of embryonic development. Genes differentially expressed between male and female hatchlings include chromogranin A, the alpha chain of glycoprotein hormones, proopiomelanocortin, prolactin releasing hormone, and somatostatin. Expression of these genes was 1.7 to 3 fold higher in females than in males, mirroring well-documented physiological and behavioral differences between the sexes (reviewed in Rhen and Lang, 2004). High-throughput studies like this will expand our knowledge of gene networks important for sexual differentiation of the hypothalamus. Identification of genes underlying morphologic and physiologic differences will allow a better understanding of the developmental causes of neuroendocrine dysfunction.

Disclosures: K.L. Gruchalla: None. **T. Rhen:** None.

Poster

245. Sexual Differentiation

Location: Hall A

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Topic: E.01. Neuroendocrine Processes

Support: NIH Grant R01MH52716

NIH Grant F32NS076327

Title: The role of microglia and mast cells in sculpting the sexually dimorphic nucleus (SDN) of the POA

Authors: *L. A. PICKETT[1], K. M. LENZ[2], M. M. MCCARTHY[1];
[1]Pharmacol., Univ. of Maryland Sch. of Med., Baltimore, MD; [2]Psychology, The Ohio State Univ., Columbus, OH

Abstract: The first neuroanatomical sex difference detected in the mammalian brain was reported in 1978 and named the sexually dimorphic nucleus (SDN) due to its larger size in males compared to females (Brain Res. 148:333, 1978). The SDN is a dense collection of calbindin-expressing neurons (J. Neurobiol. 42:315, 2000) located within the central division of the medial preoptic nucleus (MPNc) of the preoptic area (POA), a critical brain region for the control of

partner preference and maternal behaviors (Horm. Behav. 55:611, 2009; Neurosci. Bull. 30:863, 2014). Previous studies have established that both sexes generate the same number of neurons in the SDN and they selectively die off early in development in females due to a lack of the endogenous survival factor, estradiol (Brain Res. 353:7, 1985; Brain Res. Dev. Brain Res. 52:17, 1990). This system is an excellent model for naturally occurring cell death versus neuroprotection in the developing brain but the involvement of non-neuronal cells in this model system has been largely ignored. We have previously established that innate immune cells of the brain, microglia, and inflammatory mediators such as prostaglandins direct the development of sex-specific synaptic patterns in the neonatal POA that correlate with sexual behavior in the adult rat (Nat. Neurosci. 7:643, 2004; J. Neurosci. 33:2761, 2013). We now turn our attention to an additional inflammatory cell type, mast cells, which like microglia are of myeloid cell lineage with origins outside the nervous system. We determined there are more mast cells in the POA of neonatal males than females and this sex difference was mediated by estradiol. We have also found that pharmacological activation of mast cells in newborn females induces a male-typical microglial morphology, with higher numbers of ameboid microglia and lower numbers of ramified, phagocytic microglia. We are currently investigating whether mast cells modulate microglial primary phagocytosis (phagoptosis) and whether this contributes to the sexual differentiation of SDN volume by phagoptosis of neurons in the female SDN. Collectively these results indicate non-neuronal cells are crucial and unappreciated factors shaping brain development and sex-specific physiological and behavioral outcomes. Understanding the role of these cells in apoptotic and neuroprotective cascades during normal brain development will allow for further studies of novel therapeutic strategies and potential sex differences in efficacy of estradiol and/or immune cell inhibitors as neuroprotective agents.

Disclosures: **L.A. Pickett:** None. **K.M. Lenz:** None. **M.M. McCarthy:** None.

Poster

245. Sexual Differentiation

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Program#/Poster#: 245.09/P17

Topic: E.01. Neuroendocrine Processes

Support: NSERC grant RGPIN-2014-05714

CIHR grant MOP-136840

Title: Organizational influences of sex steroid hormones on glucocorticoid receptor responses in male and female Long Evan rats

Authors: ***L. INNALA**, L. HILL, Y. YANG, A. ANONUEVO, V. VIAU;
Cell. and Physiological Sci., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The sex steroid milieu during the neonatal period in the rat exerts marked effects on the development of the hypothalamic-pituitary-adrenal (HPA) endocrine axis, including programming the decline in stress HPA axis responses to repeated stimulus exposure (i.e. habituation). In Long Evan rats, neonatal ATD (aromatase inhibition) in males did not alter corticosteroid responses under acute or repeat restrain exposure, while neonatal testosterone propionate (TP) in females resulted in decreased corticosteroid output under acute conditions, and prevented corticosteroid habituation after 5 days of repeated restraint exposure. This organizational effect could involve changes in glucocorticoid-mediated negative feedback efficacy. Using Western blot analysis, we assessed hippocampal glucocorticoid receptor protein responses to single and repeated restraint exposure in adult male and female bearing postnatal implants of the aromatase blocker ATD or testosterone propionate, respectively. Analysis of receptor distribution revealed a trend towards a reduced percentage of GR in the nucleus in neonatal testosterone treated females under naïve conditions, and no effect of ATD on GR localization in males. Prompted by these findings to suggest potential interactions between stress-HPA axis habituation and the sex steroid milieu during the neonatal period on adult GR responses in the hippocampus, we are currently expanding our analysis to include other brain regions known to mediate negative feedback regulation of the HPA axis.

Disclosures: **L. Innala:** None. **L. Hill:** None. **Y. Yang:** None. **A. Anonuevo:** None. **V. Viau:** None.

Poster

245. Sexual Differentiation

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Topic: E.01. Neuroendocrine Processes

Support: CIHR Grant MOP-136840

CIHR Grant MOP-136856

Title: Stress-induced hypothalamic pituitary adrenal axis responses in male and female Long-Evans and Sprague-Dawley rats

Authors: ***Y. YANG**, L. INNALA, A. ANONUEVO, L. HILL, V. VIAU;
Cell. and Physiological Sci., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Male and female rodents show different capacities for coping with stress-induced threats to homeostasis, including stress hypothalamic-pituitary-adrenal (HPA) axis habituation. Moreover, within-sex variability in behavioral and neuroendocrine responses has also been identified in different rodent strains. To provide a framework for studying how genotype and sex may come to interact on stress HPA axis habituation, we examined plasma corticosterone and

central corticotropin-releasing hormone (CRH) mRNA responses of adult male and female Sprague-Dawley and Long-Evans rats exposed to repeated restraint (2 h day/5 consecutive days). Females in general showed higher plasma corticosterone concentrations than males under acute and repeat restraint conditions. Assessment of corticosterone output between the first and last bout of restraint indicated comparable declines in total corticosterone responses in both male and female Sprague-Dawley rats (68 % and 77%, respectively). Significant declines in corticosterone (50%) occurred in Long-Evans males (50%), but not females (109%). Repeated restraint increased CRH mRNA in the paraventricular nucleus of hypothalamus in both strains of female rats. Whereas CRH mRNA decreased in Long-Evans males, there was no change in Sprague-Dawley males. Taken together, the interactions revealed here continue to underscore the importance of sex and strain in modeling individual differences in neuroendocrine habituation and stress vulnerability.

Disclosures: Y. Yang: None. **L. Innala:** None. **A. Anonuevo:** None. **L. Hill:** None. **V. Viau:** None.

Poster

245. Sexual Differentiation

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 245.11/P19

Topic: E.01. Neuroendocrine Processes

Support: NIH MH068482

Title: Effects of DNA methyltransferase inhibition on sexually dimorphic cell groups in the preoptic area

Authors: *M. MOSLEY, J. M. WEATHINGTON, A. CASTILLO-RUIZ, N. G. FORGER; Neurosci., Georgia State Univ., Atlanta, GA

Abstract: Gonadal steroids act perinatally to orchestrate the formation of sex differences in the brain. In some cases, such sex differences are due to differential cell death, but other differences persist even when cell death is prevented. For example, the sexually dimorphic nucleus of the preoptic area (SDN-POA) is larger in males than in females in many species. Calbindin immunoreactivity (ir) defines the SDN-POA in mice (referred to as the CALB-SDN), and the sex difference in the CALB-SDN persists in Bax knockout mice, in which cell death is prevented (Gilmore RF et al., 2012). We hypothesized that sexual differentiation of the CALB-SDN is due to the hormonal control of cell phenotype. Epigenetic modifications can lead to lasting changes in gene expression and are likely candidates for such steroid-dependent, cell fate “decisions.” The best-studied epigenetic modification is the methylation of DNA, catalyzed by DNA methyltransferases (DNMTs) and usually associated with gene repression. To test whether a

disruption of DNA methylation during the neonatal critical period alters the development of the CALB-SDN, mice were injected, i.c.v., with the DNMT inhibitor zebularine, or saline alone, on the day of birth (P0) and P1. Brains were collected on P27 and immunohistochemically stained for calbindin. We find a two-fold increase in the number of CALB-SDN cells in mice neonatally treated with zebularine ($p= 0.01$). Zebularine increased CALB-SDN cell number in females to that in saline males, although zebularine-treated males had even more calbindin+ cells than control males or zebularine-treated females. We also found more calbindin+ cells just lateral to the CALB-SDN ($p< 0.04$) in zebularine-treated mice, but no effect of zebularine on calbindin+ cell number in the cortex. To determine whether other sexually dimorphic cell groups in the POA were affected by neonatal DNMT inhibition, adjacent sections were stained for estrogen receptor (ER) α ir. As expected, control females had more ERα+ cells than males in the medial POA ($p<0.01$). Neonatal zebularine treatment increased the number of ERα+ cells ($p<0.03$) and eliminated the sex difference in the medial POA: ERα+ cell counts were low in control males, and elevated to a similar level in control females and males and females treated with zebularine. In a more lateral region of the POA there was no effect of sex or zebularine treatment on ERα+ cell number. Thus, a transient disruption of DNA methylation during the neonatal period causes long-term changes in at least two sexually dimorphic markers in the mouse POA. We are currently examining other markers and brain regions, and determining possible critical periods for these effects.

Disclosures: M. Mosley: None. **J.M. Weathington:** None. **A. Castillo-Ruiz:** None. **N.G. Forger:** None.

Poster

245. Sexual Differentiation

Location: Hall A

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Program#/Poster#: 245.12/P20

Topic: E.01. Neuroendocrine Processes

Support: NC State Start Up Funds

NC State Undergraduate Research Grant

Title: Excitatory synaptic input differs by sex in the nucleus accumbens core but not shell in prepubertal rats

Authors: J. CAO, J. A. WILLETT, C. A. HAUSER, T. R. WILL, D. M. DORRIS, *J. MEITZEN;
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Abstract: Sex differences exist in how the brain mediates motivated behavior and reward, both in normal and pathological contexts. Investigations into the underlying neural mechanisms yield

accumulating evidence of sexually different dendritic spine morphology and neuromodulator and steroid sex hormone action in the striatal brain regions, including the nucleus accumbens core and shell. How these sex differences influence the electrophysiological properties of neurons in the nucleus accumbens to ultimately modulate this region's function is an area of active research. One current hypothesis is that the excitatory synaptic input onto medium spiny neurons (MSNs), the primary output neurons of the nucleus accumbens, differs by sex. Here we test this hypothesis by performing whole-cell recordings of MSNs in acute brain slices from pre-pubertal male and female nucleus accumbens core and shell. We assess intrinsic neuronal electrophysiological properties through the application of current stimuli in current-clamp and excitatory synaptic input through recording of miniature excitatory post-synaptic currents (mEPSCs) in voltage-clamp. mEPSC frequency is higher in female than in male MSNs in the core but not shell. No sex difference was found in mEPSC amplitude or time of decay. MSN intrinsic excitability and action potential properties are stable across sex in both the core and shell. This data implicates excitatory synaptic input as a potential mechanism underlying sex differences in nucleus accumbens-mediated behaviors, and that sex differences in excitatory synaptic input is generated before puberty.

Disclosures: J. Cao: None. **J.A. Willett:** None. **C.A. Hauser:** None. **T.R. Will:** None. **D.M. Dorris:** None. **J. Meitzen:** None.

Poster

246. Sexual Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 246.01/P21

Topic: E.03. Behavioral Neuroendocrinology

Support: CIHR

Title: Disruption of conditioned sexual inhibition in male rats: role of dopaminergic VTA neurons in alcohol- and cue-induced dopamine release in the nucleus accumbens core

Authors: *K. GERMÉ, L. R. GOSSIP, J. ALVAREZ-BARKHAM, N. WATSON, J. G. PFAUS;
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Abstract: When male rats are given access alternatively to a sexually nonreceptive female scented with a neutral odor (almond) and an unscented receptive female, they display a conditioned avoidance of the scented female on a final copulatory test, with two receptive females, one scented and one unscented. An acute treatment with a low dose of alcohol (0.5g/kg) before the final test disrupts this inhibition. We have shown previously that this does of alcohol

alone increases dopamine (DA) concentrations in the nucleus accumbens (NAc) compared to saline. Although almond odor alone had no effect on DA in saline-treated males, it increased DA in alcohol-treated males. Because the ventral tegmental area (VTA) sends large DA inputs to the NAc, we examined the role of VTA dopaminergic neurons in cue and alcohol-induced DA release in the NAc core. Sexually naive male Long-Evans rats were conditioned for sexual inhibition to an odor (almond) using our established paradigm and injected with saline or alcohol (0.5g/kg) before the final choice test. The retrograde tracer Fluoro-Gold (FG) was then infused into the NAc core. Male rats were exposed to alcohol and to the inhibitory odor or a novel odor for an hour. Then fluorescence immunohistochemistry was performed on VTA slices to determine the numbers of cells expressing Fos, TH and FG so the numbers of DA cells that are activated and that have axons in the NAc core. Preliminary results suggest that VTA activation may not be necessary for alcohol and cue-induced DA increase in the NAc core.

Disclosures: K. Germé: None. **L.R. Gossip:** None. **J. Alvarez-Barkham:** None. **N. Watson:** None. **J.G. Pfaus:** None.

Poster

246. Sexual Behavior

Location: Hall A

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Program#/Poster#: 246.02/P22

Topic: E.03. Behavioral Neuroendocrinology

Support: Région Centre DURAREP2

Nagoya University for YS travel grant

Title: Both populations of kisspeptin neurons are involved in the stimulation by rams of LH secretion in anestrous ewes

Authors: C. FABRE-NYS[1], *L. DUFOURNY[1], S. MARTINET[1], O. LASSERRE[2], R. P. MILLAR[3], S. OKHURA[4], Y. SUETOMI[4];
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Abstract: Sheep are seasonal breeders and are sexually quiescent during spring. However, ovulation can be induced in these females by sudden exposure to a sexually active ram a phenomenon known as “the ram effect”. The first endocrine change observed is an immediate stimulation of LH pulsatile secretion. Several studies have shown that Kisspeptins (Kiss) are potent stimulators of LH secretion. Kiss is present in 2 populations of neurons, in the arcuate nucleus (ARC) and in the preoptic area (POA). Our aim was to understand whether one or the 2 populations of Kiss neurons were implicated during the “ram effect”. Using a double immunofluorescent detection of Kiss (Kiss IR) and cFos (Fos IR) we identified Kiss neurons

activated by exposure to a sexually active ram for 2h (M2=7) or 12h (M12=12) compared to a control situation (exposure to females for 2h, n=5). Density of labelled cells were compared using Kruskall and Wallis test followed by Mann and Whitney U test. The density of Kiss IR also Fos IR and the proportion of KissFos IR differed significantly among the groups both in the ARC ($p<0.002$) and the POA ($p<0.002$) and the proportion of double labelled cells was higher in ewes exposed to males than to females but did not differ between ewes exposed to M2 and M12. Median and interquartile were respectively 1.86 ±2.45 (Cont ARC) , 21.74±2.24 (Cont POA), 26.32 ±10.49 (M2 Cont), 71.43 ± 13.53 (M2 POA), 38.36 ± 20.44 (M12 ARC), 66.65 ± 15.85 (M12 POA). By contrast the density of Kiss IR neurons or Fos IR neurons did not differ between groups. We then tested whether local administration by retrodialysis of a Kiss antagonist (P234 10-6M given for 3 h starting 1h before male introduction) either in ARC or POA would affect the LH response of anoestrus ewes to rams compared to infusion of solvant. Data were analysed as % change of LH concentration compared to the mean level during the hour before male introduction and compared using GLM ANOVA followed by Fisher post-hoc test. LH concentration changed with time in all groups. ($p<0.003$ in the POA and $p< 0.0001$ in the ARC). In the POA, there was a significant effect of treatment ($p<0.01$) and an interaction between treatment and time ($p<0.01$). Treatment differed from solvant 1 to 4 hours after male introduction ($p<0.001$ 1 to 3h after male introduction, $P<0.026$ 4 h after). In the ARC treatment had no significant effect and there is no interaction between time and treatment but post-hoc comparisons showed a significant difference between treated and control at 1h ($p<0.026$) 2h ($p<0.0016$) and tended at 3h ($p=0.038$). These results show that both populations of Kiss neurons are involved in the early stimulation of LH secretion by the “ram effect”.

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Poster

246. Sexual Behavior

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 246.03/P23

Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant MH50388

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Title: Non-ovarian aromatization regulates sexual motivation and receptivity in female quail

Authors: C. DE BOURNONVILLE[1], C. A. CORNIL[2], G. F. BALL[3], *J. H. BALTHAZART[2]; [1]Res. Group in Behav. Neuroendocrinology, Univ. of Liège, Liege, Belgium; [2]Univ. of Liege, B-

4000 Liege 1, Belgium; [3]Col. of Behavioral and Social Sci., Univ. of Maryland, College Park, MD

Abstract: Although aromatase is expressed in both male and female brains, its functional significance in females remains unclear. In male Japanese quail, we found that brain aromatase regulates both motivational and performance aspects of sexual behavior but that the latency of action of locally produced estrogens is different for the two responses. In female quail, sexual receptivity is activated by estrogens. However it is not known whether sexual motivation is similarly estrogen-dependent and whether estrogens locally produced in the brain contribute to these behavioral responses. 18 ovariectomized (OVX) females received a 20 mm long Silastic implant filled with crystalline testosterone (T) while 8 OVX females and 12 SHAM-operated females received an empty implant of the same size. Half of the T-treated females were also chronically treated with the potent aromatase inhibitor Vorozole and all other birds were injected in parallel with the vehicle solution. All birds were then tested for their motivation to approach a male sexual partner or a congener of either sex (sexual vs. social motivation) and for sexual receptivity. Ovariectomy induced a significant decrease of time spent in close proximity to the male. Treatment with testosterone partially restored this response and this effect was prevented when estradiol synthesis was inhibited by Vorozole. The total time spent near the male or the female (social motivation) was not affected by these treatments. Sexual receptivity (composite score including crouches and avoids) of OVX females did improve following T treatment and this effect was blocked by the aromatase inhibitor. These changes in receptivity reflected mostly effects on male avoidance behavior rather than on sexual crouches. Serum estradiol concentration was significantly higher in SHAM females than in all OVX groups and serum E2 was significantly higher in OVX+T than in OVX or OVX+T+Vorozole females. Together these data demonstrate that treatment of OVX females with T increases sexual motivation and aspects of sexual receptivity and that these effects are mediated at least in part by non-gonadal aromatization of the androgen. Crouching behavior was not significantly affected by T suggesting that this behavior requires exposure to higher doses of estrogens or is only activated after longer latencies. Brain aromatization likely contributes to the behavioral effects observed here following T treatment but alternative sources of estrogens (e.g. adipose tissue) should also be considered.

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Poster

246. Sexual Behavior

Location: Hall A

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Program#/Poster#: 246.04/P24

Topic: E.03. Behavioral Neuroendocrinology

Title: Depressive-like behavior in same sex preference male rats with prenatal letrozole

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Abstract: Commonly there is a sex preference difference: usually males select females, while females in estrus choose male sex partners. However, in all species studied there is a subpopulation that shows same sex preference. Prenatal administration of the aromatase inhibitor, letrozole, induces same sex preference in male rats. This effect is mediated by letrozole´s-induced decrease in brain estradiol levels along development, essential to organize the structures that mediate male sexual behavior in adulthood. In addition, there is a larger incidence in depressive symptoms in homosexual men in comparison with heterosexuals, apparently due to social factors. In this work we aim to explore the putative biological component of this difference. The experimental group received a daily prenatal letrozole (0.56µg/kg) injection to mothers from gestation day 10 to term (during 10 days) to induce same sex preference in some male rats' offspring. A control group received oil. The male offspring were left undisturbed until 3 months of age, when they were evaluated for sex preference (a box where the experimental male could choose to interact with a receptive female or a sexually experienced male) and time interacting with either stimuli. After the sex preference test all animals were subjected to the forced swim paradigm (FST) where immobility, swimming and climbing were recorded. The results showed an increased immobility (depressive-like behavior) in male rats with same sex preference as compared to males with female preference. Results also showed that there was a males' subpopulation that had a higher interaction with males (even sexual, lordosis or proceptivity), despite they spent more time with the female. In these males there was also an increased immobility in the FST. These results suggest that our method permits to discern at least two populations of males oriented to other males that consistently displayed higher levels of depressive like behavior.

Disclosures: A. Hernandez-Gonzalez: None. **A. Fernandez-Guasti:** None.

Poster

246. Sexual Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 246.05/P25

Topic: E.03. Behavioral Neuroendocrinology

Title: Hormonal response in enforced interval of copulation and copula add libitum in male rats

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Abstract: Mating behavior is under the regulation of two main axis, it is activated by the hypothalamus-pituitary-gonadal axis (HPG) and inhibited by hypothalamus-pituitary-adrenal (HPA). An increase of Corticosterone (C) and Testosterone (T) after masculine sexual behavior has been reported in several mammal species, suggesting that both axes are activated simultaneously by sexual behavior. After sexual activity, plasma levels of C and T increase in sexual experienced male rats. Enforced Interval of Copulation (EIC) was described by Larsson (1956), this model consist of interruption of mating by means of the female withdrawal during one minute each time, until ejaculation. With this approach, it has been observed that this repeated interruption of copulatory interaction in experienced male rats decrease the time needed to achieve ejaculation. The aim of this study was to investigate what response in levels of T and C are caused by different intensities of sexual stimulation in sexually experienced and inexperienced male rats. Two groups of male rats, with and without sexual experience, were divided into four subgroups according to the copulatory scheme: copula *ad libitum* until ejaculation (CAD-E); Enforced Interval of Copulation up to 3 intromissions (EIC-3I); Enforced Interval of Copulation up to ejaculation (EIC-E); copula *ad libitum* up to 3 intromissions (CAD-3I) and in presence of receptive female during 20 min physically separated by a grid between them to prevent physical contact (GRID). The different sexual conditions correlated to higher plasma levels for C in both groups. Males with prior sexual experience had higher C concentration in plasma for all sexual conditions. The greater C levels was observed for EIC-E, they were significantly higher than the levels observed for CAD-E. The animals from EIC-3I and CAD-3I had the lowest levels among all groups; also we found differences between them. Plasma levels for T increased both, in experience and naïve group, after exposure to a receptive female, but only experienced males had significant differences compared with control group. In experienced males, T levels increased significantly in all the sexual conditions as compared with naïve males, no differences between subgroup were found. Males allowed to ejaculate showed an increase in plasma levels both for C and T. Altogether, these data showed that the increase of T and C levels strongly depends on sexual experience, which corroborate the simultaneous activation of HPA and HPG axes, interaction of both axes depends on the sexual conditions which can modulates the intensity and patterns of hormonal response following sexual activity.

Disclosures: T.M. Medina: None. **M. Arteaga-Silva:** None. **H. Bonilla-Jaime:** None. **M. Hernàndez-Gonzàlez:** None.

Poster

246. Sexual Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 246.06/P26

Topic: E.03. Behavioral Neuroendocrinology

Title: Neural circuits in the medial preoptic area associated with appetitive female sexual behaviour are modified by steroid hormones

Authors: *J. W. PAYNE, M. GRAHAM, K. GERMÉ, J. G. PFAUS;
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Abstract: The medial preoptic area (mPOA) is critical for the control of appetitive sexual behaviours in the female rat, and dopamine (DA) receptor activation has been shown to mediate the display of these behaviours. We have also shown previously that DA receptors in this region are themselves dependent on the hormonal priming of the female. Specifically, in female rats primed with estradiol benzoate (EB) alone, activation of D2 receptors (D2R) in the mPOA increases appetitive behaviours, whereas in females primed with both EB and progesterone (EB+P) these behaviours increase after D1 receptor (D1R) activation (Graham & Pfaus, 2010; 2012). Modulation of DA receptors by estradiol and progesterone has been further demonstrated using Western blots, and females treated with EB alone had reduced D1R protein levels in the mPOA, whereas females treated with EB+P had increased D1R protein in the mPOA. D2R protein levels showed the reverse pattern. To further understand how ovarian steroids might affect the neural circuits in the mPOA associated with appetitive female sexual behaviour, the present experiment used fluorescence immunohistology to map pathways projecting from the mPOA to either the ventral medial hypothalamus (VMH) or the ventral tegmental area (VTA), areas known to regulate lordosis. Ovariectomized rats were infused with FluoroGold (FG), a retrograde tracer, into either the VMH or VTA 10 days prior to sacrifice. Rats were randomly assigned into one of three steroid treatment groups: EB+P (10µg and 500µg, respectively), EB+Oil, or Oil+Oil; with hormone injections administered 48h and 4h prior to sacrifice. Fluorescent immunohistology was conducted on mPOA slices to label either glutamate or GABA neurons, and neurons expressing D1R or D2R. Thus, in combination with the autofluorescence of FG-positive cells, we were able to quantify neuronal type, type of DA receptor expression, and if the neurons project from the mPOA to either the VMH or VTA. Preliminary results indicate that the administration of EB increased the number of GABA cells that expressed D2R. These data support previous findings demonstrating that ovarian steroids modulate the expression of DA receptors in the mPOA, and extend those findings to the identification of neurochemical systems that might be altered by mPOA DA release in the facilitation of appetitive sexual behaviour in the female rat. Supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada.

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Poster

246. Sexual Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: CONACYT 167101

PAPIIT IN200512

IN210215

Title: Naloxone blocks neurogenesis in the olfactory bulb induced by paced mating in the female rat

Authors: *M. SANTOYO ZEDILLO, R. G. PAREDES, W. PORTILLO MARTÍNEZ;
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Abstract: Previous research from our group, demonstrated that in the female rat a paced mating encounter increases the number of newborn cells in the accessory olfactory bulb (AOB). On the other hand, when female rats are allow to pace the sexual interaction they develop a positive affective (reward) state, evaluated by the conditioned place preference paradigm. This reward state is mediated by opioids because the intraperitoneal injection of naloxone (opioid receptor antagonist) blocks the change of preference induced by paced mating. In the present study we evaluated if blocking the opioid receptors could reduce the number of new cells that incorporate to the AOB generated during the first session of paced sexual contacts. Sexually-naïve female rats, bilaterally ovariectomized and hormonally supplemented were randomly assigned to one of five groups: 1) without sexual contact, 2)without sexual contact injected with naloxone, 3) females that mated without pacing the sexual interaction, 4) females injected with saline before paced mating and 5) females injected with naloxone before paced mating. An increase in newborn cells, BrdU inmunoreactive (BrDU IR), in the AOB of the females treated with saline that paced the sexual interaction was clearly observed when compared to the other groups. Naloxone administration before paced mating blocked the increase in the number of BrdU IR cells. These data support that opioid peptides have a fundamental role in the production of new cells in the olfactory bulb induced by paced mating in the female rat. Further research will need to determine the role of these newborn cells in the physiology of reproduction.
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Disclosures: M. Santoyo Zedillo: None. **R.G. Paredes:** None. **W. Portillo Martínez:** None.

Poster

246. Sexual Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 246.08/P28

Topic: E.03. Behavioral Neuroendocrinology

Support: UVM

Title: Effects of the endocrine disruptor 2-4 dichlorophenol in male rat sexual behavior

Authors: *H. A. HURTAZO[1], F. QUINTANILLA[1], J. P. MAC GREGOR[2], M. CRUZ-SOTO[1]; [1]Univ. Del Valle De Mexico, Queretaro, Mexico; [2]Univ. Politecnica de Santa Rosa Jauregui, Queretaro, Mexico

Abstract: Chlorophenols present in the food chain and aquatic environment have been of great concern due to their potential endocrine disrupting effects on human health and wildlife. 2,4-Dichlorophenol (2,4-DCP) is a chemical substance primarily used as an intermediate in herbicide and dye manufacture. It has also been reported that 2,4-DCP can be formed as a degradation product of another herbicide component, 2,4-Dichlorophenoxyacetic acid. Phenols and chlorines naturally react with each other to form 2,4-DCP on disinfection of drinking water or on deodorization of polluted water processes. The administration of 2,4-DCP to female rats via drinking water during the pre-mating, mating, gestation and lactation periods at a concentration of 300 ppm caused a decrease in litter size and increased liver and spleen weights of F1 pups, although 2,4-DCP is suspected of having endocrine disrupting effects, there is still a lack of understanding on whether these endocrine responses can be manifested by an impairment of sexual behavior. In the present study, the endocrine disrupting effects of (2,4-DCP) on male sexual behavior were investigated. Sexually naïve male Wistar rats (300-350 g) were kept under a reversed 12-h light/dark cycle with free access to food and water. The animals were divided into two groups, one control and one administrated with 1.25 mg/day for 45 days. After 45 days of administration male sexual behavior was evaluated. The following parameters were recorded: 1) latency to the first mount and intromission; 2) number of mounts and intromissions; 3) ejaculation latency. The results demonstrated that 2,4-DCP affected male sexual behavior. We found a decrease of mount, intromission and ejaculation latency compared with control animals. Further studies are required to find out the neural mechanism involved in the sexual behavior effect and also, an analysis of aromatase expression in areas such as the olfactory bulb and medial preoptical area in response to 2,4 DCP administration.

Disclosures: H.A. Hurtazo: None. **F. Quintanilla:** None. **J.P. Mac Gregor:** None. **M. Cruz-Soto:** None.

Poster

246. Sexual Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 246.09/P29

Topic: E.03. Behavioral Neuroendocrinology

Title: The role of experience and testing arenas on the ejaculatory threshold in male rats

Authors: *J. OLAYO-LORTIA, A. MORALES- OTAL, A. CRUZ-BENITES, A. FERREIRA- NUÑO;
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Abstract: The Multiple Partner-Choice Arena (MPCA) is a device developed in our laboratory, consisting of four acrylic cylinders arranged in a circle, with a small door located at the base of each cylinder where only the female can cross. Inside of each compartment, a sexually expert male rat (SEM) left, while in the central compartment a hormone-primed receptive female is allowed to choose the male to copulate with. Under these competitive conditions, in a previous study we demonstrate that all SEM ejaculate faster in comparison when they were tested in a standard arena (SA, a closed acrylic cylinder), since the parameters of the ejaculatory threshold (number of intromissions, and ejaculation latency), shown a significant reduction. To analyze the role of experience and testing arenas in the reduction observed on ejaculatory threshold in the MPCA, we compare two groups of sexually naïve male rats (n = 12) evaluated in both arenas (MPCA and SA) during four successive weekly sexual tests of 30-min. During the first 2 copulatory series of each test, the number of mounts (NM) and intromissions (NI) preceding each ejaculation, as well as mount (ML), intromission (IL) and ejaculation (EL) latencies, post-ejaculatory interval (PEI), ejaculation frequency (EF) and the hit rate (HR) were recorded. For the record of ML, IL, EL and PEI in the MPCA, only time those females spent in the compartment of each male considered. In comparison with the males tested in the SA, in the MPCA males shown a significant increase in ML and IL. However, a significant reduction in NM and NI on both copulatory series in most of the four tests obtained and consequently this provokes a significant reduction in the EF. No significant differences observed on EL, PEI and HR parameters in both groups of males. In conclusion, our results shown that the reduction obtained in the NI when male rats are tested in the MPCA depends on the competitive conditions, while the reduction observed in EL when SEM are tested in the MPCA depends on sexual experience.

Disclosures: J. Olayo-Lortia: None. **A. Morales- Otal:** None. **A. Cruz-Benites:** None. **A. Ferreira- Nuño:** None.

Poster

246. Sexual Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 246.10/P30

Topic: E.03. Behavioral Neuroendocrinology

Support: NSERC

Title: Investigating the role of ghrelin in male rat sexual behavior

Authors: *L. M. HYLAND[1], S. ROSENBAUM[2,3], B. WOODSIDE[3], J. PFAUS[3], A. ABIZAID[2];
[2]Neurosci., [1]Carleton Univ., Ottawa, ON, Canada; [3]Psychology, Concordia Univ., Montreal, QC, Canada

Abstract: The physiology of reproduction in animals depends on the organism`s metabolic state and energy availability. The orexigenic peptide hormone ghrelin plays an integral role in energy metabolism and is known to have an inhibitory effect on reproductive physiology. In contrast, ghrelin and its receptor, the GHSR-1a, is also key for generating motivated behaviors, including sex behaviors. The aim of the current study was to investigate the effects of a GHSR knock out genetic manipulation animal model on appetitive and consummatory male sexual behavior using bi-level chambers. Male wild type (n=10) and GHSR KO rats (n=7) were exposed to sexually receptive females for 30 minutes inside bi-level chambers and were allowed to copulate. This protocol was followed every four days for a total of five sessions. During these sessions we measured appetitive (locomotor activity in anticipation of a female) and consummatory behaviors (latency to mount, intromissions, latency to ejaculate, and number of ejaculations). Results show that GHSR KO rats have a shorter latency to approach a female during the initial exposure to the female, and this effect disappears by the fifth session. Interestingly, male GHSR KO rats displayed less anticipatory locomotor activity in the bi-level chambers than their WT littermates. These data suggest that ghrelin has a modulatory effect on male rat sexual behavior, and may differentially affect appetitive and consummatory behavior.

Disclosures: L.M. Hyland: None. **S. Rosenbaum:** None. **B. Woodside:** None. **J. Pfaus:** None. **A. Abizaid:** None.

Poster

246. Sexual Behavior

Location: Hall A

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Program#/Poster#: 246.11/P31

Topic: E.03. Behavioral Neuroendocrinology

Support: SEP-CONACYT-167773

Title: Sexually dimorphic brain nuclei in female rats that undergo same-sex preference conditioning

Authors: *M. B. TECAMACHALTZI-SILVARÁN[1], M. BARRADAS-MOCTEZUMA[2], L. I. GARCÍA[2], P. CARRILLO[2], J. MANZO[2], G. A. CORIA-AVILA[2];

[1]Ctr. De Investigaciones Cerebrales, Xalapa, Mexico; [2]Ctr. de Investigaciones Cerebrales, Univ. Veracruzana, Xalapa, Mexico

Abstract: We have previously shown that under the effects of quinpirole (QNP a D2-type agonist) male rats can be easily conditioned to develop a same-sex partner preference. In a drug-free final test our conditioned males displayed a social and sexual preference towards the male partner over a sexually-receptive female. In addition, there was an increase in the area size of the supraoptic nucleus (SON), but no changes were observed in the preoptic area (SDN-POA) Behavioural Brain Research 283 (2015) 69-77. In the present study we assessed the area size of the SON and SDN-POA of female rats with conditioned same-sex preference. We used ovariectomized Wistar females, primed exclusively with estradiol benzoate (EB) 48 h prior to every conditioning trial. On the day of conditioning they received 1.25 mg/kg of QNP (i.p.) and 1 min later they were allowed to cohabitate during 24 h with a lemon-scented female, also primed with EB. Females were returned to their single home cages and this was repeated every four days, for a total of three trials. A first QNP-free preference test occurred four days after the last conditioning. Females were primed with EB+P and chose between two female partners (lemon-scented vs. unscented). The results indicated that females preferred the lemon-scented female as observed with more body contacts, sexual solicitations, and hops and darts. Females were reconditioned for two more sessions, and four days later were tested again for partner preference between one stud male and the lemon-scented female. The results indicated that QNP females displayed a similar preference for males over females, whereas control females preferred males. Four days later their brains were obtained and processed for the Nissl dye technique to assess the area size of SON and SDN-POA. Taken together, these results indicate that female rats primed exclusively with EB can be conditioned to develop a same-sex preference under the effects of enhanced D2-type receptor activity. The preference is observed with social and sexual behaviors when females are fully primed with EB+P. Such conditioning process appears not to affect the area size of SON and SDN-POA.

Disclosures: M.B. Tecamachaltzi-Silvarán: None. **M. Barradas-Moctezuma:** None. **L.I. García:** None. **P. Carrillo:** None. **J. Manzo:** None. **G.A. Coria-Avila:** None.

Poster

246. Sexual Behavior

Location: Hall A

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Program#/Poster#: 246.12/P32

Topic: E.03. Behavioral Neuroendocrinology

Title: Hypothalamic and peripheral oxytocin in the female rat and the effect of mating experience

Authors: M. L. LANGETT, *N. CAMERON;
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Abstract: Sexual activity is known to affect the oxytocin (OT) neuron populations within the paraventricular nucleus (PVN) and the supraoptic nucleus (SON). However, specific factors influencing each nuclei's activation remain unknown. In addition, the effect of mating on plasma OT levels has not been consistently shown. We hypothesize that OT production in the brain and plasma levels are linked to the type of sexual experience, as assessed by pacing and vaginal cervical stimulations (VCS) received. To test this hypothesis Experiment 1 examined baseline OT levels in brain and plasma of proestrus or metestrus female rats. In Experiment 2, sexually-experienced ovariectomized steroid-primed females were used to study the effects of VCS and paced mating on OT levels. These females were either paced or not paced, and vaginally masked or unmasked during a 30 minute trial. For both studies, OT levels in the brain and plasma were analyzed with an enzyme-linked immunosorbent assay. In experiment 3, proestrus or metestrus females received an intraperitoneal injection of fluorogold five days prior to brain collection. OT peripheral projections from the PVN and SON were examined using immunofluorescence of the retrograde tracing of fluorogold and OT positive cells. Our results show a significant effect of estrous cycle on the SON, where OT concentrations were higher at proestrus ($p=0.02$). Estrous cycle did not affect OT concentration in PVN or plasma levels. Additionally, no significant correlation between the PVN, SON and plasma OT levels was found. In experiment 2, VCS significantly increased PVN OT concentration ($p=0.03$) but had no effect on SON or plasma OT levels. Paced mating had no effect on brain or plasma OT levels. In experiment 3, estrous cycle had no effect on the average number of dual labeled neurons in either nuclei suggesting that both the SON and PVN have the capacity to contribute equally to plasma OT levels. These results indicate the importance of VCS in PVN OT activation. However, the lack of correlation between PVN OT and plasma levels during mating suggest the PVN may not be releasing OT into the periphery. Interestingly, OT production in the SON did not increase during the two mating conditions and is not associated with sexual behavior in the experienced female rat. Additionally, only the SON OT levels are affected by estrous cycle. In conclusion, the lack of correlation between brain and plasma OT levels may imply a quick release of OT following the initiation of sexual activity that was not captured. Our findings demonstrate that in the rat the PVN and SON equally project to the periphery, and VCS only influences PVN OT production during mating in experienced female rats.

Disclosures: M.L. Langett: None. **N. Cameron:** None.

Poster

246. Sexual Behavior

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Program#/Poster#: 246.13/P33

Topic: E.03. Behavioral Neuroendocrinology

Title: Effect of sexual behavior on the prevention of testosterone-induced precancerous prostate lesions

Authors: *D. HERRERA-COVARRUBIAS[1], M. B. TECAMACHALTZI-SILVARAN[2], M. BARRADAS-MOCTEZUMA[2], J. MANZO[2], G. E. ARANDA-ABREU[2], N. ISMAIL[4], M. HERNÁNDEZ[3], G. A. CORIA-AVILA[3];
[2]Ctr. de Investigaciones Cerebrales, [1]Univ. Veracruzana, Xalapa, Mexico; [3]Ctr. de Investigaciones Cerebrales, Univ. Veracruzana, Xalapa, Veracruz, México, Mexico; [4]Sch. of Psychology, Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Prostate cancer has been classically associated with androgens like testosterone (T) because they induce cell division and spontaneous mutations that could result in more abnormal cells within the gland. In previous studies we showed that rats treated with subcutaneous (s.c.) implants of T (100 mg/kg) during four weeks expressed precancerous prostate lesions such as dysplastic epithelium, apolarity of the nucleus, and abnormal Nucleus:Cytoplasm ratio (N:C 1:1). Because some studies suggest that the lack of sexual activity may be a risk factor for the development of prostate cancer, we explored such posibility in rats under the model of testosterone-induced lesions. A total of twenty wistar rats (250 g) were divided in two groups. One group received a s.c. implant of T, while the other, the control group, received an empty implant. Both groups bore the implant during 5 weeks. During that time the two groups were further divided into two subgroups. The first subgroup was allowed to have sex for 10 trials, while the second subgroup was only exposed to a receptive female for 10 trials without access to copulation. Sexual behavior occurred twice a week and was videorecorded and scored. After 5 weeks, animals were sacrificed with a lethal dose of sodium pentobarbital and the prostates were extracted and processed for H&E dye. Results showed that rats treated with T presented precancerous histological abnormalities in the dorsolateral and ventral portions of the prostate. Moreover, compared to controls, males treated with T displayed poor sexual performance during the first few trials. Sexual activity modulated the malignancy of the prostate lesions. In conclusion, five-week exposure to a high dose of T is sufficient to induce pre-cancerous lesions in the prostate. The lesions are modulated by sexual behavior, but the latter is also affected by high levels of T.

Disclosures: D. Herrera-Covarrubias: None. **M.B. Tecamachaltzi-Silvaran:** None. **M. Barradas-Moctezuma:** None. **J. Manzo:** None. **G.E. Aranda-Abreu:** None. **N. Ismail:** None. **M. Hernández:** None. **G.A. Coria-Avila:** None.

Poster

246. Sexual Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: Canadian Institutes for Health Research (CIHR)

Comisión Nacional de Investigación Científica y Tecnológica (CONICYT)

Title: Development of a conditioned ejaculatory preference in male rats is determined by their first sexual experiences

Authors: *G. R. QUINTANA ZUNINO, L. PRIMEAU, J. PFAUS;
Psychology, Concordia Univ., Montreal, QC, Canada

Abstract: Estrous odors of female rats are salient appetitive cues for male rats that compel them to move towards females in heat during their first sexual experiences. However, after baseline rate of sexual responding has been achieved, the importance of these cues tends to diminish, and at this stage learning becomes pivotal on the establishment of new associations. We have shown previously that male rats develop a conditioned ejaculatory preference (CEP) for females scented with a neutral odor like almond or lemon (ScF) that is paired with the male's post-ejaculatory reward state during repeated sexual experiences. The present study evaluated the behavioral and neural components of CEP for a neutral odor cue after manipulating the very first sexual experience(s) of male rat. Sexually naïve males were divided into two groups that received one or five trials of copulation to ejaculation with sexually receptive females either scented with almond odor (ScF) or not scented (UnScF). Following this training, half the animals in each group were given 10 subsequent copulatory trials with the other type of female (ScF for males trained first with UnScFs, or UnScF for males trained first with ScFs). On a final test, males were placed into a large open field with two sexually receptive females, one ScF and one UnScF. Males that had their first five experiences with either ScFs or UnScFs did not show a CEP for either female, nor did males that had their first sexual experience with the ScF followed by 10 trials with UnScFs. However, males that had their first sexual experience with an UnScF followed by 10 trials with ScFs displayed significant CEP for the UnScF in the open field, indicating that the odor conditioning had been inhibited by the first experience with the UnScF. Exposure to the scent cue before perfusion revealed different patterns of Fos-IR (neural marker for general neural activation) in regions of the nucleus accumbens and medial preoptic area. Altogether, these findings show a differential role of conditioned and unconditioned cues during a male rat's first sexual learning experiences on the development of CEP.

Disclosures: G.R. Quintana Zunino: None. **L. Primeau:** None. **J. Pfaus:** None.

Poster

246. Sexual Behavior

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Program#/Poster#: 246.15/P35

Topic: E.03. Behavioral Neuroendocrinology

Support: SEP-CONACYT-167773

Title: Conditioning heterosexual preference in male rats with unconditioned same-sex preference

Authors: ***M. BARRADAS**, M. B. TECAMACHALTZI-SILVARAN, L. I. GARCÍA, P. CARRILLO, J. MANZO, G. A. CORIA-AVILA;
Neurosci., Ctr. De Investigaciones Cerebrales, Xalapa, Mexico

Abstract: Several studies in the last 50 years indicate that sexual dimorphism of the brain depends on the exposure (or not) during critical periods of development to testosterone (T) or its metabolite estradiol (E). Accordingly, demasculinized males are likely to display unconditioned same-sex preference when they reach adulthood. In addition, our group has shown that partner preference can be conditioned in adulthood when males cohabitate with males under the effects of the D2-type agonist quinpirole (QNP, 1.25 mg/kg, i.p.). In the present study we assessed the unconditioned same-sex preference of demasculinized males and then we conditioned them to prefer females. Male rats were divided in five groups, depending on whether or not they were to be demasculinized: Intact (I), sham castration at postnatal day 1 (ShPN1), castrated at PN1 (CXPN1), castrated at PN30 (CXPN30) and sham castrated at PN30 (ShPN30). At PN60 the unconditioned sexual preference was assessed before a male and a sexually-receptive female as potential partner choices. The results indicated that groups I, ShPN1 and ShPN30 displayed unconditioned sexual preference for the female. However, the group CXPN1 preferred the male, and the group CXPN30 showed a low interest towards the female but no indication of same-sex preference. Males from group CXPN1 were organized into three subgroups to undergo conditioning: a) cohabitation with a receptive female, b) cohabitation with a receptive female + QNP and c) QNP without cohabitation. Conditioning occurred every 4 days, for a total of three trials. For days after the last conditioning trial there was a second preference test to assessed conditioned preference. Male rats under the effects of QNP that cohabitated with a female did develop a heterosexual preference. However, neither cohabitation alone nor QNP alone induced a preference for the female. The results indicated that heterosexual partner preference can be conditioned in demasculinized males with unconditioned same-sex preference.

Disclosures: **M. Barradas:** None. **M.B. Tecamachaltzi-Silvaran:** None. **L.I. García:** None. **P. Carrillo:** None. **J. Manzo:** None. **G.A. Coria-Avila:** None.

Poster

246. Sexual Behavior

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Program#/Poster#: 246.16/P36

Topic: E.03. Behavioral Neuroendocrinology

Title: The role of female sexual components on mate choice of the male rat in a multiple- partner choice arena

Authors: *A. FERREIRA-NUNO[1], A. CRUZ-BENITES[2], J. OLAYO-LORTIA[2], A. MORALES-OTAL[2];
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Abstract: Rat is a polygamous species, since males and females mate with multiple partners simultaneously. To evaluate mate choice in the rat, our laboratory develop a Multiple Partner Choice Arena (MPCA), formed by 4 transparent acrylic cylinders (40 x 50 cm) placed in a circular fashion with a sexually expert male rat (MSE) in each. In the central area, an ovariectomized receptive female rat primed with estradiol benzoate and progesterone (OVX+EP) was placed, allowing the opportunity to choose the male to copulate with, entering in small doors which has the cylinders at its base. Under these conditions, we demonstrate that female rat copulate longer and receives more ejaculations from one of the four males. In order to demonstrate whether the male rat is also capable of preferring a particular female and evaluate the importance of female sexual components of attractivity (female odor), proceptivity (hopping, ear wiggling and darting) and receptivity (lordosis) in this selection, two groups of MSE (n = 7) were tested in a 6-cylinder MPCA. In the first 5-min olfactory test, in which the entries were sealed with wire mesh, sawdust from housing cages of 6 OVX+EP female rats were placed behind each door and the time spent investigating the female odor by each group of males were recorded. After the test, we selected the 2 most preferred females and 2 females less preferred by smell. Subsequently, in a second 5-min. olfactory test, we evaluated again the time spent investigating the female odor of the sawdust bedding of the 4 selected females. Finally in a third 30-min. sexual contact test, in which the wire meshes were removed and the sawdust was replaced by the corresponding female, we recorded: the time spent by male in front of each cylinder, the number of intromissions and ejaculations that each female received from each male, the number of proceptive and receptive female behaviors displayed during the visit of each male, and the order in which the 4 females were visited. The three tests were performed weekly, 3 times. Considering the most preferred female, the female that received more ejaculations, the number of ejaculations were correlated with the aforementioned parameters of the female sexual components: attractivity, proceptivity and receptivity. Males showed a significant preference for one of the females in 83% of tests and this preference was correlated with the characteristics of the female in the following order: lordosis (r = 0.76), ear wiggling (r = 0.74), darting (r = 0.62), hopping (r = 0.34), time spent in the male compartment (r = 0.21) and visitation order (r = 0.15). Therefore, male rats prefer significantly those female rats showing more lordosis and ear wiggling.

Disclosures: A. Ferreira-Nuno: None. **A. Cruz-Benites:** None. **J. Olayo-Lortia:** None. **A. Morales-Otal:** None.

Poster

246. Sexual Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: Conacyt Grant 220772 Mexico

Conacyt Scholarship 232728 Mexico

Title: Biphasic effects of intra-VTA anandamide administration on sexual behavior expression of the male rat

Authors: A. CANSECO-ALBA, *G. RODRIGUEZ-MANZO;
Cinvestav-Sede Sur, IPN, Mexico City, Mexico

Abstract: Male sexual behavior, like other rewarding behaviors, is modulated by the dopaminergic mesocorticolimbic system (MSL). The dopaminergic neurons of the MSL system originate in the ventral tegmental area (VTA) and project mainly to the nucleus accumbens and the prefrontal cortex. Endocannabinoids (eCN) are retrograde transmitters that modulate MSL activity by regulating GABA and glutamate release both at the VTA and the nucleus accumbens. VTA eCN receptors have been suggested to play a key role in the regulation of motivation, therefore eCN play an important role in the regulation of rewarding behaviors through the modulation of MSL activity. We recently reported that the systemic administration of the eCN anandamide (AEA) exerts dose-based biphasic effects on copulation, facilitating its expression at low doses. On this basis we hypothesized that AEA might exert its effects on male sexual activity by acting at the MSL system. The objective of this work was to determine if intra-VTA AEA administration to sexually experienced male rats reproduced the biphasic effects on copulatory behavior observed after its systemic administration. To this aim, independent groups of sexually experienced male Wistar rats were bilaterally infused with different AEA doses (0.1 - 3.0 μg/rat) into the VTA and subjected to a 60-min sexual behavior recording with a sexually receptive female. Results showed that the lower AEA doses (0.3 and 1.0 μg/rat) facilitated sexual behavior expression, while the highest dose tested (3.0 μg/rat) had inhibitory effects on copulation. None of the tested doses significantly modified the animals' spontaneous ambulatory behavior. It is concluded that AEA exerts its modulatory effects on male rat sexual behavior expression by interacting with the MSL dopaminergic system.

Disclosures: A. Canseco-Alba: None. **G. Rodriguez-Manzo:** None.

Poster

246. Sexual Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: Brinkman Family Foundation Grant

Title: The effects of acute prenatal exposure to valproic acid on sociosexual behaviors and anxiety in female rats

Authors: *S. M. HARDING, J. A. CAPUTO, S. R. BARRETT, E. C. MASTERS, M. M. MCDONOUGH;
Psychology Dept, Fairfield Univ., Fairfield, CT

Abstract: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that is characterized by enhanced anxiety, social impairments, and deficits in communication. In rodents, exposure to the antiepileptic drug valproic acid (VPA) has been linked to ASD-like behavioral changes in males, although studies using females have been limited and inconsistent. Therefore, the present study was conducted to examine long-term effects of early exposure to VPA on social behaviors and anxiety in female rats. Pregnant Long Evans rats were injected s.c. with saline (Control, n=1), Low VPA (350mg/kg; n=2), or High VPA (600 mg/kg; n=2) on gestational day 12.5. In adolescence, the female offspring were tested for social interactions with female conspecifics using a social preference test, and anxiety was assessed with an emergence test. Preliminary data analysis suggests that prenatal VPA exposure had no effect on social preference. In the emergence test, females exposed to High VPA spent less time in the closed portion of the apparatus compared to Low VPA ($p < .02$) and Control groups ($p < .08$), suggesting heightened anxiety. These findings were consistent with what we have reported with male rats. In adulthood, all female rats were ovariectomized and received injections of estradiol benzoate and progesterone before undergoing tests for sociosexual behavior with male rats, including test for copulation, partner preference, and 50kHz vocalizations. VPA groups were compared to saline Controls and a separate group of sexually experienced females. Preliminary data analysis reveals that High VPA and Low VPA groups showed significantly fewer ear wiggles than sexually experienced females ($p < .03$ and $p < .002$, respectively), suggesting reduced proceptivity. In addition, lordosis was observed less frequently in the High VPA group compared to Sexually experienced females ($p < .05$) suggesting reduced receptivity. After a single sexual encounter, both Low VPA and High VPA groups showed increased preference for an intact male vs. a castrate compared to Controls ($p < .05$), suggesting enhanced sexual motivation. Surprisingly, no differences between groups were seen in 50kHz vocalizations. These results suggest that acute exposure to VPA during development may be sufficient to induce anxiety in female rats, but that the effects of VPA exposure on social interactions vary based on dose and type of interaction, i.e. same sex conspecifics vs. sexual partners. These findings will be discussed in comparison to what we have reported with males, and have important implications for the study of ASD in females.

Disclosures: **S.M. Harding:** None. **J.A. Caputo:** None. **S.R. Barrett:** None. **E.C. Masters:** None. **M.M. McDonough:** None.

Poster

246. Sexual Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: Canadian Institutes of Health Research (CIHR)

Title: One of these things is not like the other: Sign-tracking for sucrose does not predict sign-tracking for sex

Authors: ***L. M. SPARKS**, V. BOUZO, V. PALLIKARAS, D. DI GREGORIO-RIFIORATI, A. SHARMA, S. KANTHASAMY, 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). Furthermore, such differences extend across different types of reward; animals that exhibit ST for food reward also ST for drug reward. Studies have yet to examine whether these differences extend to sexual reward. Objective: The present study examined whether rats identified as ST or GT following sucrose conditioning would continue to exhibit ST or GT when an ejaculatory reward state served as the US. Method, Phase 1: Sexually-naïve, male, Long-Evans rats received 16 autoshaping sessions in operant chambers, where a lever (CS) predicted the availability of sucrose (US; 10% w/v) delivered into a fluid port. Phase 2: Rats received 13 Pavlovian conditioning trials 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). Pavlovian-conditioned approach toward the CS (ST) and US (GT) was measured by the proportion of time spent in an area located around the CS or door to the female compartment, respectively, in the absence and presence of the cue. Preliminary Results, Phase 1: Sign-trackers displayed significantly greater lever contacts, significantly shorter latency to the first lever contact, and significantly longer latency to the fluid port compared to goal-trackers. Phase 2: On trials 1 and 7, rats did not display CS- or US-directed behavior; no differences were observed in the proportion of time spent in the CS- and US-designated areas. On trial 13, individual differences in PCA were detected; a subset of rats spent significantly more time in the CS-designated area (ST), another subset spent significantly more time in the US-designated area (GT), and others were identified as intermediates. For rats displaying ST and GT using sucrose reward, only one-third maintained their phenotype following sexual conditioning. Conclusions:

These results provide further evidence that conditioned cues acquire incentive motivational properties through Pavlovian conditioning using sucrose or sexual reward. Importantly, our preliminary findings suggest that individual differences in PCA responses might not extend to all types of reward. Instead, such differences are dependent on the nature of the reward itself.

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Poster

246. Sexual Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: NSF IOS 1256799

NIDA Grant T32DA007234

NIH Grant T32 GM008471

Title: Investigation of glutamate neurotransmission during sex behavior in female Syrian hamsters

Authors: *K. M. MOORE, L. E. BEEN, R. L. MEISEL;
Dept. of Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: Female sexual behavior is a fundamental motivated behavior for which there is an extensive literature on its underlying neurobiology. Despite this wealth of information, there is surprisingly little known regarding the neurotransmission associated with different components of the female's ongoing sexual interactions with the male. While dopaminergic signaling has traditionally been examined in this area, glutamate is the major excitatory neurotransmitter in the central nervous system, and is well known for its involvement in long-term potentiation and synaptic plasticity in both learned and motivated behaviors. Nonetheless, glutamatergic neurotransmission has been largely understudied in naturally motivated behaviors, particularly sex behavior, partially due to technical limitations in measuring glutamate release in real time. Classic electrochemistry based on oxidation/reduction such as fast scan cyclic voltammetry for dopamine was not possible as glutamate is non-electroactive. Our study utilized a technique involving enzymatic biosensors to elucidate the effects of male copulatory behavior on glutamate release in multiple regions of the female brain. Our goal is to determine how this patterning of glutamate release is associated with various components of sexual behavior, including its motivation and termination. Our results suggest that the pattern of glutamatergic neurotransmission in different brain regions in the female hamster may be responsible for

encoding distinct properties of the female's copulatory interaction with the male. Specifically, the prefrontal cortex appears to encode overall bouts of male copulatory behavior, which we hypothesize may be responsible in signaling to downstream regions responsible for copulatory reward. In contrast, recordings restricted to the ventrolateral portion of the ventromedial nucleus of the hypothalamus appear to encode individual vaginal cervical stimulations, which we hypothesize may be involved in signaling termination of sexual receptivity. We aim to continue to advantage of this biosensor technology and mating patterns in female Syrian hamsters to further characterize the regions examined in this study, as well as other regions of interest such as the medial amygdala, an area implicated in the endocrine cascade to set up the progestational state. These studies will develop a comprehensive picture of the relation of *in vivo* glutamate release in the female brain to individual components of sex behavior.

Disclosures: K.M. Moore: None. **L.E. Been:** None. **R.L. Meisel:** None.

Poster

246. Sexual Behavior

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Support: NSF Grant IOS 1256799

NIH Grant T32 DA07234

Title: Using fast-scan cyclic voltammetry to measure dopamine release from the nucleus accumbens of female hamsters during sex behavior

Authors: *L. E. BEEN, S. R. EBNER, B. T. HIMMLER, R. L. MEISEL;
Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: In many mammals, reproductive success depends on a female receiving an optimal pattern of vaginal stimulation during mating. Despite the evolutionary importance of this species-specific "vaginal code", the neural mechanisms underlying the female's regulation of vaginal stimulation during mating are poorly understood. Previous research in our lab suggests that dopamine release in the nucleus accumbens is an integral component of how female hamsters control the receipt of vaginal intromissions by a male. Using fast-scan cyclic voltammetry we can measure dopamine release from the nucleus accumbens of female hamsters during mating behavior to track the receipt of vaginal intromissions during mating behavior with sub-second temporal resolution. Adult female hamsters were ovariectomized and implanted with a unilateral cannula dorsal to the nucleus accumbens core. A chlorinated silver reference electrode was implanted in the contralateral forebrain and a stainless steel stimulating electrode was implanted the ipsilateral ventral tegmental area. Following recovery, females were hormone-

primed to induce sexual receptivity and an acute carbon-fiber electrode was lowered into the nucleus accumbens using a micromanipulator. Females were paired with a stimulus male and background-subtracted dopamine transients were recorded during a 10-minute sex behavior test. Voltammetry recordings were time-stamped to video recordings of behavioral tests so that dopamine transients could be correlated with individual behavioral events. Our preliminary results suggest that dopamine transients are time-locked to the receipt of vaginal stimulation by the male. Future experiments will test the hypothesis that sexual experience can shift the pattern of dopamine release such that dopamine transients anticipate the receipt of vaginal intromission by the male. These data provide the first example of measuring dopamine release in real time during female sex behavior and are the first steps in uncovering the neural mechanisms by which females regulate the timing of mating behavior.

Disclosures: **L.E. Been:** None. **S.R. Ebner:** None. **B.T. Himmler:** None. **R.L. Meisel:** None.

Poster

246. Sexual Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 246.22/P42

Topic: E.03. Behavioral Neuroendocrinology

Support: R-607-264-057-121

Title: A single dissociation: Intranasal oxytocin alters affiliative but not sexual cues

Authors: *N. M. Y. KUEK[1], J. C. J. LIU[2];
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Abstract: A common assertion amongst theories of love is that there exist distinct systems of love. More recently, anthropologist Helen Fisher suggested that these systems may have dissociable underlying mechanisms: lust is regulated by estrogens and androgens; attraction by dopamine, norepinephrine, and serotonin; and attachment by oxytocin and vasopressin. In support of this notion, one study found that natural variations in plasma oxytocin levels correlate with non-verbal expressions of attachment (affiliation cues), but not with non-verbal expressions of lust (sexual cues). To the extent that this suggests separate systems dissociable at the neuroendocrine level, we sought to investigate whether administration of oxytocin would selectively alter the expression of affiliation but not sexual cues. 102 healthy young adults were administered either intranasal oxytocin or a placebo, and were paired with an unfamiliar participant of the opposite gender. Participants were given discussion questions that induced varying degrees of intimacy (low, medium, high), and were video-taped during their discussions. Subsequently, two trained coders independently coded the video for displays of sexual cues (lip bite, lip suck, lip lick, tongue protrusion, lip pucker) and affiliation cues (nod, Duchenne smile,

gesticulation, lean). We found a significant three-way interaction between the type of non-verbal cue (affiliation vs. sexual), drug condition (oxytocin vs. placebo), and intimacy level of the discussion (low vs. medium vs. high; $F(2, 99) = 3.39$, $p = .04$). Namely, for affiliation cues, oxytocin participants displayed fewer cues than placebo participants when discussing medium and high intimacy questions. However, for sexual cues, there were no main or interaction effects involving participants' drug condition. Our finding of a single dissociation supports the idea that different love systems are independently regulated by different hormones. We note, however, that *within* the affiliation system, our findings suggest a complex, context-dependent relation between oxytocin levels and the expression of cues.

Disclosures: N.M.Y. Kuek: None. **J.C.J. Liu:** None.

Poster

246. Sexual Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 246.23/Q1

Topic: E.03. Behavioral Neuroendocrinology

Support: NSERC 341673

Title: Acute postnatal phthalate exposure results in both an immediate and long-term deficit in the masculinization of crucial neural circuits in the male rat brain

Authors: *M. R. HOLAHAN, A. WEIR, C. SMITH;
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Abstract: Phthalates are synthetically-derived chemicals used to soften and provide malleability for a variety of common household and personal care products. Because they are not chemically bound to these products, they are easily released into the environment and, due to their high oil solubility, they readily infiltrate biological systems. Studies examining the biological impact of phthalate exposure on developing organisms are critical given that estimates of phthalate exposure are considerably higher in infants and children compared to adults. In this regard, phthalate exposure has been shown to disrupt Sertoli and Leydig cell function during sensitive developmental periods. A reduction in the number or in the functioning of Sertoli and Leydig cells can lead to reduced testosterone and estradiol levels in the brain which may interfere with the organizational effect of these sex steroids resulting in long-lasting changes in the nervous system and behavioral outcomes. Our published data (to be re-illustrated) has shown that post-natal exposure to phthalates (PND16 - PND22) has an immediate (but not readily apparent long-term or organizational) impact on the masculinization of hippocampal CA3 axonal inputs and CA3 spine density arrangements. Spine density on third and fourth order basal dendritic branches and second, third and sixth order apical dendritic branches of CA3 neurons was reduced in male

rats to control levels seen in female rats following exposure to phthalates. New data have revealed that post-natal exposure to phthalates during the same timeframe (PND16-PND22) resulted in persistent, long-term deficits in the masculinization of dopamine cell densities in the ventral tegmental area and, to a lesser extent, in the substantia nigra. These data indicate that phthalate exposure during sensitive post-natal developmental periods may impede both the activational and organizational effect of sex steroids on the masculinization of critical memory- and reward-related brain circuits in the male brain. Phthalate-induced interruption of these organizational effects may have consequences on the frequency of sexually-dimorphic behaviors in males such as courtship displays, territorial aggression, mating, and parental care.

Disclosures: **M.R. Holahan:** None. **A. Weir:** None. **C. Smith:** None.

Poster

246. Sexual Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant R01 MH 50388

Title: Hypothalamic modulation of mesolimbic reward circuitry: a functional tracing study

Authors: *O. IYILIKCI[1], J. BALTHAZART[3], G. F. BALL[2,4];
[2]Psychological and Brain Sci., [1]Johns Hopkins Univ., Baltimore, MD; [3]GIGA Neurosciences, Univ. of Liege, Liege, Belgium; [4]Dept. of Psychology, Univ. of Maryland, College Park, MD

Abstract: There is ample evidence indicating that the medial preoptic nucleus (POM), Lateral Hypothalamus (LHA), paraventricular nucleus PVN and the mesolimbic reward circuitry are implicated in regulating sociosexual behaviors in vertebrates. However, there is a paucity of studies investigating the potential interplay of hypothalamic and mesolimbic reward systems, particularly in the case of male sexual behaviors. We argue that projections originating from POM, LHA and/or PVN may play a modulatory role on the mesolimbic reward circuitry related to sexual behavior. The present study, therefore, aimed to elucidate whether cells in POM, PVN and LHA that have projections to VTA are activated during sociosexual interactions. To this aim, we stereotaxically injected a biotinylated dextran amine (BDA) into VTA as a neuroanatomical tracer. At low molecular weights (3kDa), BDA retrogradely labels cell bodies that send projections to the injection site. One week after the injections, male quail in the experimental group interacted freely for 15 minutes and demonstrated stereotypical appetitive and consummatory sexual behaviors. Animals in the control condition were placed in same experimental apparatus without a female stimulus for the same amount of time. 90 minutes after testing, subjects' brains were prepared for triple immunohistochemical labeling of BDA, FOS

and Orexin. Our results demonstrated a significant increase in Fos-immunoreactivity (ir) in POM in response to sociosexual interactions, similar to the previous findings. More interestingly, among the retrogradely labeled BDA cells in POM, we found significantly more FOS labeled associated with sociosexual interactions. Our results, so far, indicate that cells with direct projections from POM to VTA exhibit enhanced Fos-ir while the birds are engaging in appetitive and consummatory sexual behaviors. These data are consistent with the known role of the POM in regulating sociosexual behaviors, especially for appetitive sexual behaviors.

Disclosures: O. Iyilikci: None. **J. Balthazart:** None. **G.F. Ball:** None.

Poster

246. Sexual Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 246.25/Q3

Topic: E.03. Behavioral Neuroendocrinology

Title: The neurobiology of reproductive competition

Authors: S. M. BOWDEN[1], M. H. FERKIN[2], G. J. DE VRIES[1], *A. PETRULIS[1]; [1]Neurosci. Inst., Georgia State Univ., Atlanta, GA; [2]Dept. of Biol. Sci., Univ. of Memphis, Memphis, TN

Abstract: To optimize reproductive fitness, brains control timing of ejaculation as well as amount of sperm per ejaculate. The latter happens when males compete to fertilize the eggs of a single female, a condition termed sperm competition. When competition is high, males can increase the amount of sperm per ejaculate, a phenomenon called sperm loading (SL). For example, meadow voles double the amount of sperm per ejaculate within 30 minutes after exposure to the odor of a competing male; they do so without changing reproductive behavior. Although the neural circuits underlying SL have not been identified, the paraventricular and supraoptic nuclei of the hypothalamus (PVN and SON) are well positioned to mediate these effects. They could do so in two ways: via hormonal release of vasopressin (AVP) and oxytocin (OT), both of which increase contractions of the myoepithelium in reproductive ducts that propel sperm, or by actions on the autonomic nervous system via descending PVN projections to the spinal cord. Our preliminary data suggests that peripheral blockade (via Manning compound) of AVP receptors significantly decreases, but does not eliminate, SL in meadow voles. This suggests that magnocellular AVP neurons in the PVN and SON are involved in SL. We are bolstering this hypothesis by using immunohistochemistry to co-localize odor-induced cFos protein expression and OT or AVP in the PVN/SON of male voles exposed to odors signifying high sperm competition (male + female odors) or low sperm competition (male only or female only).

Disclosures: **S.M. Bowden:** None. **M.H. Ferkin:** None. **G.J. de Vries:** None. **A. Petrulis:** None.

Poster

246. Sexual Behavior

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Program#/Poster#: 246.26/Q4

Topic: E.03. Behavioral Neuroendocrinology

Title: Dopamine signaling in the nucleus accumbens is necessary for sexual preference in male mice

Authors: ***Y. BENY**, T. KIMCHI;
Neurobio., Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Sexual preference to opposite-sex individuals is an innate, essential behavior for successful reproduction and propagation of all animal species. Males' sexual preference and motivation to approach female stimuli are triggered by female pheromonal signals and depends on the animals' endocrine state and internal drive. Here we tested the hypothesis that the innate attraction of males to females pheromones and sexual preference are mediated by dopamine release within the nucleus accumbens (NAc). Male mice with a mutation in the TrpC2 gene, a crucial component expressed in sensory neurons of the vomeronasal organ (VNO), fail to detect pheromones through the VNO, do not express olfactory preference for female over male odors, and exhibit sexual behavior indiscriminately toward both sexes. We applied a conditioned olfactory aversion (COA) paradigm on adult wild-type (WT) and TrpC2-/- mutant male mice. According to this paradigm, mice were exposed to female pheromones, followed by either lithium-chloride (LiCl) or saline IP injections and were tested for their sexual preference, motivation and consummatory sexual behaviors. We show that TrpC2-/- mutant males are capable of learning a specific-aversion to female pheromones and thus to discriminate between sex odors, in a similar manner to WT males. Next, we quantified dopamine release in the NAc during interaction with an alien female and male conspecifics, using an in-vivo microdialysis assay. We found that in WT males, exposure to a female conspecific, but not to a male, lead to a significant elevation in dopamine levels in the NAc. In contrast, in TrpC2-/- males, exposure to female stimuli failed to induce elevation in accumbens dopamine levels. Finally, we show that WT males bilaterally injected with D1R antagonist in the NAc failed to develop conditioned place preference for female pheromones, and lost their sexual preference. In summary, our study demonstrates that the representation of the intrinsic rewarding value of female pheromones driving the innate sexual preference of males to females is coordinated by NAc dopamine, possibly via D1 receptors and is VNO-mediated.

Disclosures: **Y. Beny:** None. **T. Kimchi:** None.

Poster

246. Sexual Behavior

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Topic: F.03. Motivation and Emotion

Support: General Grant of NSFC (31371092)

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CUHK Direct Grant (2013.1.080)

Title: Spargel overexpression in dopaminergic neurons induces male-male courtship

Authors: *K. WU[1], J. QIAO[1], C. QIAN[2], Y. KE[1];
[1]Sch. of Biomed. Sci., The Chinese Univ. of Hong Kong, Hong Kong, China; [2]Sch. of Pharm., Fudan Univ., Shanghai, China

Abstract: Peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) is well-studied in muscle and liver, and has been regarded as the master regulator of mitochondria. Its functions include mitochondrial biogenesis, regulation of cellular energy metabolism and reactive oxygen species suppression. Recently, dopaminergic (DA) neurons, which control locomotor ability, arousal, rewards and courtship, have been found to be selectively sensitive to sustained PGC-1α overexpression. In our study, we overexpressed the PGC-1α fly homolog, spargel, in DA neurons to investigate the effect of spargel on DA neurons and fly behaviors. Our results indicated that spargel overexpression did not significantly affect survival rate and locomotor ability. However, increased inter-male chain activity, which usually reflects changes in courtship behavior, was observed in spargel overexpressing flies 10 days post-eclosion. Paired courtship assays revealed that courtship towards males was significantly enhanced upon spargel overexpression but not in courtship towards female. Immunohistochemical analysis on whole-mount brain did not indicate any significant changes in DA neuron number and morphology. Increased mitochondrial DNA copy number, which indicates mitochondrial biogenesis, was detected in fly heads with spargel overexpression. To conclude, our findings showed that spargel overexpression in DA neurons could selectively upregulate male-male courtship and such behavioral change might be associated with increased mitochondrial biogenesis in DA neurons.

Disclosures: **K. Wu:** None. **J. Qiao:** None. **C. Qian:** None. **Y. Ke:** None.

Poster

246. Sexual Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Title: The incentive value of multisensory stimuli in male rats

Authors: *E. M. SNOEREN, A. ÅGMO;
Univ. of Tromso, Tromso, Norway

Abstract: Male rats prefer a hormonally primed female rat over a male rat in a sexual incentive motivation test. Since approach to a conspecific must depend on distant stimuli, the modalities involved can be vision, movement, olfaction or audition. Previous studies have shown that the playback of 50 kHz ultrasonic vocalizations (USV) have no incentive value, while the odor of a receptive female induces approach behavior. In this study, we investigated what stimulus or what combination of stimuli (multisensory) is required to induce approach behavior in males. Male rats (n=12) were placed in a sexual incentive motivation test for 10 minutes and presented with two incentives: a male rat and 'another stimulus'. The following stimuli were used in a randomized order: an empty box, a receptive female, the odor of a receptive female, an anesthetized receptive female, a devocalized receptive female or the playback of USV. The different stimuli were sometimes presented together in different combinations as multisensory stimulus. All possible combinations of stimuli were presented. When a multisensory stimulus excluded odor, an anosmic subject male rat was used. The results show that the modality of only olfaction, moving, or vision (but not audition) is enough to remove the preference for social contact with a male. Both incentives were approached equally. A multisensory stimulus of at least 2 modalities, however, was sufficient to induce approach behavior towards the stimulus. One of these modalities needed to be the smell of a receptive female. The multisensory stimulus of smell and USV, though, failed to have an incentive value over the male rat. In conclusion, the modalities moving, vision and olfaction are important for the attraction of conspecifics. But a multisensory stimulus of 2 modalities is necessary to induce preference for the stimulus over social contact with a male rat. The playback of USV, however, seems to decrease the incentive value of the different stimuli.

Disclosures: E.M. Snoeren: None. **A. Ågmo:** None.

Poster

247. Parental Behavior

Location: Hall A

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Program#/Poster#: 247.01/Q7

Topic: E.03. Behavioral Neuroendocrinology

Support: CIHR Grant MOP102568

Title: Comparing the efficacy of maternal fluoxetine and exercise in a rodent model of postpartum depression: outcome of both mother and male and female offspring

Authors: *A. R. GOBINATH[1], R. J. RICHARDSON[2], C. CHOW[2], J. L. WORKMAN[2], S. E. LIEBLICH[2], A. M. BARR[3], L. A. M. GALEA[2];
[1]Neurosci., [2]Psychology, [3]Anesthesiology, Pharmacology, & Therapeut., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Postpartum depression (PPD) affects approximately 15% of mothers and can have negative effects on the mother-infant dyad. Pharmacological antidepressants such as fluoxetine (Prozac) are commonly used to treat PPD. However, fluoxetine can remain active in breast milk and potentially impact offspring development. For this reason, non-pharmacological therapies, such as exercise, may be more agreeable. Unfortunately, it is unclear whether exercise is efficacious for treating PPD while preserving normal offspring development. To investigate this, we treated dams daily with high levels of corticosterone (40 mg/kg), to induce a depressive-like phenotype, or oil during the postpartum. Within the oil and corticosterone conditions, four additional antidepressant groups were created: 1. Fluoxetine (Prozac; 10 mg/kg) + exercise (voluntary access to running wheel); 2. Fluoxetine + no exercise; 3. Saline (vehicle for fluoxetine) + exercise; 4. Saline + no exercise. Male and female offspring were weaned and then tested in adulthood for anxiety-like behaviour using the novelty suppressed feeding task and for stress reactivity using the dexamethasone suppression test. Preliminary findings indicate that maternal postpartum fluoxetine reversed corticosterone-induced disruptions in maternal care, regardless of exercise condition. Additionally, preliminary findings indicate that exercise had a greater antidepressant-like effect than fluoxetine for the dams. For this reason, we predict that exercise will result in a protective effect particularly in the adult male offspring. Our findings will shed light on how the postpartum antidepressant treatments (Prozac, exercise) differentially affect the well-being of the mother as well as the male and female offspring. Funded by CIHR to LAMG.

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Poster

247. Parental Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant R01DC008343

NIH Grant T32GM008605-13

Title: Estrogen receptor alpha expression in the auditory cortex changes across motherhood

Authors: *A. MORENO[1], K. K. CHONG[3,2], T. N. IVANOVA[2], R. C. LIU[2];
[2]Biol., [1]Emory Univ., Atlanta, GA; [3]Biomed. Engin., Georgia Inst. of Technol., Atlanta, GA

Abstract: Maternal response to infants requires sensitivity and detection of infant specific sensory cues, yet how these social cues are processed to enhance responsiveness is not fully understood. It is hypothesized that changes in sensory neural encoding are responsible for enhanced processing of social cues (Miranda, 2009). Mouse mothers quickly learn to respond to pup-calls and display long-term neural plasticity in the auditory cortex (AC) for those calls. Because estrogen treatment enhances pup-call responsiveness in non-mothers (Koch, 1989), parturition related estrogen exposure might be responsible for the enhancement of social auditory cue processing in the AC. To determine estrogen's role in pup-call representation enhancement in the AC, it is important to determine if estrogen can have a direct effect on AC neurons. To achieve this, we characterized the distribution of the most common auditory cortical estrogen receptor, estrogen receptor alpha (ERα), over the course of parturition and motherhood. Age matched animal groups included female virgin pup-naive, late pregnancy pup-naïve, early and late mother mice. ERα immunohistochemistry was performed on 40μm thick brain sections containing the primary AC. Our results indicate a large number of ERα immunoresponsive (IR) neurons throughout the AC across motherhood. There was no change in ERα -IR density moving from rostral to caudal in the AC. Comparing across conditions using a one-way ANOVA, there is a significant difference in the density of ERα-IR neurons between conditions ($F_{3,9} = 5.9$ $p < .001$). Density of ERα-IR neurons peaked in pregnant females, similar to the endogenous estrogen peak during late pregnancy (Miranda, 2009). Not only are AC neurons sensitive to estrogen but also estrogen concentration might alter AC ERα expression. ERα-IR density in late mothers was significantly lower than pregnant females ($p = .001$), and dropped below virgin pup-naïve levels. Because late mothers are known to retain maternal responsiveness (Ehret, 1989), this suggests that AC ERαs might not be necessary for mothers to maintain enhanced neural responses to pup-calls. Our findings are consistent with previous work hypothesizing that estrogen acts as a priming mechanism, preparing the AC for social auditory learning of pup-associated cues (Banerjee, 2013). Estrogen associated molecular mechanisms are a fertile area for social auditory communication research and can determine how sensory encoding fits into the picture of maternal response.

Disclosures: **A. Moreno:** None. **K.K. Chong:** None. **T.N. Ivanova:** None. **R.C. Liu:** None.

Poster

247. Parental Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: NICHD grant RO1HD057962

Title: Effects of reproductive state on the expression of oxytocin receptor mRNA and protein in the midbrain raphe nuclei of female rats

Authors: *Z. GRIEB, J. LONSTEIN;
Neurosci. Program, Michigan State Univ., East Lansing, MI

Abstract: The female brain undergoes tremendous neurochemical changes across different reproductive states, which during the peripartum period facilitate females' ability to adjust to the increased investments of motherhood and properly care for offspring. This facilitation of maternal behavior is, in part, achieved through changes in central oxytocinergic systems. For example, oxytocin signaling is upregulated in forebrain regions important for the display of maternal behaviors, including the medial preoptic area. This is functionally relevant, as antagonizing oxytocin receptors in the medial preoptic area interferes with normal maternal behaviors. This area of research, though, has largely ignored the midbrain. Given some recent evidence demonstrating an interaction between the oxytocinergic and serotonergic systems in mediating social reward, we are investigating the expression of oxytocin receptors in the midbrain raphe nuclei (the source of most central serotonin) in female rats across different reproductive states. To accomplish this, we are using qRT-PCR to examine oxytocin receptor mRNA expression in the raphe nuclei across four different reproductive states: diestrus virgin, pregnancy day 10, ~3 hours of parturition, and postpartum day 7. We are also conducting autoradiography using a highly-selective oxytocin receptor ligand to quantify the density of oxytocin receptor binding in the raphe at these same time points. We predict that oxytocin receptor mRNA and protein will be upregulated in the raphe nuclei soon after parturition, which would be consistent with what is found in some forebrain regions regulating maternal behavior. We also predict that while the pregnancy day 10 and postpartum day 7 groups will have lower oxytocin receptor expression compared to the day of parturition group, both groups will have higher expression than the virgin controls. This research will contribute to the growing literature examining how interactions between the oxytocinergic and serotonergic systems influence social behavior, and as far as we are aware, the first to investigate the expression of oxytocin receptors in the midbrain raphe nuclei across numerous reproductive states in the brain of any female animal.

Disclosures: Z. Grieb: None. **J. Lonstein:** None.

Poster

247. Parental Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: 2013 NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation

NICHD grant HD073710

Title: Ultrasonic vocalizations and parenting in a rat model of postpartum depression

Authors: *M. PEREIRA;
Dept. of Psychological and Brain Sci., Univ. of Massachusetts Amherst, Amherst, MA

Abstract: Postpartum depression is a serious psychiatric condition that has deleterious effects on the mother, and poses a risk for the mother-infant relationship and ultimately the infant's development. Maternal anhedonia is a major clinical feature central to postpartum depression and likely contribute to deficits in parenting. The present study used Wistar Kyoto (WKY) mother rats, an animal model of depression-like symptomatology which we have developed to examine the postpartum disorder (Pereira et al. 2012), to investigate the relationship between depressive-like symptoms and parenting disturbances. WKY and Sprague Dawley (SD) postpartum females were examined for their affective responses to the cues and behaviors of the pups, as measured by their ultrasonic vocalizations (USVs) during a maternal behavior test. Both WKY and SD mothers predominantly produced ~50 kHz USVs when interacting with the pups in the maternal behavior test. Preliminary analysis of results indicates that WKY mothers exhibited fewer vocalizations in response to social cues from pups as compared with SD mothers. Similarly, WKY mothers exhibited substantial disturbances in their maternal behavior. Taken together, these results provide evidence for the presence of maternal USVs during mother-litter interactions, and further suggest that variations in USVs produced by mothers during social interaction with their pups may function as an index of their positive maternal affect. Rat USVs may be used for to study the neurobiological mechanisms underlying maternal affect in animal models of postpartum disorders.

Disclosures: M. Pereira: None.

Poster

247. Parental Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 247.05/Q11

Topic: E.03. Behavioral Neuroendocrinology

Title: Immediate early gene activation in post parturient oxytocin receptor forebrain knockout mice

Authors: *S. K. WITCHEY[1], K. GERBIG[1], H. K. CALDWELL[1,2];
[1]Biol. Sci., Kent State Univ., Kent, OH; [2]Sch. of Biomed. Sciences, Kent State Univ., Kent, OH

Abstract: In females, the oxytocin (Oxt) system is not only important to parturition and milk ejection but plays a role in the initiation of maternal behavior. Previous work from our lab has shown that disruption of Oxt signaling in total body Oxt receptor knockout (Oxtr−/−) mice and forebrain-specific oxytocin receptor knockout (OxtrFB/FB) mice results in significant increases in the abandonment of first litters relative to control mice. These data suggest that Oxt, signaling through the Oxtr, lowers the threshold for the initiation of maternal care. To determine where Oxt may be acting in the brain to affect maternal care in our mice, we examined immediate early gene activation, i.e. cFos, in OxtrFB/FB and wildtype brains that were collected approximately one hour after parturition. We hypothesized that there would be differences in cFos immunoreactivity in brain areas known to be important for the onset of maternal care, such as the paraventricular nucleus, the supraoptic nucleus, the medial preoptic area, the bed nucleus of the stria terminalis, and the lateral septum. We predicted that OxtrFB/FB would show reduced c-Fos activation in some of these brain areas. We found that c-Fos differed between the genotypes in only one brain area, the nucleus accumbens shell (NAcS)- with OxtrFB/FB mice having increased c-Fos immunoreactivity compared to wildtype controls. The NAcS, which expresses the Oxtr, is important for the rewarding properties of motivated behaviors, including maternal behavior. In rats and voles, Oxt in the NAcS has been implicated in the consolidation of maternal memory and the regulation of "spontaneous" maternal behavior, respectively. Our data suggest that at least in mice, Oxt signaling in the NAcS may also impact the responsiveness of dams to pups, perhaps altering the rewarding properties of their interaction, which could alter the likelihood that a dam will initiate maternal care.

Disclosures: S.K. Witchey: None. **K. Gerbig:** None. **H.K. Caldwell:** None.

Poster

247. Parental Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: UNMH PEDS RAC 9971NA

Title: Child executive functioning is predicted by parent hypothalamic pituitary adrenal (hpa) function in both cancer survivors and healthy control children

Authors: *R. RIEGER[1], S. M. DINCES[1], S. N. HILE[1], L. N. ROWELL[1], J. F. L. PINNER[1], N. C. MOSS[1], R. S. ALLEN[1], M. EMERY THOMPSON[2], A. C. TANG[1,3,4], R. D. ANNETT[5]; [1]Dept. of Psychology, [2]Dept. of Anthrop., [3]Dept. of Neurosciences, Univ. of New Mexico, Albuquerque, NM; [4]Office of Intl. Sci. and Engin., Natl. Sci. Fndn., Arlington, VA; [5]Dept. of Pediatrics, Univ. of Colorado Denver, Denver, CO

Abstract: Exposure of infant rats to mild stress associated with a relatively novel non-home environment can modify the influence of maternal HPA function on offspring cognitive function. To determine whether the impact of these converging early life influences generalize to humans, we investigated whether experience of cancer, a major chronic stressor, can change the influence of parent HPA function on child executive function (EF). Eighty-one children, ages 5-18, from 63 families (Healthy Controls=32, Cancer Survivors=31) were included in a nested-design study. To identify major dimensions underlying EF, principle component analysis was performed on 9 measures of EF (NIH Examiner). The first three components accounted for 78.47% variance with the first interpreted as attention control, the second as responsivity to stimuli, and the third as response inhibition. Parent HPA function was indexed by normalized evoked salivary cortisol response (CORTne=PreStress -PostStress)/PreStress) to the Trier Social Stressor Task (TSST). Multilevel modeling was applied to each of these three components with cancer experience and parental cortisol as predictor variables controlling for child age, showing a significant main effect of parent CORTne on the component of child attention control ($F(1,51.35)=4.08, p=.049$)---parents with higher rise in cortisol response to the TSST had children with higher levels of attention control. This result suggests that parent ability to increase cortisol output in response to a stressful event can predict child attention control across different stress conditions. Therefore improving parent self-stress regulation may positively contribute to cognitive development in children experiencing different levels of stress.

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Poster

247. Parental Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 247.07/Q13

Topic: E.03. Behavioral Neuroendocrinology

Title: The role of sensitization and stress on maternal behavior and neuronal activation in lactating mice

Authors: *M. CHAVEZ[1], D. MERCADO[1], L. JAQUES[1], M. MCCREARY[2], K. D'ANNA-HERNANDEZ[1];
[2]Psychology, [1]California State Univ. San Marcos, San Marcos, CA

Abstract: Lactating dams display a reduction in the hormonal and behavioral response to stress relative to virgin dams. However, it is unknown whether these effects are driven by perinatal hormonal changes or exposure to pups. As maternal behavior is vital for offspring survival and sensitive to the effects of stress, this study proposes to answer this question by analyzing the effect of a stressor on maternal behavior in lactating dams compared to sensitized virgins mice (virgins females exposed to pups). The mice were divided into two groups; lactating dams (LD; n=10) and sensitized virgins (SV; n=10). From PND 1-4, virgin mice were sensitized to four donor pups that were scattered along with the nesting material in the home cage for two hours/day. On the last day of sensitization, the virgins and lactating dams underwent a 15 minute pup retrieval and maternal behavior test. On PND 5, LD and SV were placed on the elevated zero maze to induce a mild stressor. Maternal behavior was again analyzed for 15 minutes immediately after the EZM. After the maternal behavior test and ninety minutes following the stressor, dams were perfused and are being assessed for c-Fos as an indirect marker for neuronal activity to address brain differences in response to the stressor. For the behavioral results, two way repeated measures ANOVAs revealed differences in maternal behaviors between sensitized virgins and lactating dams following the EZM mild stressor. Overall, lactating dams were quicker to retrieve pups and spent less time nursing than sensitized virgins. However, following the stressor, sensitized virgins displayed shorter latencies to both self-groom and lick and groom pups compared to the lactating dams. There is also a strong trend towards increased cFos activation in the paraventricular nucleus of the hypothalamus in SV vs. LD, suggesting altered neuronal activation in response to stress in brain regions important for stress regulation. This work suggests pregnancy hormones rather than pup exposure may underlie an adaptive maternal behavioral and neural response to stress as high levels of licking and grooming, as seen in SV, has previously been shown to reduce pup stress reactivity and may have adverse consequences for offspring raised in adverse conditions.

Disclosures: **M. Chavez:** None. **D. Mercado:** None. **L. Jaques:** None. **M. McCreary:** None. **K. D'Anna-Hernandez:** None.

Poster

247. Parental Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: Z01-MH-002498-24

NIMH (ZIA-MH-002498-24)

Title: Absence of the father during pre-weaning does not impact adult offspring anxiety, social recognition or parental care in C57Bl/6J mice

Authors: *S. WILLIAMS[1], W. S. YOUNG[2];
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Abstract: Variations in neonatal and pre-weaning environments have important and enduring effects on neurobiology and behavior throughout life. Rodent studies have shown that changes in maternal care received can result in altered stress responses and anxiety, as well as social behaviors including parental care of offspring. The presence or absence of the father in the pre-weaning environment has had mixed effects of adult offspring, likely dependent on the species and/or strain. Since parental care is a major contributor to the early environment, the neurobiology underlying the transition to and maintenance of parental behavior has been investigated for several decades. Studies investigating the genetic basis of parenting have used mice primarily from the C57Bl/6J background strain. The breeding of genetic mutants often occurs from permanently co-housing heterozygous male and female breeder pairs, resulting in the presence of the father during rearing. Our observations show that the male contributes to the care of pups in this procedure. However, maternal behavior studies typically remove the male prior to the birth of the pups, resulting in a novel parenting environment for the females to transition to maternal care compared to what they experienced as pups. Here we investigated the adult behavior of offspring reared with their fathers (WF) or without theirs fathers (NF) present during the pre-weaning phase of development. Male and female C57BL/6J mice were paired and once pregnancy was confirmed divided into 2 groups: WF and NF. The NF group had the male removed 1 week prior to birth, and in the WF group fathers remained with the mothers throughout gestation and weaning. Adult male and female offspring were tested for anxiety using an elevated O maze. Pup retrieval was assessed in both male and female offspring in the first postpartum week. Social recognition was measured using a 30 minute 2-session habitation test in males only. We found no differences between WF and NF groups on anxiety-like behavior, social recognition or likelihood to retrieve pups. These results indicate that the presence or absence of the father does not have major effects on baseline behaviors in offspring of the C57Bl/6J strain. Importantly, these results suggest that differences in parental care observed in genetic mutants from the C57BL/6J background most likely result from the genetic disturbance and not from the female rearing young in a different social environment than she was reared, especially when the heterozygous parents are behaviorally normal. This research was supported by the NIMH (Z01-MH-002498-24).

Disclosures: S. Williams: None. **W.S. Young:** None.

Poster

247. Parental Behavior

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Program#/Poster#: 247.09/Q15

Topic: E.03. Behavioral Neuroendocrinology

Support: UNMH PEDS RAC 9971NA

Title: The experience of cancer affects the relationship between parent and child hypothalamic pituitary adrenal (hpa) function

Authors: *S. M. DINCES[1], S. H. HILE[1], L. N. ROWELL[1], J. F. L. PINNER[1], R. E. RIEGER[1], N. C. MOSS[1], R. S. ALLEN[1], M. EMERY THOMPSON[2], A. C. TANG[1,3,4], R. D. ANNETT[5]; [1]Dept. of Psychology, [2]Dept. of Anthrop., [3]Dept. of Neurosciences, Univ. of New Mexico, Albuquerque, NM; [4]Intl. Sci. and Engin., Natl. Sci. Foundatation, Arlington, VA; [5]Dept. of Pediatrics, Univ. of Colorado, Denver, Aurora, CO

Abstract: Rodent studies show that offspring experience of moderate stress can modify the association between the infant's and the mother's HPA function. Therefore, having a mother with poor HPA function does not mean that her offspring will necessarily have similarly poor function. Here we examine whether this modifiability of the relation between parental and child HPA function generalizes to humans. HPA function was examined in parents and their children from healthy control (HC) and pediatric cancer survivor (CS) families. Multilevel modeling was applied to data from a nested-design study (85 children, 5-18 years old, from 64 families, HC:n=32; CS:n=32) to characterize the relation between parent (baseline and post-stress salivary) and child (hair) cortisol measures within HC and CS groups, controlling for child age, sex and parent ethnicity. A second analysis was performed; examining whether corticosteroid exposure as part of cancer treatment affects the association between parent and child cortisol. The interaction effect between cancer experience and parental cortisol on child HPA function was significant (Baseline:$F(1,46.23)=4.53$,p=.039; Post-Stress:$F(1,47.21)=8.99$,p=.004) with a more positive association between parent and child cortisol in HC compared to CS families. The interaction effect between child corticosteroid exposure and parent cortisol was significant (Baseline:$F(1,61.02)=4.76$,p=.033; Post-stress:$F(1,61.42)=6.04$,p=.017) with a more positive association between parent and child cortisol in the non-exposed (N=67), compared to the exposed group (N=11). Parallel to findings from the rodent model, these results reveal a modifiability of the relation between parent and child HPA function by child corticosteroid exposure and indicate plasticity of HPA function beyond infancy.

Disclosures: **S.M. Dinces:** None. **S.H. Hile:** None. **L.N. Rowell:** None. **J.F.L. Pinner:** None. **R.E. Rieger:** None. **N.C. Moss:** None. **R.S. Allen:** None. **M. Emery Thompson:** None. **A.C. Tang:** None. **R.D. Annett:** None.

Poster

247. Parental Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: IUP Start-Up Funds.

Title: Effects of maternal 5-HT2C inhibition and mouse pup handling on maternal behavior

Authors: *D. V. WIDZOWSKI[1], D. S. AMPOFO[2];
[2]Biol. & Psychology, [1]Indiana Univ. of Pennsylvania, Indiana, PA

Abstract: Many genetic, physiological and environmental factors have an impact on maternal behavior including care for offspring and on subsequent structural and functional development of the offspring. For example, pharmacological manipulation of serotonergic systems can alter numerous aspects of rodent maternal behavior including retrieval, nursing, and licking of pups. In rodents, the quantity and quality of maternal care can influence resilience to stress and cognitive performance of the offspring as adults. Despite recent studies focusing on the role of serotonin in maternal behavior, the function of specific receptor subtypes in maternal behavior remains unclear. Selective activation of 5-HT2C receptors has been reported to impair maternal behavior but few studies have focused on the effects of selective 5-HT2C receptor antagonists on maternal behaviors. Since activation of 5-HT2C receptors by an agonist impairs aspects of maternal behavior (e.g., pup retrieval, nursing) we hypothesized that selective inhibition of these receptors would have an opposite effect. To test this hypothesis, in this pilot study we evaluated the effects of two factors on mouse mothers' allocation of behavioral time: 1.) Sub-chronic selective 5-HT2C inhibition (SB242084 1 mg/kg ip) or vehicle/placebo during the days one to seven post-natal and 2.) Transient (5 minute) handling or no-handling of pups on the same days. Daily ten-minute behavioral observations were made approximately 30 minutes before administration of drug (baseline) and 15 minutes after (acute drug effect). Outcome measures included quantification of time of various maternal behaviors (e.g., nursing, licking and grooming) as well as other maternal behaviors (e.g., self-grooming, nest building) following drug administration. Results showed a significant effect of handling and postnatal day on maternal licking and grooming but no main effect of 5-HT2C inhibition and no interactive effect at the dose tested. A marginally significant ($p = 0.054$) effect of 5-HT2C antagonism was noted on maternal self-grooming. No effect of 5-HT2C antagonism or handling was noted on baseline behaviors. These results do not support the hypothesis that 5-HT2C inhibition increases maternal licking and grooming of pups.

Disclosures: D.V. Widzowski: None. **D.S. Ampofo:** None.

Poster

247. Parental Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: NSF Grant IOS 1256898

UNK RSC URCA Grant

Title: Biparental behavior in the burying beetle, Nicrophorus orbicollis: a role for dopamine?

Authors: J. P. SPEER[1], J. W. YAEGER[2], K. J. RENNER[2], *S. C. PANAITOF[1];
[1]Biol., Univ. of Nebraska at Kearney, Kearney, NE; [2]Biol., Univ. of South Dakota, Vermillion, SD

Abstract: Burying beetles, *Nicrophorus orbicollis*, exhibit facultative biparental care of young. While the ecological and evolutionary factors that have helped shape the remarkable plasticity of their reproductive behavior are well characterized, the neuromodulation of biparental care in this species remains poorly understood. Male-female beetle pairs bury a small vertebrate carcass as food for its altricial young. During a breeding bout, male and female behavior changes synchronously at appropriate times and is coordinated to provide effective care for offspring. Previous studies show that juvenile hormone (JH) levels rise dramatically at the time beetle parents first accept and feed larvae and stay elevated during the period of active larval care. Levels of JH then fall abruptly when parental care is terminated. However, fluctuations in reproductive hormone levels alone cannot account for the elaborate control of *N. orbicollis* reproductive behavior. The biogenic amines octopamine (OA), dopamine (DA) and serotonin (5-HT) mediate a wide range of insect behaviors, including a plethora of reproductive social behaviors. Thus, these neurotransmitters represent potential candidates for the neuromodulation of burying beetle reproductive behavior. In this study, we measured whole brain monoamine levels in individual insects, and established the OA, DA and 5-HT profiles of male and female burying beetles during a breeding bout. Remarkably, after 24 hr of parental care, when parental feeding rates begin to peak, levels (pg/μg protein) of DA (mean ± SEM) increase in breeding beetles (19.8 ± 2.6) compared to nonbreeding, unmated controls (16.5 ± 2.1) matched for age and colony background ($P<0.05$). In contrast, OA and 5-HT levels remained unchanged. These findings provide the first evidence for a potential role of DA in the modulation of burying beetle parental care. To better understand the effect of these central monoamines on beetle reproductive behavior, we have set out to clone and characterize several subtypes of OA, DA and 5-HT receptors. Additional studies will take advantage of the experimental tractability of this invertebrate model of biparental care and use *in vivo* pharmacological approaches to alter brain monoamine function via receptor antagonists or agonists and assess behavioral changes associated with the onset, maintenance and termination of care.

Disclosures: **J.P. Speer:** None. **J.W. Yaeger:** None. **K.J. Renner:** None. **S.C. Panaitof:** None.

Poster

247. Parental Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Title: Bubble nesting behavior is disrupted in male Betta splendens exposed to sub-toxic levels estradiol

Authors: *A. J. VELKEY[1], K. WOOLWINE[2], H. KAY[2], N. KHAN[2], A. EDWARDS[2], K. GUDYKA[2], K. CARTER[2], H. PREMO[2];
[1]Psychology, [2]Neurosci., Christopher Newport Univ., Newport News, VA

Abstract: Bubble nesting is an important component of the reproductive cycle of Betta splendens. Many environmental factors can influence bubble nesting behavior and nest construction, including exposure to xenoestrogenic substances. As nest size is an indicator of mate quality, and nest density is an important factor that influences the viability of eggs, endocrine disruption of nesting behavior can have substantial impact on the life cycle of exposed organisms. The present study examines the effect of low-level estradiol exposure on the size of bubble nests formed and maintained by adult male Betta splendens. Males housed in solitary conditions were exposed to varying estradiol levels (0 µg/L, 30 µg/L, or 60 µg/L). Images of nest sizes were taken three times a day (9 AM, 12 PM, and 3 PM) for one week pre-exposure and one week post-exposure. The nest areas were quantified using grid counts. Results indicate reduced bubble nest area and maintenance in animals exposed to the low, but not the high, dose of estradiol. These findings can have important ramifications in the determination of environmental liability of xenoestrogenic substances.

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Poster

247. Parental Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Title: Hypocretin receptors 1 and 2 differentially regulate materal motivation and maternal depressive behavior in lactating dams

Authors: *Y. CABRERA, H. NORRIS, N. GRENIER, A. KENT, E. LANE, K. D'ANNA-HERNANDEZ;
California State Univ. San Marcos, San Marcos, CA

Abstract: Postpartum depression is a serious illness that contributes to disruptions in maternal care. Postnatal neuromechanistic changes in the arousal and reward systems may contribute to the onset of this disorder. This study will address the role of both hypocretin (HCRT) receptors (HCRTR) 1 and 2, both activated by the neuropeptide HCRT-1, which is related to both arousal and reward, on depressive behavior in the postpartum period. HCRT is associated with general wakefulness in rats and humans and also enhances signaling in the mesolimbic pathways associated with increased motivation and reward-seeking behavior. During lactation, there is an increase in HCRT-1 neuronal activity in both rodent and human mothers associated with time awake caring for offspring, a rewarding behavior. This study will administer varying doses of HCRTR1 antagonist SB-334867 (n=10 each group, vehicle and 30 mg/kg) as well as HCRT-2 antagonist EMPA (n=8-9 each group, vehicle and 30, 75 mg/kg) on postnatal day 4. Separate groups of dams were tested for depressive-like symptoms on the forced swim and tail suspension test after which they underwent a 20 min test for pup retrieval and initiation of maternal behavior. An additional group of dams were tested for pup retrieval on the Tmaze as a proxy for maternal motivation. Parity was also considered as an additional factor. There was no effect of parity or HCRTR1 antagonism on depressive-like symptoms in the forced swim or tail suspension tests. However, primiparous dams that received 75 mg/kg of the HCRT-2 antagonist floated more and swam less than 30 mg/kg dams ($p=0.036$), suggesting blocking HCRTR2, but not HCRTR1, facilitates postpartum depressive symptoms. In addition, in the Tmaze, multiparous dams that received the HCRTR1 antagonist increased time to approach pups relative to primiparous dams ($p<0.05$) and HCRTR1 receptor antagonist decreased overall maternal behaviors, including time licking and grooming pups ($p<0.05$) and latency to retrieve pups ($p<0.001$). Together this work suggests that depressive symptoms in the postpartum period may be regulated via the HCRTR2 receptor while pup-directed behaviors may be driven by changes in the HCRTR1 system.

Disclosures: Y. Cabrera: None. **H. Norris:** None. **N. Grenier:** None. **A. Kent:** None. **E. Lane:** None. **K. D'Anna-Hernandez:** None.

Poster

247. Parental Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant F32HD075758-02

NIH Grant R01HD057962

Title: Trait anxiety is associated with medial prefrontal cortex GABA system protein expression in postpartum rats

Authors: *C. M. RAGAN, J. S. LONSTEIN;
Neurosci., Michigan State Univ., East Lansing, MI

Abstract: Typically, early postpartum females have lower anxiety compared to nulliparous females, but some postpartum women (and laboratory rats) experience elevated anxiety after giving birth that interferes with mother-infant interactions and socioemotional development of offspring. This peripartum anxiety is strongly predicted by previous high anxiety in humans and maternal rats. Although the neurobiological mechanisms associated with differences in anxiety among mothers is unclear, central GABA neurotransmission has been implicated in anxiety in nulliparous and randomly-selected postpartum females. In the current study, we selected 8 low-anxious and 8 high-anxious female rats based on the time spent in the open arms of an elevated plus maze on postpartum day 7. We then analyzed protein expression of GAD65 (responsible for synthesis of GABA released from terminals), and the vesicular GABA transporter (vGAT; responsible for uptake and storage of GABA into vesicles) in the medial prefrontal cortex (mPFC), an area associated with emotion regulation. We found a negative correlation between vGAT expression in the mPFC and the number of open arm entries driven by the high-anxious females. We also found a trend suggesting a positive correlation between GAD65 expression and open arm time in the low-anxious females. There was no relationship between vGAT expression and open arm entries, nor between GAD65 expression and anxiety, in the high-anxious females. There was no association between vGAT expression and open arm time nor GAD65 and anxiety in the low-anxious females. These data suggest that differential cortical GABA regulation may contribute to behavioral differences between high- vs. low-anxious mothers.

Disclosures: C.M. Ragan: None. **J.S. Lonstein:** None.

Poster

247. Parental Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant R0084148

Title: Gestational stress impairs maternal motivation and induces anhedonia: role of dopamine signaling in the nucleus accumbens shell

Authors: *B. LEUNER[1,2,3], A. HAIM[3], C. ALBIN-BROOKS[1], M. SHERER[1], E. MILLS[1]; [1]Dept. of Psychology, [2]Behavioral Neuroendocrinology Group, The Ohio State Univ., Columbus, OH; [3]Dept. of Neurosci., The Ohio State Univ. Wexner Med. Ctr., Columbus, OH

Abstract: Postpartum depression is a common complication following childbirth experienced by as many as 20% of all new mothers. Mothers diagnosed with postpartum depression often experience anhedonia and impaired mother-infant interactions which can compromise infant development and yet the underlying neural mechanisms mediating these symptoms of postpartum depression remain unspecified. Maternal care is a goal-oriented, highly motivated behavior, which in conjunction with increased anhedonia suggests that deficits in reward and motivation processing might play a role. Here, we investigated whether gestational stress, a risk factor for postpartum depression, would similarly induce anhedonia in postpartum rats using the sucrose preference test. We also evaluated the extent to which gestational stress disrupted maternal motivation as assessed in both a pup retrieval test and the conditioned place preference test. Lastly, we investigated the impact of gestational stress on various components of dopamine signaling in the nucleus accumbens (NAc) shell, a critical component of the brain's reward circuit. Specifically, immunohistochemical staining and densitometry were used to detect and quantify tyrosine hydroxylase (TH; rate limiting enzyme of dopamine synthesis), dopamine D1 receptor (D1), dopamine D2 receptor (D2), and the dopamine transporter (DAT; protein responsible for DA reuptake and termination of dopamine signaling). Our results demonstrate that gestational stress induces anhedonia in postpartum rats and leads to deficits in maternal motivation. These behavioral effects in gestationally stressed mothers were accompanied by decreased density of TH, D2 receptor, and DAT in the NAc shell but no change in D1 receptor density. Overall, gestational stress represents an important translational risk factor that can be used to investigate the neurobiological mechanisms underlying postpartum depression which as suggested by our results may involve altered DA signaling in the NAc.

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Poster

247. Parental Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: DGAPA/UNAM IN200413

Title: Effects of the environmental context on the maternal behavior of early undernourished rats

Authors: M. REGALADO, *M. GIORDANO, N. SERAFIN, L. RUBIO, C. TORRERO, M. SALAS;
Univ. Nacional Autónoma De México, Querétaro, Qro., Mexico

Abstract: The complex maternal motivational state occurring during the early postpartum not only promotes the expression of nursing and care of the offspring, but also the display of vigorous pup-seeking behavior when the pups are spread out of the nest or under unfamiliar conditions. In this study we assess the long-term effects of perinatal undernutrition (PU) on the behavioural performance of lactating dams, versus the well-fed mothers to a new nest environmental signals that may interfere with the maternal care of the progeny. The unfamiliar paradigm, consisted of two chambers connected by a horizontal tunnel (10 cm in diameter) as a device to provoke maternal novelty. The day before testing the litter was separated 12h from the pups and thereafter, the maternal behavioral tests were evaluated at 4, 8, and 12 days of the lactating period. In all subjects the frequencies of approaching to the young, sniffing and licking pups, digging sawdust, rearing, and self-grooming bouts were sampled. Early underfed mothers reduced ($p<0.05$) the approaching and their licking to the pups, digging sawdust, and increased the number of pups sniffing, and self-grooming bouts on day 8 ($p<0.05$) vs. the control dams. The results indicated that PU interferes with the maternal responsiveness to novel cues elicited by the pups in a non-familiar conditions. These findings, suggest that the motivational response of PU dams to the pups is poorly focused to protect and give care to the progeny, and presumably results in newborn careless and delayed brain development.

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Poster

247. Parental Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant RO1HD057962

NIH Grant R1F31MH099892

Title: Postpartum lesions targeting serotonergic neurons in the dorsal raphe alter various aspects of maternal behavior

Authors: *M. A. HOLSCHBACH[1], E. M. VITALE[2], J. S. LONSTEIN[1,2];
[1]Neurosci. Program, [2]Psychology, Michigan State Univ., East Lansing, MI

Abstract: The survival and wellbeing of mothers and their young require high levels of maternal care, aggression toward conspecifics, and low anxiety. These behaviors are affected by pharmacological manipulation of serotonin signaling, but no experiments have analyzed in detail the effects of serotonin-specific lesions of the midbrain on all of these postpartum behaviors. We performed serotonin-specific lesions of the dorsal raphe using a saporin-conjugated toxin targeting the serotonin transporter. After dorsal raphe infusion of the toxin or an inactive control conjugate on postpartum day 2, undisturbed maternal behavior was observed daily and retrieval of scattered pups observed every other day for one week after surgery. Anxiety-like behavior was measured in an elevated plus maze and light dark box on postpartum days 8 and 9, respectively, followed by tests of aggression toward a male intruder in the home cage. Serotonergic lesions of the dorsal raphe altered numerous postpartum behaviors. During undisturbed observations, lesioned animals groomed themselves less and showed more crouching over and less licking of pups. Lesions did not greatly affect pup retrieval or anxiety-like behavior, but did reduce the average duration of attack bouts during aggression testing. This experiment indicates new roles for DR serotonin in the suite of behavioral changes occurring during the postpartum period.

Disclosures: **M.A. Holschbach:** None. **E.M. Vitale:** None. **J.S. Lonstein:** None.

Poster

247. Parental Behavior

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Topic: E.03. Behavioral Neuroendocrinology

Support: NIH Grant HD075750

Title: Impacts of perinatal oxytocin exposure on maternal behavior, physiology, and epigenetic regulation

Authors: *A. M. PERKEYBILE[1], W. M. KENKEL[1], J. R. YEE[1], T. S. LILLARD[3], T. ROSENSTEIN[1], C. F. FERRIS[1,2], C. S. CARTER[4], J. J. CONNELLY[3];
[1]Psychology, [2]Ctr. for Translational Neuroimaging, Northeastern Univ., Boston, MA; [3]Psychology, Univ. of Virginia, Charlottesville, VA; [4]The Kinsey Inst., Indiana Univ., Bloomington, IN

Abstract: The use of synthetic oxytocin (OXT) in the birth process is very prevalent in obstetric practices today as a way to induce or augment labor. The long-term consequences for mother and offspring, however, are not fully understood. Here we investigate the post-birth impacts of this birth intervention on behavior, autonomic functioning, and epigenetic regulation in the mother using the prairie vole (Microtus ochrogaster). The prairie vole is uniquely suited to answer these.

This species engages in biparental care of offspring, with the father displaying the same suite of parental behaviors as the mother aside from lactation, and also has a human-like pattern of autonomic system regulation. In addition, there are 4 identified CpG sites in the promoter region of the OXT receptor (OXTR) in prairie voles that are conserved from human OXTR and these sites are not homologous in mice or rats. In this study, pregnant females were injected with one of three doses of OXT (0.05, 0.25, or 0.5 mg/kg) or saline vehicle or were not injected on the day of expected delivery of the litter. Ninety minutes after injection, the female was sacrificed and brains were collected to examine changes in methylation of the OXTR at several CpG sites. A second cohort of adult females was implanted with radiotelemetry devices prior to mating. On the expected day of birth of the first litter, females were injected with either OXT (0.25 mg/kg) or saline vehicle, with the other drug injected just prior to the expected birth of the second litter. Order of injection was counterbalanced across subjects. Parental behavior was observed four times between postnatal days 1-3 after the birth of each litter. During each of these four observations, autonomic functioning of the mother was also recorded. Methylation changes in OXTR were seen in regions of the brain involved in maternal behavior, including an increase in methylation levels following the low dose of perinatal OXT exposure in the medial preoptic area and following the high dose of OXT in the amygdala compared to treatment with saline vehicle. Preliminary behavioral results indicate that treatment with OXT compared to saline vehicle prior to birth does not affect maternal care of offspring in the first postnatal days. Maternal autonomic function data will also be presented. Results here indicate peripartum exposure to OXT may have consequences for maternal methylation of the OXTR but does not appear to impact care of offspring and adds to our understanding of the impact of birth intervention on the mother.

Disclosures: **A.M. Perkeybile:** None. **W.M. Kenkel:** None. **J.R. Yee:** None. **T.S. Lillard:** None. **T. Rosenstein:** None. **C.F. Ferris:** None. **C.S. Carter:** None. **J.J. Connelly:** None.

Poster

247. Parental Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 247.19/R5

Topic: E.03. Behavioral Neuroendocrinology

Support: NICHD P01-HD075750

Title: A little extra push: exploring the consequences of oxytocin administration on neonatal physiology, behavior and epigenetics

Authors: ***W. KENKEL**[1], A. M. PERKEYBILE[1], J. R. YEE[1], T. ROSENSTEIN[2], C. F. FERRIS[2], C. S. CARTER[1], J. CONNELLY[3];
[1]Indiana Univ., Bloomington, IN; [2]Psychology, Northeastern Unniversity, Boston, MA; [3]Virginia Univ., Charlottesville, VA

Abstract: The majority of births in the U.S. now involve the administration of exogenous oxytocin (OXT) to induce and/or augment labor. There is evidence that such OXT can cross the placenta and reach fetal circulation, but the long-term effects on brain, body and behavior remain to be explored. Given that direct OXT exposure in early life can produce brain and behavior changes in adulthood, we sought to evaluate whether OXT administered to the expectant female can influence offspring development. Here, we present data from our model of peripartum OXT administration, using the monogamous prairie vole (Microtus ochrogaster). In this paradigm, pregnant female voles were treated with one of three doses of OXT (0.05, 0.25, or 0.5 mg/kg) or saline vehicle on the expected day of delivery. Effects on the neonate were evaluated in terms of: prenatal heart rate, epigenetic regulation and transcription of the OXT receptor, and several aspects of postnatal behavior including ultrasonic vocalizations and anxiety-related behavior in the open field test. Peripartum OXT administered to the expectant dam results in dose-dependent decreases in prenatal heart rate in both sexes and increases in OXT receptor methylation in females. Following cross-fostering, the analysis of behavioral changes brought on by peripartum OXT will contribute to a growing body of knowledge concerning the consequences of this commonplace obstetric practice.

Disclosures: W. Kenkel: None. **A.M. Perkeybile:** None. **J.R. Yee:** None. **T. Rosenstein:** None. **C.F. Ferris:** None. **C.S. Carter:** None. **J. Connelly:** None.

Poster

247. Parental Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 247.20/R6

Topic: F.02. Animal Cognition and Behavior

Title: Changes in maternal behavior due to fetal ethanol exposure and stressed environment

Authors: *V. A. DONAHUE;
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Abstract: Early-life trauma, such as exposure to drugs or maternal maltreatment, leave lasting imprints which can cause behavioral deficits. Maternal maltreatment during development can cause physical deformities, behavioral and social deficiencies, problems with executive functioning, and various molecular changes, including changes to neurotrophic factors. Brain-derived neurotrophic factor (BDNF) acts in the central and peripheral nervous system to increase the survival of neurons. Increased signaling or decreased signaling can interfere with proper neuronal development. Through the use of aUsing a Long-Evans rat model, the effect of maltreatment and ethanol exposure during development on maternal behavior, cognition and the epigenetic effects on the BDNF gene. Previous studies have indicated that Pre-impregnated dams were placed in either a stressed or non-stressed environment. aA stressfuled environment causes

dams to exhibit poor maternal behavior. Ethanol treatments were given to neo-natal rat pups which were separated into three treatment groups, ethanol-treated, intubation-control, and non-treated control. An ethanol/milk mixture was given to the ethanol-treated group, whereas the control groups received only intubation or no treatment. Ethanol treatments were administered on post-natal days 4 through 9. Dam maternal behavior such as licking and grooming, nursing posture and pup retrieval time were recorded on post-natal days 2, 4, 6, 8, and 10. On day 25, pups underwent pup drop testing to view differences in maternal behavior, a stress test, to view fear response, and a radial arm maze to view processing capacity in a new environment. RT-PCR was done to look at the levels of BDNF mRNA. In pup drop tests, there was no significant differences in retrieval time between ET and Control groups, however pups from stressed dams showed less time to retrieve than the non-stressed counterparts. In the stress test and radial arm maze, ET rats showed less fear and were quicker to choose an arm than the other treatment groups.

Disclosures: V.A. Donahue: None.

Poster

247. Parental Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 247.21/R7

Topic: E.03. Behavioral Neuroendocrinology

Support: NIMHR01-058616 to ZXW

Title: Parental environment affects social behaviors and neurochemical systems in the offspring of socially monogamous prairie voles

Authors: *M. TABBAA[1], K. LEI[2], Y. LIU[1], Z. X. WANG[1];
[1]Florida State Univ., Tallahassee, FL; [2]Univ. of California, San Francisco, San Francisco, CA

Abstract: Although it has been well documented in humans that paternal absence has detrimental effects on offspring development and wellbeing, the underlying mechanism is still unknown. In the present study using the socially monogamous, bi-parental prairie vole (Microtus ochrogaster), we examined the effects of father absence during early development on the offspring's social and anxiety-like behaviors as well as neurochemical systems in adulthood. Paired voles were checked daily. On the day of litter birth, fathers were either continuously housed with the mother and offspring to create bi-parental group (BP) or were removed to create single mother group (SM). Three times a day during the entire 20 day postnatal period, ten spot checks were conducted with a 5 minute interval between each spot check. SM offspring were licked, groomed, and carried less by a parent, and were left alone in the nest more frequently than BP offspring. At weaning (21 days of age), SM and BP offspring were housed in pairs with

a same sex and treatment sibling until 90 days of age and then tested for parental, affiliative, and anxiety-like behaviors. More SM subjects displayed parental behaviors than BP subjects. Interestingly, SM subjects engaged in more huddling behaviors compared to BP subjects. SM subjects also engaged less in non-parental behaviors, such as self-grooming, compared to BP subjects. Further, SM males, but not females, engaged in more side by side contact with a stimulus conspecific, compared to PB males, during the social affiliation test. In the elevated plus maze (EPM) test, SM subjects entered the arms of the EPM less frequently than PB subjects. Brain tissues were processed for neurochemical marker labeling. Western blot analysis revealed that SM subjects had lower levels of oxytocin receptor as well as alpha and beta glucocorticoid receptor expression in the hippocampus than BP subjects. Together, our data indicate that lack of paternal exposure during early development has long-lasting effects on the behavior and brain of this socially monogamous rodent species. The effects on other neurochemical systems, including BDNF and histone acetylation, are currently under investigation.

Disclosures: **M. Tabbaa:** None. **K. Lei:** None. **Y. Liu:** None. **Z.X. Wang:** None.

Poster

247. Parental Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 247.22/R8

Topic: E.03. Behavioral Neuroendocrinology

Support: Ludmer Centre for Neuroinformatics and Mental Health

Hope for Depression Research foundation

CIHR

Title: Individual differences in maternal care associate with variations in dendritic morphology and Rem2 within the medial preoptic area of rats

Authors: ***T. ZHANG**, C. I. PARENT, X. WEN, S. K. DHIR, R. RYAN, J. DIORIO, M. J. MEANEY;
Douglas Mental Hlth. Univ. Institute, Sackler Program for Epigenetics & Ps, McGill Univ., Verdun, QC, Canada

Abstract: The medial preoptic area (MPOA) mediates maternal behavior and is implicated in the expression of individual differences in maternal behavior such as the frequency of pup licking/grooming (LG). We sought to determine if variations in maternal behavior were associated with underlying MPOA morphology. We examined phosphorylated cyclic adenosine monophosphate (cAMP) responsive element-binding protein (pCREB) expression and dendritic

morphology within the MPOA of lactating mothers. We found a significantly higher number of pCREB-immunoreactive cells in the MPOA of high-LG mothers compared to low-LG mothers immediately after a nursing bout on postnatal day 5 (PND 5). High-LG mothers also displayed a significantly reduced population of greater dendritic complexity index (DCI) neurons compared with low-LG mothers in the MPOA. Rem2, a small GTPase, that reduces dendritic complexity while enhancing glutamatergic synapse formation was significantly increased in the MPOA of high-LG mothers compared to low-LG mothers. CREB overexpression in MPOA neuronal cultures increased the expression of genes implicated in dendritic pruning including brain-derived neurotrophic factor (BDNF) exon IX and Rem2 gene expression. CREB overexpression also reduced the DCI of cultured MPOA neurons. The enhanced expression of Rem2 following CREB overexpression in cultured MPOA neurons is a novel finding that suggests CREB's ability to regulate neuronal dendritic growth by enhancing the expression of a negative regulator of dendritic complexity. The enhanced expression of pCREB and Rem2 in the MPOA of high LG mothers compared to low-LG mothers associates with a reduced DCI and suggests enhanced glutamatergic excitatory synapses in the MPOA of high-LG mothers.

Disclosures: **T. Zhang:** None. **C.I. Parent:** None. **X. Wen:** None. **S.K. Dhir:** None. **R. Ryan:** None. **J. Diorio:** None. **M.J. Meaney:** None.

Poster

247. Parental Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 247.23/R9

Topic: F.03. Motivation and Emotion

Support: TrygFonden Charitable Foundation

MRC Studentship

Title: Heightened medial orbitofrontal cortex activity in mothers' responses to infant vocal cues: an fMRI comparison of women with and without young infants

Authors: ***C. PARSONS**[1,2], K. YOUNG[4], E.-M. JEGINDØ[3], A. STEIN[1], M. KRINGELBACH[1,2];
[1]Univ. of Oxford, Oxford, United Kingdom; [2]Ctr. for Functionally Integrative Neurosci., [3]Aarhus Univ., Aarhus, Denmark; [4]Psychology, UCLA, L.A., CA

Abstract: Becoming a mother is assumed to bring about a profound change in a woman's capacity to respond to an infant. The neural mechanisms underlying such a change are not well understood in humans. This study investigated differences in how women with and without young infants respond to salient infant vocal cues. Twenty-nine mothers of young infants and twenty-nine non-mothers participated in this study. Participants were scanned using fMRI while

they performed a task designed to ensure auditory attention (tone detection), with infant and other sounds played incidentally. The infant cues consisted of negative (cry) and neutral ('babble') sounds produced by infants aged between 6 and 8 months. Participants also listened to adult negative and neutral vocalisations. Mothers showed heightened activity relative to non-mothers in the left medial orbitofrontal cortex (mOFC) when listening to infant sounds compared to adult sounds. Among the mothers, the magnitude of the mOFC response was positively correlated with the age of their own infants. Other regions that showed differential patterns of activity in the maternal and non-maternal groups included the left inferior frontal gyrus and the right middle frontal gyrus. We propose that this differential neural activity is an important aspect of the changes associated with becoming a mother. For mothers, activity in the medial OFC appeared to be important in differentiating infant from adult vocal cues. These findings further suggest that caregiving experience may be important in shaping the extent of medial OFC activity. Heightened responsiveness to infant cues in a key reward region in mothers may support early parenting behaviours.

Disclosures: C. Parsons: None. **K. Young:** None. **E. Jegindø:** None. **A. Stein:** None. **M. Kringelbach:** None.

Poster

247. Parental Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 247.24/R10

Topic: E.03. Behavioral Neuroendocrinology

Title: Maternal care and oxytocin secretion are controlled by a sexually dimorphic hypothalamic circuit

Authors: N. SCOTT, M. PRIGGE, O. YIZHAR, *T. KIMCHI;
Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Parental care is a complex stereotypic behaviour toward offspring that is shared by numerous species. In laboratory mice, there are profound differences in offspring directed behaviours between the sexes. At their first encounter, nulliparous females behave maternally toward alien pups while males will usually ignore the pups or attack them. Here we show that tyrosine hydroxylase (TH)-expressing neurons in the anteroventral periventricular nucleus (AVPV) of the mouse hypothalamus are more numerous in mothers than in virgin females and males, and govern parental behaviours in a sex-specific manner. In females, ablating the AVPV TH+ neurons impairs maternal behaviour whereas optogenetic stimulation or increased TH expression in these cells enhance maternal care. In males, however, this same neuronal cluster has no effect on parental care but rather suppresses adult-directed aggression. Strikingly, optogenetic activation or increased TH expression in the AVPV TH+ neurons of female mice

increases circulating oxytocin, whereas their ablation reduces oxytocin levels. Using neuroanatomical tracing and optogenetic stimulation, we found that AVPV TH+ cells relay a onosynaptic input to oxytocin-expressing neurons in the paraventricular nucleus. Our findings uncover a previously unknown role for this neuronal cluster in the control of maternal care and oxytocin secretion, and provide a causal relationship between sexual dimorphism in the adult brain and sex differences in parental behaviour.

Disclosures: **N. Scott:** None. **M. Prigge:** None. **O. Yizhar:** None. **T. Kimchi:** None.

Poster

247. Parental Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 247.25/R11

Topic: E.03. Behavioral Neuroendocrinology

Support: JSPS Research Fellowship for Young Scientists (KAKENHI13J02670)

Title: Individual differences in male mice ultrasonic vocalizations to females are correlated with sexual motivation and neuronal activity in the ventral tegmental area

Authors: *K. KOUTA[1], T. KIKUSUI[2];
[2]Companion Animal Res., [1]Azabu Univ., 1-17-71 Fuchinobe, Sagamihara, Kanagawa, Japan

Abstract: Individual differences in male mice ultrasonic vocalizations to females are correlated with sexual motivation and neuronal activity in the ventral tegmental area Sexually dimorphic displays are observed in various animals, which are thought to be shaped in the process of evolution. In rodents, male mice emit ultrasounds when encountering females as courtship vocalizations. The ultrasonic vocalizations (USVs) contain song-like structure and recent studies have focused on the detail analysis of the characteristics of the songs, such as syllable compositions or temporal sequence of syllables, which are regarded as a biolinguistic model of syntax and models of social behaviors. However, biological or behavioral significance and neural mechanisms of the USVs has been poorly understood. Here, we demonstrate that amount of emission in the USVs in male mice reflected sexual motivation. That is primarily because the amount of the emission was negatively correlated with mount latency; males that emitted more showed mounting on a female with a shorter latency. In addition, comparing males before and after castration, USVs was significantly decreased after castration and testosterone replacement restored the USVs. Furthermore, males exhibited almost no vocalizations after ejaculation. Next, we screened the responsible brain regions for the USVs emission using immunohistochemistry for neuronal activity maker c-Fos. As a result, the number of c-Fos immunoreactive (ir) cells in the lateral amygdala, ventral tegmental area (VTA), and dorsal raphe nucleus were significantly higher in the males that exhibited USVs than that in exhibited no USVs. Finally, we further

investigated cell type of the c-Fos-ir cells in the VTA. Approximately 25 % of the c-Fos-ir cells were dopaminergic in mice which exhibited the USVs on average, and the dopaminergic c-Fos-ir cells were correlated with amount of the USVs. The present study suggests that courtship vocalization is expression of sexual motivation related to sex hormones rather than general sociality, and that the dopamine-related systems in the VTA is responsible for the USVs emission and its individual differences.

Disclosures: K. Kouta: None. **T. Kikusui:** None.

Poster

247. Parental Behavior

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 247.26/R12

Topic: E.03. Behavioral Neuroendocrinology

Support: JSPS Grant 13J08901

Title: Male mice ultrasonic vocalizations enhance female reproductive function

Authors: *A. ASABA, K. MOGI, T. KIKUSUI;
Azabu Univ., Kanagawa, Japan

Abstract: Male-female vocal communication is important for selecting a suitable mating partner and we previously suggested that ultrasonic vocalizations (USVs) from male mice, which were emitted when encountering females, were contributed to kin recognition and used to avoid inbreeding, on the basis of concomitant presentation with a male pheromone, ESP1 (Asaba et al, 2014). On the other hand, there have been reported that male vocalizations could promote female fertility in some animals. However, there have not been intensive studies about it in mice USVs. In the present study, we first investigated the relationship between the numbers of delivery in breeding pairs for 4 months and of USVs syllables emitted from those paired males during 3 minutes of sexual encounter with unfamiliar females. Interestingly, there was a positive correlation between these two indices, suggesting a possibility that male USVs could promote female's fertility. Next, we examined the effect of male USVs on sexual behavior in females. As results, females showed more approach behavior towards vocalizing-males than devocalized-males. Finally, to examine whether male USVs could activate neural system governing reproductive function in females, the activation of Kisspeptin neurons, key neurons to drive gonadotropin-releasing hormone neurons in the hypothalamus, were examined using dual-label immunocytochemistry with cAMP response element-binding protein phosphorylation (pCREB). In the anteroventral periventricular nucleus (AVPV), there tended to be increased in the number of Kisspeptin neurons expressing pCREB after simultaneous exposure to male-USVs and ESP1 as compared with other groups; male-USVs and vehicle, background noise and ESP1, or

background noise and vehicle. In conclusion, we suggest here that male USVs could promote fertility in female mice, by activating both their sexual motivation and central Kisspeptin neurons. It is also suggested that multisensory integration with pheromonal cue is involved in this putative function.

Disclosures: **A. Asaba:** None. **K. Mogi:** None. **T. Kikusui:** None.

Poster

247. Parental Behavior

Location: Hall A

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Program#/Poster#: 247.27/R13

Topic: E.03. Behavioral Neuroendocrinology

Title: Non-breeder's alloparenting behaviors are enhanced by coprophagy in eusocial rodents, the naked mole-rats

Authors: ***A. WATARAI**[1], N. ARAI[2], S. MIYAWAKI[3], K. MIURA[3], K. MOGI[1], T. KIKUSUI[1];
[1]Companion Animal Res., Azabu Univ., Kanagawa, Japan; [2]IPCR, Saitama, Japan; [3]IGM, Hokkaido Univ., Hokkaido, Japan

Abstract: Naked mole-rats have eusociality. Their colonies consist of a single breeding female (the queen) and some breeding males. All the other adults in a naked mole-rat colony are sexually immature (non-breeders). Non-breeders are altruistic and support parturition of the queen and nursing pups. It has also been found that naked mole-rats frequently perform coprophagy, which is a habit of eating both their own and other's feces. Hamilton's rule explains why non-breeders perform altruistic alloparenting behaviors. On the other hand, physiological mechanism promoting alloparenting behaviors of non-breeders has not been discovered. In the present study, we examined how non-breeders acquired motivation of alloparenting behaviors towards the pups. First, we assessed preference to pups and measured fecal and urinary gonadal steroid hormone concentrations of non-breeders in each reproductive stages of the queen; pregnancy, postpartum and non-lactating. Non-breeders showed preference to pups in the postpartum stage of the queen. We also found that fecal and urinary estradiol concentration increased in the pregnancy stage of the queen. Secondly, to confirm whether coprophagy was involved in motivation of alloparenting behaviors, we assessed preference to pups of non-breeders after they ate feces of the pregnant queen or impregnating with estradiol of the non-pregnant queen. As result, their preference to pups was facilitated by both the treatments. These results suggest that non-breeders acquire motivating of alloparenting behaviors by coprophagy, eating feces including estradiol from the pregnant queen.

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Poster

248. Parental and Gestational Influences on Stress Vulnerability

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 248.01/R14

Topic: E.05. Stress and the Brain

Support: NIH Grant MH104184

NIH Grant MH091258

NIG Grant MH087597

Title: Fumbling the maternal-fetal microbial handoff: maternal stress reprogramming of the developing gut-brain axis

Authors: ***E. JAŠAREVIC**, C. D. HOWARD, A. MISIC, D. P. BEITING, T. L. BALE; Univ. of Pennsylvania, Philadelphia, PA

Abstract: Prenatal stress is associated with an increased risk for neurodevelopmental disorders. In our established mouse model of early prenatal stress (EPS), long-term programming effects on offspring development have been demonstrated, including reprogramming of the hypothalamic-pituitary-adrenal (HPA) axis, dysregulation of HPA stress axis responsivity, cognitive dysfunction, and post-pubertal growth. Mounting evidence points to a likely influence of maternal stress experience on reprogramming of the gut-brain axis via the maternal vaginal microbiome. As the neonate acquires its founding population of gut microbes during passage through the birth canal, changes in the vaginal microbiome produced by stress during pregnancy alters the composition and function of the microbiota colonizing the neonate gut. Such programming exerts lasting effects on the neonate brain by metabolically altering the neonate gut environment, and ultimately contributing to aspects of our EPS phenotype. We have found that EPS changes in the microbiome composition are paralleled by plasma and gut metabolite profiles related to impaired energy metabolism and availability, and sex- and brain region-specific changes in amino acid transport by postnatal day 2 (PN2). This early life disruption in neonate microbiota composition is associated with long-term and sex-specific impact on microbial composition, metabolism, and gastrointestinal barrier deficits into adulthood as well. To provide a causal link of the stress-altered vaginal microbiota with reprogramming of the developing gut-brain axis, we used cesarean delivery (CD) of neonate mice colonized with vaginal microbiota from either control or EPS dams, and assessed microbial composition, the gut metabolome, and the developing hypothalamic translatome at PN2. We confirmed that colonizing CD offspring with maternal vaginal microbiota restored microbial diversity and abundance similar to vaginally

delivered offspring, providing validation that vertical transmission of maternal vaginal microbiota is a critical source of microbial diversity in the neonate gut. To determine whether colonization by a stress-altered microbiota recapitulates key features of our EPS phenotype, gastrointestinal barrier function and HPA stress responsivity in these offspring was assessed in adulthood. Remarkably, aspects of the EPS phenotype are transferrable by colonization of a stress-altered vaginal microbiota, validating the importance of the vaginal microbiome in neurodevelopmental programming. Together, these studies provide valuable insight into the novel role of maternal stress in driving the developing gut-brain axis.

Disclosures: E. Jašarevic: None. **C.D. Howard:** None. **A. Misic:** None. **D.P. Beiting:** None. **T.L. Bale:** None.

Poster

248. Parental and Gestational Influences on Stress Vulnerability

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 248.02/R15

Topic: E.05. Stress and the Brain

Support: MH091258

MH087597

MH099910

MH104184

Title: Placental OGT serves as an epigenetic dispatcher of prenatal stress signals to the developing fetal brain

Authors: B. M. NUGENT[1], C. L. HOWERTON[1], *T. L. BALE[2];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]Dept Animal Biol, Univ. Pennsylvania, Philadelphia, PA

Abstract: Prenatal stress is a risk factor for several neurodevelopmental disorders, including schizophrenia and autism, which display marked sex differences in age of onset, severity, and prevalence. In our mouse model of early prenatal stress (EPS), stress exposure during the first week of gestation imparts long-term neurodevelopmental programming deficits in male offspring resulting in hypersensitivity to stress, cognitive impairments, and alterations in metabolic programming. The placenta acts as an arbitrator between the mother and fetus, providing all necessary nutrients and gasses needed for fetal growth and is now widely regarded as an important contributor to healthy neurodevelopment. Previously, we identified O-linked-N-acetylglucosamine transferase (OGT) as a sex-specific placental biomarker of prenatal stress. OGT acts as a nutrient sensor that modifies numerous proteins to alter various cellular signals,

including major epigenetic processes like histone methylation and DNA demethylation. OGT is an X-linked gene with dramatically higher expression in the female placenta compared to males and expression is further decreased in males following EPS. Importantly, placental-specific knockout of OGT recapitulates the EPS phenotype, validating the important role of OGT signaling in the placenta for neurodevelopmental programming. To determine the mechanistic contributions of OGT in the placenta, we quantified expression levels of important epigenetic regulators following placenta-specific knockout of OGT and found large changes in histone modifiers and modifications associated with epigenetic repression. In addition, we used Chip-Seq to analyze activational and repressive chromatin marks to compare and contrast gene sets impacted by changes in histone methylation following placental OGT knockout. These studies provide further mechanistic insight into the importance of OGT signaling in epigenetic gene regulation in the placenta in response to stress. *Supported by MH091258, MH087597, MH099910, MH104184*

Disclosures: **B.M. Nugent:** None. **C.L. Howerton:** None. **T.L. Bale:** None.

Poster

248. Parental and Gestational Influences on Stress Vulnerability

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 248.03/R16

Topic: E.05. Stress and the Brain

Support: MH091258

MH087597

MH099910

MH104184

Title: Maternal and fetal exosomes: a message in the bottle for neurodevelopmental programming

Authors: *B. M. NUGENT, T. L. BALE;
Dept. of Biomed. Sci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Maternal and fetal exosomes: a message in the bottle for neurodevelopmental programming Bridget M. Nugent & Tracy L. Bale Department of Biomedical Sciences, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA, 19104, USA. Understanding how maternal stress affects the maternal and fetal milieu is important for identifying mechanisms involved in perturbations of brain programming, which are often associated with neurodevelopmental disorders. In our well-established mouse model, male offspring exposed to early prenatal stress (EPS) have altered hypothalamic pituitary axis (HPA)

programming, resulting in increased stress sensitivity and dysregulation in hypothalamic metabolism, similar to endophenotypes identified in boys with autism and men with early-onset schizophrenia. Previously, our lab found that gene sets important for endo- and exosomal cellular processes are down-regulated in the male placenta in response to EPS, suggesting that EPS alters maternal and fetal exosome signaling. Exosomes are small vesicles secreted locally and into the bloodstream by most tissues transferring proteins, microRNAs (miRNAs), and other signaling factors between cells and tissues as a means of short and long-distance communication. Importantly, exosomes can cross the blood-brain barrier to impact neural gene expression, and potentially alter brain development. To explore the effect of EPS on exosome signaling, we collected maternal and fetal serum and tissues on embryonic day 18.5, and isolated proteins and miRNAs for 'omics analyses. Using proteomics and small RNA-Seq, we found that maternal stress during the first week of pregnancy produced lasting and robust effects on exosome signaling, as well as sex differences in the overall number of exosomes in fetal and neonatal circulation. Our proteomics data suggest that EPS induces long-term changes in exosome production and cargo from a variety of maternal sources, including maternal immune cells and the placenta. Since miRNAs have the potential to broadly impact gene expression by targeting mRNAs for degradation, and exosomes can have widespread targets throughout the body, stress-induced alterations in exosomal miRNAs may produce extensive changes in tissue function including changes in the fetal brain. *Supported by MH091258, MH087597, MH099910, MH104184*

Disclosures: **B.M. Nugent:** None. **T.L. Bale:** None.

Poster

248. Parental and Gestational Influences on Stress Vulnerability

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Topic: E.05. Stress and the Brain

Support: MH073030

MH091258

MH087597

MH099910

MH104184

Title: Lasting and dose-responsive reprogramming of sperm microRNA content by paternal stress

Authors: *J. CHAN, A. B. RODGERS, T. L. BALE;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Epidemiological studies suggest that epigenetic inheritance of paternal lifetime experiences can influence neuropsychiatric disease risk in subsequent generations. Rodent models examining transmission via the paternal lineage, where males do not participate in offspring rearing, provide an exciting model for examining the unique epigenetic germ cell contribution to offspring development. Recent studies have demonstrated that paternal exposure to a variety of perturbations can impact offspring behavior and physiology, and have identified changes in histone modifications, DNA methylation, and/or microRNA (miRs) populations as potential mechanisms of transmission. We have developed a paternal stress model in which both male and female offspring show a significantly hyporesponsive HPA stress axis. . Paternal sperm analyses detected a significant increase in 9 specific miRs following stress. Zygote microinjection of these miRs was able to recapitulate the blunted stress response seen in paternal stress offspring. However, the timing by which sperm epigenetic marks can respond to environmental perturbations such as stress and how long these changes last is not known. Our current studies examined the maintenance and specificity of stress-sensitive sperm miRs following chronic stress exposure and their ability to reprogram offspring stress responsivity. Sires were exposed to stress either once or twice per day for four weeks, and were then bred multiple times over a 3-month period to examine the acute and long-term programming effects of paternal stress on offspring neurodevelopment. Interestingly, offspring exhibited differences in stress regulation and body weight, with the magnitude of offspring body weight differences increased with greater paternal stress experience, suggesting that germ cells can encode specific information about stress severity. miR-sequencing was used to identify the total miR population altered in sperm following stress, and to assess the relationship between stress severity and offspring phenotypic reprogramming. Ongoing studies examining the temporal responsiveness of sperm miRs following stress experience promise insight into the dynamic regulation of germ cell epigenetic marks in response to environmental perturbations, and will provide novel and exciting insight into how the paternal environment can impact offspring neurodevelopmental disease risk.

Disclosures: J. Chan: None. **A.B. Rodgers:** None. **T.L. Bale:** None.

Poster

248. Parental and Gestational Influences on Stress Vulnerability

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 248.05/R18

Topic: E.05. Stress and the Brain

Support: NIH MH073030

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NIH MH091258

NIH MH099910

NIH MH104184

Title: Placental insulin receptors as novel orchestrators of sex-specific neurodevelopmental programming

Authors: *S. L. BRONSON, T. L. BALE;
Sch. of Vet. Med., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Adverse prenatal experiences remodel the developing brain and confer lifelong neuropsychiatric disease susceptibility. Males are uniquely vulnerable to intrauterine perturbations such as maternal stress and metabolic dysfunction, which predispose them to neurodevelopmental disorders including autism, attention-deficit hyperactivity disorder, and schizophrenia. The mechanisms by which prenatal adversity produces sex differences in vulnerability to disease remain largely unclear. Recent studies have emphasized the emerging role of the placenta as an orchestrator of sex-specific fetal programming. Human studies and animal models examining the effects of maternal stressors have pointed to disruption of key homeostatic pathways within the placenta, including that of insulin. We hypothesize that impaired insulin receptor signaling within the placenta in response to diverse perturbations of the maternal milieu drives sex-specific neurodevelopmental reprogramming. To demonstrate the importance of placental insulin in neurodevelopment, we utilized the conditional Cre-Lox transgenic mice to selectively target insulin receptors (InsR) in trophoblastic cells of the placenta. Sex specificity and behavioral stress phenotypes were then examined in wildtype and placental-knockout mice as adults. Intriguingly, placental InsR deletion significantly increased the hypothalamic-pituitary-adrenal axis stress response and impaired sensorimotor gating in males, thereby recapitulating endophenotypes of sex-biased neurodevelopmental disorders. Lack of these programmatic changes in females suggests distinct vulnerability of the male placenta. To determine the changes in placental function related to InsR deletion, we conducted a transcriptomics analysis comparing genotype x sex by Affymetrix microarray. Consistent with the heightened behavioral vulnerability of males, InsR deletion elicited dramatic changes in gene expression in male placentas, indicative of vascular dysfunction and impaired lipid transport. As the fetus relies on placental transfer of maternally derived lipids, we assessed lipid composition in the fetal brain. Extensive shifts in fetal brain phospholipid and fatty acid composition were associated with enduring changes in myelination in placental InsR deficient mice, and suggest that preservation of placental lipid transfer may contribute to female resilience. Together, these data provide compelling evidence demonstrating the programmatic capacity of the placenta and reveal the emerging role of placental insulin signaling in sex-specific reprogramming in response to intrauterine adversity.

Disclosures: **S.L. Bronson:** None. **T.L. Bale:** None.

Poster

248. Parental and Gestational Influences on Stress Vulnerability

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Topic: E.05. Stress and the Brain

Support: NSERC

AIHS

Canadian Institute for Advanced Research Program in Child Brain Development

Title: Father: An essential element The impact of preconception paternal experience on offspring neurodevelopment and behaviour

Authors: *A. F. HARKER, S. RAZA, K. WILLIAMSON, B. KOLB, R. GIBB;
Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: A rich literature has been amassed demonstrating the impact of early life events on the structure and function of the developing brain. While a plethora of research has shown that maternal experience during the prenatal period of life has the ability to alter neurodevelopment and behavioural outcomes of offspring, far less in understood regarding the impact of preconception paternal experience on developing brain architecture. The goal of this research was to examine the effect of two independent preconception paternal experiences on subsequent neurodevelopment and behaviour of male and female offspring. Research has shown that stress during the prenatal period can alter brain morphology in the developing brain, and is thought to be a factor in the development of some adult psychopathologies. Our first experiment examined the impact of preconception paternal stress on offspring brain and behaviour. Our hypothesis was that paternal stress in the preconception period would negatively impact brain development in offspring, leading to behavioural abnormalities. While stress and environmental enrichment have been shown to have opposing effects on brain architecture and behavioural outcomes in offspring, we decided to explore the impact of environmental enrichment provided to fathers during the preconception period. We hypothesized that preconception paternal enrichment would alter brain development and positively impact behaviour of offspring. Both experiments followed the same experimental design. Male Long Evans rats were exposed to either a stressing paradigm or a complex environment for 27 days prior to mating. Developmental assays, anatomical measurements, and brain morphology analyses were conducted throughout offspring lifespan.

Disclosures: A.F. Harker: None. **S. Raza:** None. **K. Williamson:** None. **B. Kolb:** None. **R. Gibb:** None.

Poster

248. Parental and Gestational Influences on Stress Vulnerability

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 248.07/R20

Topic: E.05. Stress and the Brain

Support: NIH P01 HL028972

Title: Whole genome response of developing baboon fetal hypothalamus to the effects of maternal nutrient restriction (MNR) in late gestation

Authors: *T. NURMAMAT[1], L. A. COX[1], P. W. NATHANIELSZ[2], S. YANG[2], C. LI[2], J. GLENN[1];
[1]Genet., Texas Biomed. Res. Inst., San Antonio, TX; [2]Univ. of Wyoming, Laramie, WY

Abstract: Introduction: Moderate malnutrition during pregnancy is widespread around the world and it impacts large number of pregnancies in both developing and developed countries. Maternal nutrient restriction (MNR) can lead to programming of the fetal hypothalamic-pituitary-adrenal (HPA) axis, predisposing offspring to obesity and psychiatric diseases in later life (Reviewed by Matthew SG, Nature Rev Endo 2014). We have developed a baboon nonhuman primate (NHP) model of MNR and previously reported up regulation of the fetal baboon HPA axis (Li et al., 2013). Hypothalamus is one of the most sensitive regions of the brain to the effect of environmental stimuli, but the hypothalamic response to nutritional stress induced by MNR at the whole genomic level is lacking. In this study, our aim was to determine the whole genome response of fetal baboon hypothalamus to MNR in late gestation (0.9 G). Methods: Randomly selected pregnant baboons were fed either ad libitum (control; CTR; n=5) or a globally reduced diet (70% of CON; n=5) from 0.16 G through 0.9 G that produces IUGR (14% reduction in fetal weight). Fetuses were removed by Cesarean section at 0.9 G, and fetal hypothalamus samples were collected for genomic analysis. Gene arrays were performed for determining the whole genome profiles and the Ingenuity Pathway Analysis (IPA) software was used for data analysis. Results: We detected total of 10400 genes expressed in the fetal baboon hypothalamus and 745 genes accounting for 7.1% of the total genes expressed were differentially expressed in CON vs. MNR. Of those 745 differentially expressed genes, 425 genes were up regulated ($P < 0.05$) and 320 genes were down regulated ($P < 0.05$) in MNR fetuses. Canonical pathway analysis results revealed that multiple cellular growth pathways, including IGF-1, Akt, CREB, PDGF, STAT3, etc. were up regulated. Cellular stress response pathways, including p38 MAPK and EIF2 were also up regulated. Further, several metabolic pathways (Noradrenalin and adrenalin degradation and TCA cycle II) and apoptosis signaling pathways were dysregulated in response to MNR. Network analysis results show that gene networks involved in cell death and survival, embryonic development and organ development were affected Conclusions: The results show that non-human primate hypothalamus is widely susceptible to the impact of MNR in late gestation. The increase in multiple cell growth and stress response related pathways indicate highly activated fetal hypothalamic response to MNR at the molecular level, which may

contribute to the programming of HPA axis. These results need further validation by immunohistochemistry to determine the effect at the protein level.

Disclosures: **T. Nurmamat:** None. **L.A. Cox:** None. **P.W. Nathanielsz:** None. **S. Yang:** None. **C. Li:** None. **J. Glenn:** None.

Poster

248. Parental and Gestational Influences on Stress Vulnerability

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Topic: E.05. Stress and the Brain

Support: NIH Grant RO1 MH068482

GSU Brains and Behavior Seed Grant

Title: Birth: an overlooked event in brain development?

Authors: ***A. CASTILLO-RUIZ**, M. MOSLEY, N. G. FORGER;
Georgia State Univ., Atlanta, GA

Abstract: Birth involves dramatic changes in a newborn's environment and the processes associated with birth (labor and parturition) trigger an 'adaptive stress' response which prepares key peripheral organs for the transition to postnatal life. However, little is known about how birth influences the brain. Cell death is an important feature of nervous system development. In mice, there is increased cell death across many brain regions around the time of birth. Whether cell death is induced by parturition has not been addressed. In addition, mode of birth might be important for the normal course of cell death in the brain, because 19 days post-conception, Cesarean (C) born mice show decreased cell death in several brain regions (e.g., dentate gyrus, oriens layer of the hippocampus, and suprachiasmatic nucleus) relative to mice born vaginally (V) the same day. To systematically study how birth influences cell death, we manipulated birth mode (V vs C) in timed-pregnant mice and collected the brains of male and female offspring *in utero* at embryonic day (E)18.5 and E19 and *ex utero* (V and C birth carefully matched for time of delivery) at postnatal day (P)0 (3h after birth), P1, P3, and P23 (the latter three groups were cross-fostered to unrelated dams). We monitored the morphometric development (body weight and eye-opening) of these mice and assessed affective state by measuring ultrasonic vocalizations in an isolation test at P9. Birth mode did not affect gross development of newborn or juvenile mice. We did observe slightly increased body weight in C mice at weaning, which is consistent with clinical reports of higher body mass index in humans born by C-section. We also found that birth mode may alter neurobehavioral/affective development because C mice had significantly softer (lower amplitude) calls than V mice in the isolation test. Interestingly, call amplitude is reported to be the most salient feature of an infant's call for eliciting maternal

behavior in mice. We are currently processing the brains for the histochemical detection of cell death (activated caspase-3) and microglia (Iba1) markers. Microglia, the brain's resident immune cells, may play an active role in neuronal cell death. Parturition activates the peripheral immune system and birth mode influences the degree of this activation. Whether the immune activation at birth extends to the brain it is not known and will be investigated here. Taken together our work (previous and current) suggests that birth may be an important event for brain development and deviations from the natural mode or timing of birth may alter brain development and behavior.

Disclosures: A. Castillo-Ruiz: None. **M. Mosley:** None. **N.G. Forger:** None.

Poster

248. Parental and Gestational Influences on Stress Vulnerability

Location: Hall A

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Program#/Poster#: 248.09/S2

Topic: E.05. Stress and the Brain

Support: NIH Grant NS28912

NIH Grant P50 MH096889

Title: Chronic CRH stunts cortical neuron dendritic arborization: A basis for reduced cortical thickness in children exposed to high CRH *in utero*?

Authors: *M. M. CURRAN, C. SANDMAN, E. DAVIS, L. GLYNN, A. ANDRES, T. Z. BARAM;
Univ. of California Irvine, Irvine, CA

Abstract: Rationale: Hypothalamic corticotropin releasing hormone (CRH) initiates neuroendocrine responses to stress. CRH levels are low to undetectable in human plasma, but increase during pregnancy when primate placenta uniquely produces large amounts of CRH and releases it into maternal (and fetal) circulation. Placental CRH production and consequent maternal plasma levels are augmented by stress during pregnancy. We measured plasma CRH levels in a group of pregnant women every 5 weeks during gestation weeks 15-35 and correlated them to structural brain MRI scans of their children at 6-9 years. A 12% reduction in whole brain cortical thickness was found in the group of children who were exposed to higher levels of maternal CRH throughout gestation. How might increased maternal CRH levels lead to cortical thinning in children? Is gestational CRH merely a marker of stress and other stress hormones affect cortical development? Or might CRH directly influence cortical integrity and growth? Here we probed a potential causal role of CRH in decreased cortical thickness. We reasoned that: 1) cortical thickness is largely comprised of neuronal dendritic branches (Paus 2009); 2) chronic exposure to CRH reduces dendritic branching of hippocampal neurons (Chen 2004, 2008). Here we tested the biological plausibility of CRH-induced dendritic impoverishment as a direct

mediator of reduced cortical thickness. **Methods**: Sprague Dawley rat primary cortical cultures were used. Cortical neurons were isolated within 36h of birth and grown for 7d followed by 7d of CRH. Three experiments were performed with CRH concentrations of 0.01, 0.1, 1, 10 & 100nM. Cultures were fixed and stained for the dendritic marker MAP2 and individual neurons imaged by confocal microscopy without knowledge of treatment groups. Dendrites were manually traced, and dendritic arbors were compared using Sholl analysis. Statistical comparisons employed 2-way repeated measures-ANOVA, followed by Šídák's *post-hoc* multiple comparisons test. **Results:** Exposure to nM CRH levels decreased dendritic branching of cortical neurons in a dose dependent manner. Higher CRH concentrations (10nM, 100nM) reduced arborization significantly at 40-80µm from somata. Lower CRH levels (≤1 nM) had no significant effect. **Conclusions:** These results are consistent with a direct contribution of CRH to impoverished dendritic branching which may underlie reduced cortical thickness in children exposed *in utero* to higher peptide levels. Together with the imaging data, the results provide a novel potential mechanism for the influence of high maternal stress during pregnancy on the developing brain of her child.

Disclosures: **M.M. Curran:** None. **C. Sandman:** None. **E. Davis:** None. **L. Glynn:** None. **A. Andres:** None. **T.Z. Baram:** None.

Poster

248. Parental and Gestational Influences on Stress Vulnerability

Location: Hall A

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Topic: E.05. Stress and the Brain

Support: ANPCYT

UNSAM

FUNDACION FIORINI

ISN-CAEN

Title: Prenatal stress induces epigenetic changes in the rat offspring brain

Authors: *M. C. ANTONELLI[1], M. C. MONTELEONE[2], M. E. PALLARES[1], E. ADROVER[1], A. C. FRASCH[2], M. A. BROCCO[2];
[1]Inst. de Biologia Celular y Neurociencia, Buenos Aires, Argentina; [2]Univ. de San Martin, Inst. de Investigaciones Biotecnologicas, Buenos Aires, Argentina

Abstract: The period of intrauterine development represents a sensitive window during which disruption or modification of the environment can influence fetal development and might lead to altered health throughout life course. Moderate to severe stressful life-events, in combination

with inadequate social network, are closely associated with increased child morbidity, neurological dysfunction, attention-deficit hyperactivity disorder (ADHD) and sleep disturbance during infancy, which if persist in adulthood might result in depression and vulnerability to psychotic disorders. The process of 'fetal programming' is mediated by the impact of prenatal experience on the developing hypothalamic-pituitary-adrenal (HPA) axis, a dynamic metabolic system that regulates homeostatic mechanisms, including the ability to respond to stressors, and which is highly sensitive to adverse early life experiences. Several studies support the hypothesis that parental programming is mediated by epigenetic mechanisms that stably alter gene transcription affecting physiology and behavior. In this study, we analyzed the effect of prenatal stress (PS) on gpm6aexpression and the epigenetic mechanism involved.gpm6aencodesthe neuronal glycoprotein M6a involved in filopodium extension. Hippocampus and prefrontal cortex (PFC) samples were analyzed for gene expression (qPCR for mRNAs and microRNAs), methylation status (bisulfite conversion) and protein levels in male offspring at postnatal days 28 and 60.Hippocampal neurons in culture were used to analyze microRNAoverexpression effects. Prenatal stress induced changes in gpm6alevels in both tissues and at both ages analyzed, indicating a persistent effect. Two CpG islands in the gpm6agene were identified. Variations in the methylation pattern at three specific CpGs were found in hippocampus, but not in PFCsamples from PS offspring. MicroRNAs predicted to target gpm6awere identified in silico. qPCR measurements showed that PS modified the expression of several microRNAs in both tissues, being microRNA-133b the most significantly altered. Further studies overexpressing this microRNAin neuronal cultures showed a reduction in gmp6amRNAand protein level. Moreover filopodium density was also reduced, suggesting that GPM6afunction was affected. Gestational stress affected gpm6agene expression in offspring likely through changes in methylation status and in posttranscriptional regulation by microRNAs. Thus, our findings propose gpm6aas a novel target for epigenetic regulation during prenatal stress.

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Poster

248. Parental and Gestational Influences on Stress Vulnerability

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: E.05. Stress and the Brain

Support: National Research Foundation (NRF) SA

Title: Involvement of the serotonergic and orexinergic systems in the antidepressant effects of light of maternally separated male Sprague-Dawley rats

Authors: *J. J. DIMATELIS[1], A. MTINTSILANA[2], D. J. STEIN[3], V. A. RUSSELL[2];
[2]Human Biol., [3]Psychiatry and Mental Hlth., [1]Univ. of Cape Town, Cape Town, South Africa

Abstract: Depression is a debilitating mood disorder that requires more effective treatment strategies because conventional treatment does not relieve depressive symptoms in all affected individuals. Maternal separation (MS) is a well-established rodent model of depression/anxiety. Chronic light exposure during adolescence has been shown to reverse the depression-like behavior induced by the early life trauma of maternal separation. We aimed to further delineate the molecular machinery involved in the anti-depressant effect of light exposure. Specifically, we investigated the involvement of the dopaminergic (DA), serotonergic (5-HT) and orexinergic systems as they have been suggested to be involved in depression. The MS paradigm (removal of the dam from the litter for 3 h/day from postnatal day (P) 2-14) was used to induce behavioral changes in male Sprague-Dawley rats, some of whom were also treated with chronic constant light (CCL) for 3 weeks during adolescence. At P80 (adulthood), rats were decapitated and brain tissue collected for analysis of glutamate- and potassium-stimulated [3H]DA release in the nucleus accumbens (NAc) using an *in vitro* superfusion technique. Enzyme-linked immunosorbent assays were employed to measure 5-HT levels in the hypothalamus and prefrontal cortex (PFC). Polyacrylamide gel electrophoresis and Western blotting was used to measure orexin receptor 1 (OXR-1) and 2 (OXR-2) in the PFC. MS rats had significantly reduced glutamate-stimulated [3H]DA release in the NAc in comparison to non-MS animals. CCL exacerbated the MS-induced reduction of [3H]DA release in the NAc. CCL increased hypothalamic 5-HT levels and reduced 5HT levels in the PFC of MS and non-MS rats. MS rats had increased OXR-1 and OXR-2 protein levels in the PFC compared to non-MS rats, an effect that was reversed by CCL. Therefore, this study has demonstrated that the antidepressant effect of CCL treatment of MS animals may be attributable to alterations in the serotonergic system and reversal of the MS-induced up-regulation of orexin receptors in the PFC.

Disclosures: J.J. Dimatelis: None. **A. Mtintsilana:** None. **D.J. Stein:** None. **V.A. Russell:** None.

Poster

248. Parental and Gestational Influences on Stress Vulnerability

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Topic: E.05. Stress and the Brain

Support: FACMED-DIB HERMES 27889

Title: Maternal separation during breastfeeding affects eating behavior by generating a greater preference for sweetener products consumption, increase body weigh and alters locomotion activity

Authors: L. B. AYA-RAMOS[1], C. A. CONTRERAS-VARGAS[2], *Z. DUENAS[1];
[2]Med. Sch., [1]Univ. Nacional De Colombia, Bogota DC, Colombia

Abstract: It has been shown that disruption mother-breeding interaction has adverse effects on regulating physiological, neuroendocrine and behavioral systems; thus, by generating phenotypic adjustments in the eating behavior structure that alters intake patterns, especially for the highly palatable food. The aim of this study was to determine whether maternal separation during lactation (MSDL) in Wistar rats influences the sweeteners consumption, body weight gain, blood glucose level and behavioral responses such as locomotion. MSDL protocol was performed from 1 to 21 postnatal days during of 360 minutes per day, 180 in the morning and 180 in the afternoon, under the dark phase. Control group corresponds to animals that had no MSDL. On postnatal day 22 animals were distributed by sex and treatment. On postnatal day 26 experimental process started with sweeteners (sucrose, stevia, sucralose and aspartame). Sweetener consumption was taken every 24 hours, body weight gain every four days, blood glucose every five days and, on postnatal day 50 rats locomotion was tested in an open field. It was found that the maternal separation protocol generates a preference for sucrose consumption in both females and males $p<0.05$. Related with stevia and aspartame the preference was find in the females group; likewise, neonatal stress protocol generated a greater body weight gain in males with sucrose and in females with aspartame, as well as higher blood glucose levels in females with sucralose and stevia; finally, it was determined that MSDL affects locomotion in both males and females. These results indicate that neonatal stress as MSDL affects eating behavior by generating a greater preference for sweetener products consumption; besides, it may becomes a risk factor for developing chronic diseases such as diabetes and obesity.

Disclosures: L.B. Aya-Ramos: None. **C.A. Contreras-Vargas:** None. **Z. Duenas:** None.

Poster

248. Parental and Gestational Influences on Stress Vulnerability

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Program#/Poster#: 248.14/S7

Topic: E.05. Stress and the Brain

Support: FIS (Fondo de Investigacion en Salud) grant to N.L. No: FIS/IMSS/PROT/G13/1223

Title: Long term consequences of maternal separation on integration of postnatally generated granule cells

Authors: M. LÓPEZ-VARGAS, A. ROQUE, L. TORNER, E. OLVERA - CORTÉZ, *N. LAJUD;
Inst. Mexicano del Seguro Social, Morelia, Mexico

Abstract: Early life stress (ELS) increases hypothalamus - pituitary - adrenal (HPA) axis reactivity and programs for psychopathology later in life. Periodic maternal separation (MS180) is widely used ELS rodent model that mimics long term consequences observed in humans, increases basal glucocorticoid levels, depressive like behavior and impairs memory and learning. MS180 decreases survival of developmentally generated granule cells at postnatal day 15 (P5); however the long term effects on these cells have not been evaluated. Therefore, the aim of this study was to test the hypothesis that maternal separation affects granule cell integration and hippocampus development. Control (CONT) and maternally separated pups (3 hours a day from P1 -14) were injected twice with BrdU at P5 (25 mg / kg) and left to survive for 2 months. At P60 animals were perfused, brains were dissected and we performed a systematic random sampling of 40μm slices (240μm apart) for Immunostaining. We estimated the number of BrdU+ cells within the granular (GC) and sub granular (SGC) layers of the hippocampal dentate gyrus. Different sets of animals were tested in the object recognition test (ORT) and the Morris water maze (MWM). MS180 decreased GC and SGC layers volume but had no effect on total BrdU+ nuclei number; however we observed a highly significant effect of MS180 on BrdU+ nuclei density wen data were plotted along the septo - temporal axis ($F_{1,125}$:26.6 $p \leq 0.0001$). Moreover, a significant increase of BrdU+ nuclei density was observed in the temporal but not the medial and septal thirds of the dorsal hippocampus. MS180 had no effect on the ORT test or the acquisition phase of the MWM but impaired memory on the probe trial. In conclusion, MS180 selectively increased integration of granule cells originated during the postnatal period and impaired memory retrieval. The work was supported by FIS (Fondo de Investigacion en Salud) grant to N.L. No: FIS/IMSS/PROT/G13/1223

Disclosures: **M. López-Vargas:** None. **A. Roque:** None. **L. Torner:** None. **E. Olvera - Cortéz:** None. **N. Lajud:** None.

Poster

248. Parental and Gestational Influences on Stress Vulnerability

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 248.15/S8

Topic: E.05. Stress and the Brain

Title: Alterations in learning of C7BL/6 mice following daily maternal separation

Authors: *J. D. KARP[1], N. RINALDI[2], M. FRIEDFELD[2], C. ROACH[2];
[2]Biol., [1]Rider Univ., Lawrenceville, NJ

Abstract: Interactions between young rodents and their mother can influence the physiology and behavior of the offspring when they become adults. Many studies indicate that early life experiences can predispose animals to potential anxiety-related maladaptive behaviors later in life. Early life experiences that alter reactivity to the environment may also influence the ability

of animals to learn. To investigate this possibility, we separated litters of C57BL/6 mice from their mothers daily for short periods before weaning. We evaluated the behavior of both male and female mice in two learning tasks, namely a stressful step-down test and a non-stressful novel object recognition test, after the mice became adults. We observed maternally separated male and female mice displayed longer learned step-down latencies in the stress-learning task than non-maternally separated mice. This showed maternal separation increased context-dependent anxiety. Among the same animals, maternal separation increased the amount of time male and female mice would explore a novel object in a non-stressful learning task. This showed manipulation of mother-pup interactions increases the motivation of mice to interact with their environment. Together, the results of these behavioral tests suggest that maternal separation makes both male and female mice more emotionally reactive to their environment as adults and those alterations in reactivity can influence learning both non-stressful and stressful situations. In addition, we collected brain tissue samples of the mice and assayed their prefrontal cortex, striatum, and hippocampus for dopamine and norepinephrine levels using high-pressure liquid chromatography with electrochemical detection. We found a tendency for baseline brain dopamine to norepinephrine ratios to be elevated in the prefrontal cortex of maternally separated mice. These results suggest interactions between mother mice and her pups influence long lasting neurochemical and behavioral responses presumably through epigenetic mechanisms. We are currently examining the possibility that these behavior phenotypes and/or brain neurochemical attributes can permanently influence bidirectional communication between the nervous system and other systems of the body.

Disclosures: **J.D. Karp:** None. **N. Rinaldi:** None. **M. Friedfeld:** None. **C. Roach:** None.

Poster

248. Parental and Gestational Influences on Stress Vulnerability

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Topic: E.05. Stress and the Brain

Support: NIGMS (1P20GM103653)

University of Delaware Graduate Fellowship

Title: Characterizing Bdnf DNA methylation in the mPFC throughout development in healthy and early-life stressed rats

Authors: ***J. BLAZE**, T. L. ROTH;
Psychological and Brain Sci., Univ. of Delaware, Newark, DE

Abstract: Negative experiences with a caregiver during infancy can result in long-lasting changes in brain function and behavior. Victims of child abuse have increased incidences of

cognitive abnormalities and psychiatric disorders correlating to aberrant neural development, but underlying mechanisms are not well-understood. It is our central hypothesis that brain and behavior changes are conferred by early childhood adversity through epigenetic changes such as DNA methylation. DNA methylation is an epigenetic modification that alters gene transcription without changing the DNA sequence. Using a rodent model of early-life caregiver maltreatment (involving exposure to an adverse caregiving environment for postnatal days [PN]1-7), we have previously demonstrated abnormal methylation of DNA associated with the Brain-derived neurotrophic factor (Bdnf) gene in the medial prefrontal cortex (mPFC) of developing and adult male and female rats along with changes in gene expression of epigenetic regulators. BDNF is crucial for neuroplasticity and development, and aberrant Bdnf regulation has been implicated in several psychiatric disorders linked to early-life adversity. The current study aimed to more fully characterize Bdnf DNA methylation (cite-specific and 5-methylcytosine vs. 5-hydroxymethylctyosine) across multiple time-points during development in the healthy and stressed mPFC. Using a within-litter design, pups were exposed to an adverse (maltreatment condition) or nurturing (cross-foster condition) caregiving environment outside the homecage for 30 minutes each day during the first postnatal week. Remaining pups in a litter were left with the biological mother during each session (providing normal care controls). Brains were removed at postnatal day (PN) 8, 30, or 90, and bisulfite-sequencing PCR was used to quantify percentages of methylation at each CG site within Bdnf exon IV. Data indicate that early-life stress alters the normal trajectory of Bdnf methylation and confirm previous findings that methylation patterns are age- and sex- specific. We are also investigating cell-type specificity to identify methylation patterns in neurons vs. non-neurons in rats that experienced early-life maltreatment. Funded by: NIGMS (1P20GM103653) University of Delaware Graduate Fellowship

Disclosures: J. Blaze: None. **T.L. Roth:** None.

Poster

248. Parental and Gestational Influences on Stress Vulnerability

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CONACyT PhD Scholarship

PAPIIT-DGAPA-UNAM IN 216214

CONACyT 179616

CONACYT 238744

Title: Neonatal maternal separation modifies vasopressin receptor Va1 and V1b expression during development in rat amygdala and cerebellum: it possible role in anxiogenesis and alcohol intake

Authors: *A. T. NAVA KOPP, L. ZHANG;
Dept. of Physiology, Fac. of Medicine, Natl. Autonomous Univ. of, Mexico City, Mexico

Abstract: During ontogenesis, vasopressin (VP) contributes to the regulation of proliferation and morphogenesis of the target cells and organs (brain, pituitary, kidney and liver. VP system is known to be activated around birth when VP contributes to the establishment of a new equilibrium in the body fluids and the adaptation of the fetuses to the stress of the labor. Following birth, VP induces a redistribution of the blood flow via the cardiovascular system in order to increase blood volume in the vital organs and those responsible for stress reaction (brain, pituitary gland, heart, adrenals), while reducing the blood flow in other peripheral organs. Afterwards, the physiological role of VP extends to the regulation of the cardiovascular system, water re-absorption in kidney and glucogenolysis in liver. Vasopressin's effects on the central nervous system are being extensively investigated. On the other hand, using neonatal maternal separation (MS), a well-validated rodent model used to assess the effect of early postnatal stress on cognition and emotionality in adulthood, we have previously reported that in response MS, the rat hypothalamic vasopressin system becomes up-regulated, showing increased expression of mRNA and enlarged volume of the hypothalamic paraventricular and supraoptic vasopressin nuclei. The sensitivity to acute stressors of these subjects is also increased in adulthood. In order to further investigate the mechanisms underlying this stress hyper-responsiveness, we devised a series of experiments to evaluate the expression dynamics of the VP's two major receptor types, V1a and V1b, in the MS's rat brains, compared with control, using the newly available method RNAscope (ACD, San Francisco, CA). Our preliminary data indicate a down regulation of V1a expression on the GABAergic neurons in the centro-medial nucleus of the amygdala and an up-regulation of V1b in the cerebellar cortex. The role of this modification on alcohol preference and Fos expression during anxioigenic behavioral tests will be discussed.

Disclosures: **A.T. Nava Kopp:** None. **L. Zhang:** None.

Poster

248. Parental and Gestational Influences on Stress Vulnerability

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 248.18/S11

Topic: E.05. Stress and the Brain

Support: Project PRIN 20107MSMA4

Title: Prenatal exposure to ethanol and early maternal separation alters HPA axis responsiveness and emotional state in young adult rats

Authors: *F. BIGGIO[1], V. LOCCI[2], M. G. PISU[3], M. C. MOSTALLINO[3], M. SERRA[1]; [1]Univ. of Cagliari, Cagliari, Italy; [2]Univ. of Sassari, Sassari, Italy; [3]Inst. of Neuroscience, Natl. Res. Council, Cagliari, Italy

Abstract: It's wide reported that adverse stress events during pregnancy as well as in early or adolescence period may fall in long-term effects on developement and emotional/behavioral states inducing deep, and sometimes irreversible changes in the adulthood (Weinstock ., 2008). Thus, the quality of prenatal environment and postnatal experiences has been shown to predict vulnerability to psychopathologies in the adult. We studied whether a moderate dose of ethanol (1g/kg,), during the pregnancy (GD17-20) and stress induced by daily maternal separation (MS 3h, PDN 3-15) alters emotional behavior and sensitivity to acute stress in adult offspring. Because maternal separation stimulates HPA activity of the dams and changes of corticosterone levels influence maternal care (Rees et al., 2004), we evaluated if exposure to ethanol during pregnancy may influence maternal behaviour. Thus, dam corticosterone levels and maternal care level were assessed during at weaning and at the first two postnatal weeks, respectively. The responsiveness of the HPA axis to stressful conditions was evaluated in young adult male rats by measuring the basal and foot shock-stimulated plasma levels of corticosterone as well as allopregnanolone. We found that prenatal treatment with ethanol and subsequent maternal separation (EtOH-MS) induced a decrease in plasmatic corticosterone and allopregnanolone content respect to counterpart not subjected to maternal separation (EtOH-NMS). Moreover, the enhancement of corticosterone and allopregnanolone levels induced by foot-shock stress in EtOH-MS was markedly increased compared to that observed in EtOH-NMS group. In addition, elevated plus maze test revealed an increase in anxiety state in EtOH-MS group respect to EtOH-NMS. As expected, (Biggio et al., 2014) we found that maternal separation induced an increase in arched-back nursing and pup-licking in mothers not exposed to ethanol. Interestingly, the exposure to ethanol during late pregnancy did not affect the maternal care of pup-separated dams. This results suggests that the alteration in emotional state and stress response in animals subjected to prenatal stress and subsequent maternal separation is not related to the quality of maternal care received. In conclusion, these results suggest that stressful experiences during pregnancy and childhood may change the programming stress-responsiveness of HPA axis involved in emotional regulation in adulthood. Wenstock M., 2008. Neurosci Biobehav Rev. 32(6):1073-86 Rees S.L., et al, 2004. Horm.Behav.46, 411-419. Biggio F., et al, 2014. Eur Neuropsychopharmacol. 24(7):1152-61

Disclosures: F. Biggio: None. **V. Locci:** None. **M.G. Pisu:** None. **M.C. Mostallino:** None. **M. Serra:** None.

Poster

248. Parental and Gestational Influences on Stress Vulnerability

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 248.19/S12

Topic: E.05. Stress and the Brain

Support: NSF IGERT Grant 0006461

HUCM Pilot Grant U400023

HUCM Pilot Grant U400045

Title: Effects of maternal separation and post-weaning housing on social and depressive-like behaviors and neuronal oxytocin expression in rats

Authors: Y. GILLES[1], A. NTIM-ADDAE[1], L. APOLONIO[2], K. F. MANAYE[1], *E. K. POLSTON[1];
[1]Dept. of Physiol., Howard Univ. Col. of Med., Washington, DC; [2]Col. of Computing, Georgia Inst. of Technol., Atlanta, GA

Abstract: Maternal separation (MS) is a well-established rodent model for depression. The adverse effects of MS can be ameliorated by environmental enrichment. We investigated whether social enrichment in the form of group housing could also mitigate the effects of MS. Male and female Sprague-Dawley rats were assigned to two neonatal groups, MS or non- MS. MS pups were separated from their mothers for four hours each day for the first 21 days of life. Non-MS pups remained undisturbed with their mothers. On postnatal day 21, half of the animals from each treatment group were weaned into either single or same-sex group housing conditions to yield four experimental groups: maternal separated/single housed (SSH), maternal separated/group housed (SGH), non-separated/single housed (NSH), and non-separated/group housed (NGH). A social play test was performed at 5 weeks of age, followed in adulthood by two tests for depressive-like behaviors, the forced swim test (FST), and the sucrose preference test (SPT). The FST measures helplessness and the SPT tests for anhedonia, traits associated with depression in humans. All animals were euthanized and brains were removed. Sections through the hypothalamus were immunostained for oxytocin (OT), a neuropeptide that is implicated in social bonding and depression. Numbers of OT-positive neurons in the paraventricular and supraoptic nuclei of the hypothalamus (PVN, SON) were quantified stereologically. A significant main effect of post-weaning housing was observed in almost every behavioral measure. Single housed animals exhibited more social behaviors than group housed animals, irrespective of whether the behaviors were categorized as ‘prosocial’ (sniffing grooming, approach) or ‘antisocial’ (boxing, pinning, rejecting). Effects of sex and MS on social behaviors were relatively minor. Results from the FST and SPT were similar. Post-weaning housing exerted the most dominant effect on depressive-like behaviors, while the effects of sex and MS were less significant. OT cell numbers in the main cell groups of the PVN and SON did not reflect the behavioral findings; no group differences were observed in either of these regions. However, in the anterior parvocellular division of the PVN, there was a significant interaction between housing and MS treatment. In both sexes, SGH animals had significantly more OT cells than any other group. Our results show that while sex and MS treatment can influence social and

depressive-like behaviors in this model, social isolation in adulthood plays a more significant role. The effects of MS and social isolation may be related to a change in OT neuronal number in the anterior parvocellular division of the PVN.

Disclosures: Y. Gilles: None. **A. Ntim-Addae:** None. **L. Apolonio:** None. **K.F. Manaye:** None. **E.K. Polston:** None.

Poster

248. Parental and Gestational Influences on Stress Vulnerability

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 248.20/S13

Topic: E.05. Stress and the Brain

Support: CNPq 454429/2014-2

CNPq 400850/2014-1

CNPq 306271/2014-1

Title: Maternal separation reduces NFKB1 and miRNA-155 expression in hippocampus of female adolescent mice with no effect of physical training

Authors: *L. WEARICK-SILVA[1], T. W. VIOLA[1], L. A. AZEREDO[1], A. CENTENO-SILVA[1], T. W. BREDY[2], R. GRASSI-OLIVEIRA[1];
[1]Biomed. Res. Inst., Pontifical Univ. of Rio Grande Do Sul, Porto Alegre, Brazil; [2]Univ. of California Irvine, Irvine, CA

Abstract: MicroRNAs belong to a family of non-coding RNAs that regulates the expression of their target mRNAs post-transcriptionally, playing important roles in development, differentiation and autoimmunity. Recent identifying identified that miRNA-155 can transcriptionally control NFKB1 gene expression in brain tissues. In addition, immune re-programming has been related to early life stress (ELS) exposure, impacting neurodevelopment. Moreover, evidences suggest that ELS activates intracellular pathways of NF-kB. Here, we investigate the expression of NFKB1 and miRNA-155 in the hippocampus of female mice exposed to maternal separation (MS). We also investigate whether physical training during infancy and adolescence could impact the expression of these targets. Newborn BALB/c litters were randomly assigned to MS or Standard Rearing (SR - Controls) at birth and were exposed to daily 3-h maternal separation from post-natal day (PND) 2 to PND15. Another set of mice were assigned to exercise group that run 60 min/day in a treadmill for 3 weeks from PND31 to PND52. At PND52 mice were tested in the Open Field Test (OFT) for anxiety-like behavior and immediately sacrificed to hippocampus extraction and NFKB1 mRNA and miRNA-155 assessed by qPCR. We found that adolescents female mice exposed to ELS exhibited lower expression of

NFKB1 mRNA and miRNA-155 in hippocampus when compared to animals not exposed to MS. Furthermore, we found that anxiety-like behavior was positively correlated with NFKB1 expression. In addition, we conducted another experiment using a moderate-intensity physical exercise during infancy and adolescence as intervention in both MS and SR animals, but we did not find any effect on those parameters previously described. Taking into account the role of that NFKB1 and miRNA-155 in immune response, our results suggests MS can modify immune-related genes expression and this could not be reverted by physical training.

Disclosures: **L. Wearick-Silva:** None. **T.W. Viola:** None. **L.A. Azeredo:** None. **A. Centeno-Silva:** None. **T.W. Bredy:** None. **R. Grassi-Oliveira:** None.

Poster

248. Parental and Gestational Influences on Stress Vulnerability

Location: Hall A

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Topic: E.05. Stress and the Brain

Support: NIH grant MH078105

NIH grant MH078105-S1

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YNPRC base grant OD P51 OD011132

NIH grant MH086633

Title: Effects of maternal care on amygdala, prefrontal cortex, and hippocampal development in infant rhesus monkeys

Authors: C. L. FU[1], V. K. WING[1], D. L. PADMANABHAN[1], B. R. HOWELL[3], D. G. GUZMAN[1], J. R. GODFREY[1], Y. SHI[4], M. STYNER[4], *M. SANCHEZ[2,1];
[2]Yerkes Natl. Primate Res. Ctr., [1]Emory Univ., Atlanta, GA; [3]Univ. of Minnesota, Minneapolis, MN; [4]Univ. of North Carolina, Chapel Hill, NC

Abstract: Childhood maltreatment is a detrimental experience with neurobehavioral consequences. Using a naturalistic rhesus monkey model of infant maltreatment, we have reported long-term socioemotional alterations (e.g. low affiliation; high levels of aggression, anxiety, and fear), elevated levels of stress hormones (cortisol) and brain structural alterations during adolescence (effects on brain white matter (WM) using diffusion tensor imaging and

bigger amygdala volumes) linked to poor emotional regulation. In this study we examined the unfolding of brain structural effects during infancy and the juvenile period (6-12 months of age), focusing on the amygdala, prefrontal cortex (PFC), and hippocampus, due to their critical roles in emotional and stress regulation. We also examined whether exposure to maltreatment-induced elevated cortisol levels predicted structural changes. To disentangle the effects of experience from those of inheritance on brain development, we used a cross-fostering experimental design with random assignment of infants to control or maltreating foster mothers at birth. This resulted in 42 infants (20 Control -11 females, 9 males; 22 Maltreated -8 females, 14 males-) balanced by biological mother group, sex and social rank. Structural magnetic resonance imaging (MRI) scans were acquired at 6 and 12 months of age, and rhesus infant atlases and AutoSeg software were used to generate automatic tissue segmentations (i.e. WM, gray matter -GM, cerebrospinal fluid -CSF-). These segmentation outputs were manually adjusted by 3 raters blind to group. Total volumes of the hippocampus, amygdala, and PFC GM and WM were calculated and correlated with hair cortisol prenatal exposure (from hair shaved from the back of the neck at birth) and postnatal accumulation (from birth-6 months). Smaller amygdala and PFC GM volumes were detected in maltreated animals than in controls at 12 and 6 months of age, respectively. Significant correlations were also found between amygdala and PFC volumes and levels of the stress hormone cortisol during infancy and pregnancy, respectively. No significant maternal care effects were found in the hippocampus, though, but these could emerge later in life. Our findings suggest that the neurodevelopmental effects of maltreatment emerge during infancy, but the directionality, magnitude and timing differ among brain regions, potentially due to their different developmental trajectories.

Disclosures: C.L. Fu: None. **V.K. Wing:** None. **D.L. Padmanabhan:** None. **B.R. Howell:** None. **D.G. Guzman:** None. **J.R. Godfrey:** None. **Y. Shi:** None. **M. Styner:** None. **M. Sanchez:** None.

Poster

248. Parental and Gestational Influences on Stress Vulnerability

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 248.22/S15

Topic: E.05. Stress and the Brain

Support: NSERC

Princess Nourah bint Abdul Rahman University

Title: Impacts of maternal stress and high fat diet on hpa axis programming in offspring

Authors: *S. ABUAISH[1,2], C. M. W. LUM[1,2], P. O. MCGOWAN[1,2,3,4];
[1]Biol. Sci., Univ. of Toronto, Scarborough, ON, Canada; [2]Cell and Systems Biol., [3]Psychology, [4]Physiol., Univ. of Toronto, Toronto, ON, Canada

Abstract: The hypothalamic pituitary adrenal (HPA) axis is a neural system responsible for maintaining endocrine homeostasis, including during psychosocial stress. This system is known to be highly impacted by early life cues that later shapes the organism's response to stress, consequently modulating mental health. Early life environments, including maternal stress and overnutrition, have been independently reported to program the HPA axis, however there are no reports of their joint effect on the development of the HPA axis. This study investigated the effects of maternal stress using a chronic variable stress exposure during the last half of gestation and maternal overnutrition in the form of high fat diet (HFD) exposure during gestation and lactation on HPA-related behavioural and physiological measures in rat adult offspring. Preliminary results show that maternal chronic variable stress and maternal HFD induced an increase in HPA axis reactivity in dams during pregnancy, as indicated by increased corticosterone levels in response to a restraint challenge. In addition, HFD increased maternal behaviour, a known mediator of HPA programming in offspring. Adult offspring showed sexually dimorphic alterations in anxiety-like behaviour. Male offspring from HFD litters showed decreased anxiety-like behaviour on the elevated plus maze test regardless of their exposure to prenatal stress. Female offspring from prenatally stressed HFD litters showed increased anxiety-like behaviour in the open field test. This was comparable to the increased anxiety-like behaviour exhibited by female offspring from either maternal stress or HFD litters. In addition, anxiety-like behaviour on the elevated plus maze test and corticosterone levels in response to a restraint challenge were significantly correlated with the amount of maternal care provided to the male offspring. These behavioural changes could be mediated through differential gene expression and underlying epigenetic modification of HPA-related genes in limbic regions, which will be examined in ongoing studies.

Disclosures: S. Abuaish: None. **C.M.W. Lum:** None. **P.O. McGowan:** None.

Poster

248. Parental and Gestational Influences on Stress Vulnerability

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 248.23/S16

Topic: F.02. Animal Cognition and Behavior

Title: Impact of Early Life Stress during Stress Hyporesponsive period (SHRP) attenuates both fear retention and extinction in rats

Authors: *P. K. MISHRA, JR, L. T. RAO, B. M. KUTTY;
NIMHANS, Bangalore, India

Abstract: Purpose: The long-term impact of 10-days of maternal separation stress on the fear memory and fear extinction was evaluated in male Wistar rats. **Methods:** Early maternal separation and isolation stress (EMS) were carried out in rat pups during the Stress Hypo Responsive Period (SHRP) for 6 hours daily for 10 days, while effects on fear retention and extinction were ensured 2 months later. 2 months after EMS, rats were exposed to cued fear conditioning session. 24 hours, 48 hours and 72 hours after fear conditioning, both normal control and EMS groups of rats were received extinction training sessions respectively. Percentage freezing was assessed during all stages of fear retention and fear extinction training including session of retention of fear extinction. **Results:** The retention of fear memory was stronger in EMS rats than controls. 10 days after extinction training session, EMS rats showed increased freezing to the conditioned stimulus than controls. **Conclusions:** Our data suggested that EMS causes a long-term behavioural disposition that is activated by acute stressors like fear conditioning. **Affiliation:** CSIR, ICMR and NIMHANS, Government of India for the financial support. **Key Words:** Maternal separation stress, SHRP, Fear conditioning, Extinction

Disclosures: P.K. Mishra: None. **L.T. Rao:** None. **B.M. Kutty:** None.

Poster

248. Parental and Gestational Influences on Stress Vulnerability

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 248.24/S17

Topic: E.05. Stress and the Brain

Support: Mead Johnson Nutrition Collaborative Research Grant

Title: A blend of dietary prebiotics and the probiotic LGG modulate behavioral and cognitive responses to maternal separation stress

Authors: R. V. WAWORUNTU[1], K.-A. M. NEUFELD[2], *B. M. BERG[1], S. M. O'MAHONY[3], T. G. DINAN[4], J. F. CRYAN[3];
[1]Mead Johnson Nutr., Evansville, IN; [2]Alimentary Pharmabiotic Ctr., [3]Dept. of Anat. and Neurosci., [4]Dept. of Psychiatry, Univ. Col. Cork, Cork, Ireland

Abstract: Maternal separation (MS) of rat pups is a robust and reliable model of early life stressed-induced alterations in behavior. Changes in the gut microbiota of MS rat pups have also been found, suggesting gut-brain axis signaling to be an important mechanism of action. Since certain dietary factors are known to impact composition and diversity of the gut microbiota, this study assessed the impact of consuming prebiotics polydextrose (PDX) and galactooligosaccharide (GOS) with or without the probiotic Lactobacillus rhamnosus GG (LGG) during early development on cognition, social- and anxiety-related behaviors in rodents. Rats were separated from their mothers between postnatal day (PD) 2 to 12. Both MS and non-

separated (NS) rats (N=5-9 each) were fed control or prebiotic diet (7 g/kg PDX-GOS) with or without LGG (108 cfu/ml) in drinking water from PD 21 throughout behavioral testing to PD 100. Overall there were no differences in body weight or food intake across diets. However, the open field test revealed that MS rats traveled a shorter distance with reduced velocity than NS rats ($p<0.05$). These MS effects were ameliorated by prebiotic feeding ($p<0.01$) and LGG ($p<0.01$), but interestingly not when combined. MS rats displayed deficits in spatial memory in the Morris water maze ($p<0.05$) while rats fed prebiotic plus LGG showed a reversal of this impairment ($p=0.05$). In conclusion, these results demonstrate that both prebiotics and LGG ameliorate early life stress-induced changes in adult behavior and when combined can improve memory performance.

Disclosures: **R.V. Waworuntu:** A. Employment/Salary (full or part-time):; Mead Johnson Nutrition. **K.M. Neufeld:** 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.; Mead Johnson Nutrition. **B.M. Berg:** A. Employment/Salary (full or part-time):; Mead Johnson Nutrition. **S.M. O'Mahony:** 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.; Mead Johnson Nutrition. **T.G. Dinan:** 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.; Mead Johnson Nutrition. **J.F. Cryan:** 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.; Mead Johnson Nutrition.

Poster

248. Parental and Gestational Influences on Stress Vulnerability

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 248.25/S18

Topic: E.05. Stress and the Brain

Support: VIEP-BUAP SAL-2015

CONACYT grant No. 243333 to JRE and CC

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CAUP is fellowship of CONACYT No. 213985

Title: Cross-fostered high-yawning dams increased stress responses in the outbred Sprague-Dawley rats: a base of atypical maternal care

Authors: *C. URIBE[1], C. CORTES[1], J. R. EGUIBAR[1,2];
[1]Inst. De Fisiología BUAP, Puebla, Mexico; [2]Res. Office-VIEP, Benemérita Univ. Autónoma De Puebla, Puebla, Mexico

Abstract: Maternal care in rats determined stress responses in adulthood. So, lower maternal licking-grooming (LG) and arched-back nursing (ABN), increase the responses in the hypothalamic-pituitary-adrenal (HPA) axis after middle-stress maneuvers. Anxiety-like responses also dependent on the maternal care received. Pups that receive less maternal care showed epigenetic changes that can be transfer from the mothers to daughters. In our laboratory, we selectively bred a high-yawning (HY) subline with a mean of 20 yawns/h that showed lower nest quality, lower latency to retrieve the pups but showed atypical retrieving and re-retrieving. The aim of this study was to analyze the effect of maternal care on stress responses using the cross-fostering technique. The subjects (Ss) were maintained under standard conditions. In the first 12 h after delivery we adjusted the litters to eight pups and culled by sex and exchange all the litter between HY and Sprague-Dawley (SD) dams, and in-fostering used as a control group. At 90 of age, all Ss were tested in light-dark box and in the open-field arena. Twenty min later we obtained blood in a tube by decapitation. Plasma was recovered by centrifugation (3500 r.p.m.) during 10 min. Plasma was separate using Pasteur pipettes, stored in freezer at -20oC until ELISA assays were done. Corticosterone and adrenocorticotropic hormones were measured using ELISA kits (Corticosterone ELISA Kit Catalog No. ADI-900-097, Enzo®; Adrenocorticotropic Hormone ELISA Catalog No. 21-ACTHU-E01 ALPCO®). Results were summarized in the table 1. Results show that HY subline showed less anxiety levels, but SD offspring raised by HY dams showed higher anxiety levels and more HPA activation after open field test. Therefore, HY dams are likely model of maternal maltreatment because mimics human inadequate maternal care that is elevated anxiety responses and higher responses in the HPA axis.

Table 1. Effect of cross-fostering between HY and SD dams in anxiety-like behavior and HPA axis

.	HY offspring		SD offspring	
.	HY dams	SD dams	HY dams	SD dams
Open field Test				
Time in corners (s)	405 ± 16a	428 ± 23a	509 ± 14b	457 ± 9
Time in center (s)	12.5 ± 3.6a	7.7 ± 3.2a	0.6 ± 0.4	3.4 ± 1.5
Traveled distance (m)	20.3 ± 1.2a	13.7 ± 2.2a	20.3 ± 4.2	24.7 ± 2.5
ACTH (pg/mL)	18.4 ± 7.1	45.3 ± 26.6	34.3 ± 21.8	41.0 ± 16.8
Corticosterone (µg/dL)	5.9 ± 0.6a	5.4 ± 0.6a	17.4 ± 2.4b	10.2 ± 1.8

Light-dark box Test				
Time in light (s)	40.9 ± 8.5a	27.9 ± 6.1a	6.8 ± 1.1b	20.8 ± 3.6
Number of transitions	2.2 ± 0.8	1.8 ± 0.5	1.0 ± 0.0	1.4 ± 0.4
ACTH (pg/mL)	12.0 ± 6.4	26.0 ± 17.6	25.4 ± 12.6	74.4 ± 23.8
Corticosterone (µg/dL)	8.6 ± 1.4	6.5 ± 1.5	9.3 ± 1.6	12.4 ± 1.9
Basal levels				
ACTH (pg/mL)	29.3 ± 14.3	24.3 ± 12.3	26.0 ± 15.0	24.5 ± 9.3
Corticosterone (µg/dL)	4.3 ± 1.2	6.8 ± 0.8	8.6 ± 2.0	7.3 ± 1.5
a (P				

Disclosures: **C. Uribe:** None. **C. Cortes:** None. **J.R. Eguibar:** None.

Poster

248. Parental and Gestational Influences on Stress Vulnerability

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 248.26/S19

Topic: F.02. Animal Cognition and Behavior

Title: Effect of VNS-paired extinction of conditioned fear in a VPA-induced animal model of autism

Authors: ***A. ALVAREZ-DIEPPA**, K. GRIFFIN, S. CAVALIER, P. SEKAR, C. MCINTYRE; Univ. of Texas At Dallas, Richardson, TX

Abstract: Administration of vagus nerve stimulation (VNS) during trials of fear extinction reduces expression of conditioned fear faster than extinction training alone. Our preliminary studies show that VNS pairing with extinction training affects expression of markers of synaptic plasticity in the basolateral complex of the amygdala (BLA), an area of the brain that is important for extinction learning, leading to an increase in P-CaMKII and GluN2B expression and a decrease in Arc protein levels. Although we've shown that VNS can enhance extinction of fear and affect plasticity in the BLA of healthy rats, the ability of VNS to rescue impaired extinction learning is yet to be tested. Rats prenatally exposed to VPA on day 12.5 of gestation

(VPA-exposed rats) are used as an animal model of autism, and they exhibit impaired extinction of conditioned fear. This study was designed to test the potential of VNS to reverse extinction impairments in the VPA-exposed rat model of autism. VPA-exposed and saline-control rats were subjected to auditory fear conditioning followed 24 hours later by extinction training that was paired with either VNS or Sham stimulation. During a conditioned fear response test on day 3, VPA-exposed rats that received VNS during extinction training showed freezing levels that were similar to those of saline-control rats, and significantly lower than those of VPA-exposed rats that received sham stimulation during extinction training. Results suggest that VNS-paired extinction can rescue impaired extinction learning in the VPA-exposed animal model of autism.

Disclosures: A. Alvarez-Dieppa: None. **K. Griffin:** None. **S. Cavalier:** None. **P. Sekar:** None. **C. McIntyre:** None.

Poster

249. Food Intake and Energy Balance: Integration of Peripheral Signals I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 249.01/S20

Topic: E.07. Food Intake and Energy Balance

Support: F30CA177170

T32GM007753

Harvard/MIT Joint Program in Neuroscience

Title: *In vivo* imaging of vagal afferent neurons to decode internal senses

Authors: *E. K. WILLIAMS, D. E. STROCHLIC, R. CHANG, B. D. UMANS, S. LIBERLES; Harvard Med. Sch., Boston, MA

Abstract: The vagus nerve controls diverse physiological processes, including feeding behavior, respiration, blood pressure, heart rate, nausea, and cough. However, little is known about the repertoire of sensory mechanisms residing in vagal sensory neurons, or the diversity of vagal cell types that transmit signals from the periphery to the brain. We developed an *in vivo* imaging technique involving genetically encoded reporters of neural activity that enables a massively parallel analysis of individual vagal sensory neuron responses. We found discrete vagal sensory neuron subsets responsive to different physiological stimuli. Furthermore, using genetic tools, we have identified molecular marks for subsets preferentially responsive to particular stimuli. These molecularly defined subsets exhibit specific peripheral and central projection patterns, and control specific physiological functions. These studies provide the first direct insights into single-neuron receptive fields, and the molecular identities of functional subsets in this key body-

to-brain sensory system. Determining response properties of individual neuron types is a critical first step towards understanding sensory transduction mechanisms.

Disclosures: **E.K. Williams:** None. **D.E. Strochlic:** None. **R. Chang:** None. **B.D. Umans:** None. **S. Liberles:** None.

Poster

249. Food Intake and Energy Balance: Integration of Peripheral Signals I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 249.02/T1

Topic: E.07. Food Intake and Energy Balance

Support: NIH R037 DK35254 to TJB

Title: Lipolysis activates sensory nerves in white fat

Authors: ***J. T. GARRETSON**, V. RYU, L. A. SZYMANSKI, T. J. BARTNESS;
Biol., Georgia State Univ., Atlanta, GA

Abstract: Background: White adipose tissue (WAT) lipolysis is principally initiated by central signals via the sympathetic nervous system (SNS). Sensory circuits innervating fat multisynaptically connect into central feedback loops back toward WAT and elsewhere throughout the CNS in areas dense with metabolic function (e.g. nucleus of the solitary tract, rostral ventral lateral medulla, paraventricular hypothalamus) yet the physiological role of WAT afferents in metabolism is still unknown; therefore, we tested if lipolysis triggers increases in WAT sensory nerve activity. Method: Siberian hamsters were anesthetized, nerves innervating left and right inguinal WAT (IWAT) were resected, cut, and decentralized distal trunks attached to electrodes for afferent nerve electrophysiological multiunit measures. CL316,243, a specific β3 receptor agonist, was unilaterally infused into IWAT simultaneously with saline into the contralateral fat pad to test if β3 agonism-induced lipolysis increases IWAT afferent nerve activity using each animal as its own control. Glycerol (0.13mM) and eicosopentanoic acid (EPA, 0.29mM), products highly released during natural lipolysis, also were tested to answer which specifically affect IWAT afferents. Fat pad-specific spike frequency was assessed and analyzed as percent change from baseline activity over time compared with saline vehicle-injected contralateral recording. Results: CL316,243 (a dose and injection method confirmed to cause lipolysis as indicated by increased WAT phosphorylated hormone-sensitive lipase (pHSL)) trigged rapid (<10 min) increases in IWAT afferent nerve multiunit activity nearly 2-fold versus the saline vehicle control in the contralateral IWAT. Glycerol and EPA caused similar patterns of activation. Conclusions: These results indicate that β3-induced lipolysis and products of this process are sensed by IWAT sensory nerves rapidly. Afferent transduction of lipolysis likely

feeds back though spinal and central circuits to affect SNS drive. Future studies will elucidate the physiological role of this activation.

Disclosures: J.T. Garretson: None. **V. Ryu:** None. **L.A. Szymanski:** None. **T.J. Bartness:** None.

Poster

249. Food Intake and Energy Balance: Integration of Peripheral Signals I

Location: Hall A

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Topic: E.07. Food Intake and Energy Balance

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CONACyT 24784-M

CONACYT 118673

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PAPIIT IN216907

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Title: Differential distribution of adiponectin receptors 1 and 2 in olfactory bulb and hippocampus in the rat and insulin receptor induction by adiponectin

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Abstract: Introduction: Adiponectin (APN) is an adipocyte-derived hormone that has peripheral beneficial effects as anti-inflammatory, cardioprotective, anti-atherogenic, also as metabolism modulator and insulin pathway sensitizer. Even though there is evidence of the presence of adiponectin and its receptors in the brain, little is known about its influence in Central Nervous System. Furthermore, brain is an insulin-sensitive organ with wide expression of insulin receptor (InsR) particularly in areas related to cognitive processing and feeding regulation, however there are no reports about the induction of this receptor by means of APN. Objective: The aims of this work were to describe the presence and distribution of Adiponectin Receptor 1 and 2 (AdipoR1 and AdipoR2) in the olfactory bulb (OB) and hippocampus (HIPP) of rats and evaluate the effects of APN administration in brain over InsR expression in these two brain structures. Methodology: Intact male Wistar rats weighting 250-300 grs were used for immunofluorescence

and PCR assay. For Western Blot, animals were injected in OB or HIPP with 1µg of APN (1µg/µl); control animals followed the same procedure receiving only isotonic saline solution. OB and HIPP were dissected, homogenized and supernatants collected. 60 micrograms of protein was electrophoresed for InsR protein content. Results: Our data have showed presence of AdipoR1 and AdipoR2 in periglomerular, mitral and granulose cell layers in OB; also in pyramidal cells of CA1 and CA3 and granular cells of dentate gyrus in HIPP. Moreover, a significant increase in the amount of InsR in the OB of APN hippocampal treated group versus control was observed. Histological analysis shown that there are more InsR positive cells in OB and HIPP in animals treated with APN compared with control. Conclusion: Our results indicate that AdipoR1 and AdipoR2 are expressed in different cellular groups in OB as well as in HIPP in rat brain. These receptors correspond with those described for skeletal muscle and liver respectively. In addition, we also showed that APN regulates InsR content mainly in the OB than in HIPP.

Disclosures: A. Miranda-Martinez: None. **R. Guevara-Guzmán:** None.

Poster

249. Food Intake and Energy Balance: Integration of Peripheral Signals I

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Topic: E.07. Food Intake and Energy Balance

Support: KNAW

CSC

Title: Effects of long-term estrogen replacement in the ventromedial nucleus of the hypothalamus on ovariectomy-induced changes in body weight, fat distribution and bone mass in rats

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Abstract: Estrogens play an essential role in energy and bone metabolism. In rodents, ovariectomy (OVX) causes increased body adiposity and bone loss which is reversible with systemic estradiol (E2) treatment. Recent studies have indicated that within the brain the hypothalamus, in particular its ventromedial nucleus (VMH) is involved in the effect of E2 on energy homeostasis. Moreover, the VMH regulates bone metabolism via the sympathetic

nervous system. In our study we aimed to determine in rats: 1) the effect of chronic intracerebroventricular (icv) administration of E2 on white adipose tissue (WAT) metabolism after OVX, and 2) the effect of E2 in the VMH on WAT metabolism, calorie ingestion, energy expenditure and bone mass. Icv E2 for 4 weeks decreased body weight and reduced visceral but not subcutaneous fat, confirming that central E2 administration affects body fat distribution. Next we administered E2 into the VMH for 3 hours by bilateral microdialysis. E2 in the VMH increased LPL and HSL gene expression in gonadal WAT, indicating a role for the VMH in peripheral WAT metabolism. Finally, we administered E2 into the VMH of OVX rats for 4 weeks by using slow-releasing E2 containing beeswax pellets. Food and water intake during the light period were reduced by E2 administration in the VMH. Metabolic cages data showed a lower respiratory exchange rate (RER) in the E2 group during the light period indicating increased fat oxidation in the E2 treated group. Although, total food intake was not significantly different, food efficiency (gram body weight gain per gram chow) was decreased in the E2 VMH group compared to the control group. The effect of E2 administration on bone metabolism was determined 8 weeks after E2 containing pellets were implanted in the VMH. Femur bone mass and structure was evaluated by high resolution micro CT scanning. Both trabecular bone number and bone thickness were decreased after OVX. However, E2 administration in the VMH did not change total or trabecular bone number. In conclusion, we showed that chronic E2 administration in the VMH of ovariectomized rats affects body fat distribution and energy balance, but does not prevent bone loss.

Disclosures: **Z. Zhang:** None. **A. Kalsbeek:** None. **E. Fliers:** None. **A. Boelen:** None. **J. Liu:** None. **Y. Su:** None. **E. Foppen:** None. **B.C.J. van der Eerden:** None. **N. Bravenboer:** None. **P.H. Bisschop:** None.

Poster

249. Food Intake and Energy Balance: Integration of Peripheral Signals I

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Support: Productos Medix ZIP0268

Consejo Nacional de Ciencia y Tecnologia de Mexico Grants 179484

Title: The combination phentermine + 5hydroxytryptophan/carbidopa showed a synergistic effect on food intake and weight loss in rats: a new drug combination therapy for obesity

Authors: ***C. PEREZ DIAZ**[1], B. KALYANASUNDAR[1], M. G. MORENO[1], A. LUNA[1], S. A. SIMON[2], R. GUTIERREZ[1];

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Abstract: Obesity is a major public health problem. Although the main treatment for obesity should be diet and exercise, for some people these activities are frequently supplemented with the use of appetite suppressants. Recently Hendricks et. al. 2009 reported that physicians frequently prescribed a new drug combination of appetite suppressants for the treatment of overweight: phentermine(PHEN) plus 5hydroxytriptophan/carbidopa(5HTP/CB). 5HTP is a precursor of serotonin, carbidopa is a peripheral L-aromatic decarboxylase inhibitor, leading to more 5HTP available to enter the brain; and PHEN acts inhibiting recapture of dopamine, norepinephrine and serotonin. Despite that this seems to be a common practice, the use of this combination has not extensively study and currently it is not authorized by the FDA. Furthermore, it is not known if this combination is synergistic at the behavioral level(body weight-loss, food suppression and locomotor activity) and modulating single-unit activity in the nucleus accumbens shell(NAcS). The main goal is to determine the efficacy of this combination at the behavioral and electrophysiological level in rats. To address this issued, we used i.p injections of PHEN and 5HTP/CB either alone or in combination for 7 days daily treatment. For PHEN and 5HTP/CB the ED50 for weight loss was 15 and 31 mg/kg, respectively. We then combined the ED50 of 5HTP31 and CB75mg/Kg against the ED25, ED50 and ED75 of PHEN(10, 15 and 20 mg/kg, respectively). We found that the combination significantly produced a larger increase in weight-loss, strongly decreased food intake and a reduction in locomotor side effects relative to its compounds by themselves. This data demonstrate at the behavioral level that the combination PHEN+5HTP/CB are synergistic. Now we record single-unit activity of the NAcS in order to shed light of the neuronal correlates of this synergism(only CB75+5HTP31+PHEN15). We found that PHEN15 alone exerts a strong inhibitory imbalance towards inhibition(n=74, inh 62%: act 9%), whereas PHEN15 in the presence of 5HTP/CB induced a quite similar inhibition in 57% inhibited neurons (out of 140) and 7% was activated. These data indicates that the combination do not interfere with the ability of PHEN15 to inhibits the NAcS activity. Our pilot studies indicated that PHEN+5HTP/CB combination exhibits synergism of its pharmacological effects and reduced their adverse effects. Hence this preclinical evidence demonstrated for the first time a synergistic combination with its possible neuronal mechanism in the brain reward region(NAcS) supporting the use of this combination as future therapy for the treatment of obesity.

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Poster

249. Food Intake and Energy Balance: Integration of Peripheral Signals I

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Program#/Poster#: 249.06/T5

Topic: E.07. Food Intake and Energy Balance

Title: Genetic ablation and pharmacological blockade of FKBP51 in mice reduces obesity and metabolic dysfunction

Authors: *G. BALSEVICH[1], N. C. GASSEN[1], A. HÄUSL[1], C. W. MEYER[2], X. FENG[1], C. DOURNES[1], A. URIBE[1], M. THEODOROPOULOU[1], M. PAEZ-PEREDA[1], T. REIN[1], F. HAUSCH[1], A. CHEN[1], M. H. TSCHÖP[2], M. V. SCHMIDT[1];
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Abstract: FKBP51 is an immunophilin protein best known as a regulator of the glucocorticoid receptor and consequently the physiological stress response. Additional functions of FKBP51 include the regulation of AKT signaling, which represents a central node within the insulin signaling pathway and a key pathway found to be deregulated in type 2 diabetes (T2D) and obesity. In mice, genetic ablation of FKBP51 furthermore results in a reduced body weight phenotype. Nevertheless, whether FKBP51 plays a critical role in whole body energy and glucose homeostasis remains to be elucidated. We aimed to characterize the role of FKBP51 in energy and glucose homeostasis using FKBP51 knockout (51KO) mice. We found that 51KO mice were protected from high fat diet-induced weight gain and glucose intolerance. In addition 51KO mice showed a prolonged response to insulin examined in an insulin tolerance test. At a molecular level, we found that insulin signaling was enhanced specifically within skeletal muscle of 51KO mice. In line with these findings, glucose uptake was significantly increased by FKBP5 knockdown in differentiated myotubes. In order to assess whether pharmacological blockade of FKBP51 may serve as novel therapeutic possibility, we treated mice with a highly selective FKBP51 antagonist (SAFit2) for either 10 or 30 days. We report that systemic treatment with SAFit2 lowered body weight gain and glucose tolerance. Finally, in C2C12 myotubes, SAFit2 treatment increased glucose uptake. These data directly implicate FKBP51 in metabolic regulation and provide evidence for the therapeutic potential of FKBP51 in the treatment of obesity and T2D.

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Poster

249. Food Intake and Energy Balance: Integration of Peripheral Signals I

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Topic: E.07. Food Intake and Energy Balance

Support: Swiss National Science Foundation (SNSF)

Forschungskredit UZH

Title: Central GLP-1 signaling contributes to cancer anorexia and body weight loss in hepatoma tumor bearing rats

Authors: *T. BORNER, T. A. LUTZ, T. RIEDIGER;
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Abstract: The cancer-anorexia-cachexia syndrome (CACS) negatively affects therapy success and quality of life in cancer patients. The area postrema (AP) and the nucleus of the solitary tract (NTS) are involved in the control of food intake and implicated in the mediation of emesis and nausea. Neurons in the AP/NTS are activated in tumor-bearing (TB) animals. In our recent study, a specific surgical lesion of the AP attenuated anorexia and body weight loss induced by tumor growth in rats. While this clearly supported a role of the AP in the mediation of CACS, the exact mechanism still needs to be elucidated. In the brainstem, the neuropeptide glucagon-like peptide-1 (GLP-1) mediates food aversions and anorexia induced by toxins and endotoxins. Therefore, we hypothesized that GLP-1 signaling could also play a role in tumor-dependent anorexia. Using a rat hepatoma tumor model, we investigated if a pharmacological blockade of brainstem GLP-1 receptors (GLP-1R) ameliorates cancer anorexia. In addition, we tested if tumor growth was paralleled by a conditioned food aversion. Chronic fourth ventricular administration of the GLP-1R antagonist exendin-9 (100 µg/day, $n = 8$) partially reversed the anorexia in TB rats leading to a significantly higher mean daily food intake compared to vehicle-treated TB animals (16.3 ± 0.4 vs. 13.9 ± 0.6 g, $n = 8$, $p < 0.01$). Furthermore, exendin-9 treatment attenuated tumor-induced body weight loss in these animals (-16 ± 2.4 vs. -27.4 ± 4.7 g, $p < 0.05$). In non-tumor-bearing (NTB) rats, the same exendin-9 treatment did not affect food intake or body weight. Hence, the anti-anorectic effect of GLP-1R blockade in TB animals appears to be unrelated to alterations of baseline food intake. To examine the presence of tumor-induced food aversion, a two diet choice paradigm was used. Food preference for two flavored diets was tested before tumor induction. During tumor growth, animals received their preferred diet for 8 days; non-tumor-bearing rats were included as controls. A second preference test was conducted for 5 consecutive days during the anorectic phase. While diet preference was preserved in NTB rats ($n = 7$), the preference for the previously preferred diet decreased from 83 to 8% in TB rats ($n = 8$, $p < 0.001$). The conversion of diet preference into diet avoidance in hepatoma tumor-bearing rats suggests the development of a tumor-induced food aversion. This study provides first evidence for a role of central GLP-1 signaling in the mediation of cancer-anorexia and body weight loss. Tumor-induced food aversion might be a contributing factor to cancer anorexia in this tumor model. GLP-1R antagonists may be therapeutically useful for the treatment of CACS.

Disclosures: **T. Borner:** None. **T.A. Lutz:** None. **T. Riediger:** None.

Poster

249. Food Intake and Energy Balance: Integration of Peripheral Signals I

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Topic: E.07. Food Intake and Energy Balance

Support: NIH Grant 1R01DC013904-01A1

Title: High fat diet results in activation of microglia and reorganization of the nucleus of the solitary tract as well as a change in the intestinal microbiota

Authors: *K. CZAJA[1], A. VAUGHN[2], E. M. COOPER[2], P. M. DILORENZO[3], L. J. O'LOUGHLIN[2], M. E. KONKEL[2], J. H. PETERS[2];
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Abstract: In the current study, we studied whether high fat diet results in microglia activation and reorganization of nucleus of the solitary tract (NTS) and alters gut microbiota in Sprague Dawley rats. One group of rats was fed regular rodent diet (RD; Teklad F6; 3.1 kcal/g; 6.4 % fat) for the entire experiment and a second group received RD for two weeks followed by a high fat diet (HFD; Open Source D12492; 5.24 kcal/g; 34.9 % fat) for four weeks. Body weight and food intake were monitored three times a week. Fresh fecal pellets containing the intestinal microbiota were collected from all rats before introducing the HFD at day 7 and again at day 28. Body fat composition was determined by Dual-Energy X-ray Absorptiometry (DEXA) scans before and after introduction of the HFD. At the completion of the study (animals demonstrated diet-induced obesity), rats were perfused (4% paraformaldehyde) and the hindbrains were collected, sectioned, and stained using standard immunofluorescence methods. Additionally, sections of the cecum, duodenum, and jejunum were collected for microbiome analysis. Microglia activation was determined using an antibody against Isolectin 4 to label vagal afferents and by quantifying changes in the density of fluorescent staining with an antibody against ionizing calcium adapter binding molecule 1 (Iba1). We found that HFD-induced obesity triggered a shift in the intestinal microbiota. Specifically, we observed an increase in Streptococcus mitis in the distal jejunum and an increase in Proteus mirabilis, Lactobacillus animalis, and Enterococcus faecalis in the caecum, as determined by 16S ribosomal sequencing. In addition, HFD-induced obesity resulted in withdrawal of vagal afferents as well as microglia activation in the intermediate NTS of rats. Neural damage was also observed when neurons were treated *ex vivo* with each of the bacteria, as judged by fluorescence staining with Beta III-tubulin antibody. The alteration in intestinal microbiota, degeneration of vagal neurons innervating the gut, and inflammation with reorganization of the hindbrain feeding centers in response to diet-induced obesity is consistent with a previous report of neuroanatomical alterations in the gut-brain communication following damage to the peripheral vagal nerve. This model system provides a foundation to investigate the relationship between microbiota and neural damage in animals with diet-induced obesity and sets

the stage to address whether specific bacterial species contribute to the disruption of vagal signaling and obesity.

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Poster

249. Food Intake and Energy Balance: Integration of Peripheral Signals I

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Topic: E.07. Food Intake and Energy Balance

Title: Effects of ethanol administration on energy homeostasis in male Long Evans rats

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Abstract: Ethyl alcohol is an energy-containing (7 kcal/g) beverage widely consumed for its relaxing and mood elating effects. In developed countries, alcohol-derived energy comprises 10-15% of total energy intake by adults. Many epidemiological and laboratory studies with humans have often reported opposing findings regarding the relationship between alcohol and energy balance. Reports from rodent studies are also inconclusive. The inconsistencies necessitate well-controlled animal studies. Here, we hypothesized that the effects of alcohol on food intake and body weight (BW) are dose-dependent. Male *Long Evans* rats were first habituated to i.p. injections of saline every other day for one week (3 injections total). All injections occurred 2hrs before light onset. Then they received either ethanol (EtOH) or saline (SAL) injections. The SAL group received only saline injections at the same time when the EtOH rats were injected with ethanol. The EtOH group received the dose of 1 g/Kg every other day for 2 weeks (6 injections total), followed by the dose of 3 g/Kg administered once per week for 4 weeks (4 injections total). Initially, rats had *ad lib* access to standard laboratory chow and tap water, and subsequently a 45% high fat (HF) diet was introduced on the second week of 1 g/Kg EtOH injections. The data indicated that SAL and 1 g/Kg EtOH injections had no effect on food and water intake. When both chow and HF diets were available, the SAL and EtOH treated rats all preferred the HF diet and did not differ in their diet preference. However, EtOH at the dose of 3 g/Kg significantly suppressed food and water intake up to 85% and 36%, respectively ($P<0.001$). Food intake suppression lasted for at least 72h post-injection. As a result of reduction in food intake, weight gain in the EtOH group was suppressed, and thus the BW of EtOH rats (382.5±6.7 g) was significantly lower than that of the SAL controls (511.7±17.3 g) after the last injection ($P<0.05$). The lower BW of the EtOH group was sustained even after two weeks without EtOH treatment (SAL 503.8±17.0 g vs EtOH 413.1±8.4 g; $P<0.05$). At the end of the experiment,

visceral fat was dissected and weighed. Compared to the SAL controls (4.3±0.5%), the EtOH rats (1.7±0.3%) had less percent visceral fat composition (P<0.05). Overall, these results indicate that 3 but not 1 g/Kg EtOH suppress appetite and weight gain and support the hypothesis that the effects of ethanol on energy homeostasis are dose dependent.

Disclosures: **N. Nelson:** None. **N. Liang:** None.

Poster

249. Food Intake and Energy Balance: Integration of Peripheral Signals I

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Title: Effects of Roux-en-Y gastric bypass surgery on lingual fatty acid receptor expression in high fat diet-induced obese female rats

Authors: ***A. HAJNAL**[1], A. M. ROGERS[2], S. D. PRIMEAUX[3,4];
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Abstract: The fatty acid receptor, CD36, on the circumvallate papillae of the tongue has been implicated in the preference for and intake of dietary fat. CD36 knock-out mice exhibit a reduced preference for linoleic acid and selective decreases in CD36 expression on the circumvallate papillae attenuates the preference for linoleic acid in rats (Chen et al., 2013; Sclafani et al., 2007). Lingual CD36 expression is hypothesized to alter fat intake and fat preference, thereby leading to changes in body weight. Roux-en-Y gastric bypass (RYGB) surgery has been successfully used as a treatment for obesity. Following gastric bypass surgery, the preference for and sensitivity to dietary fat, as well as other tastants, is altered. The goal of the current experiment was to assess fatty acid receptor mRNA levels on the circumvallate papillae following RYGB in female rats. All rats undergoing surgery were fed a high fat diet (HFD, 60%kcal of fat) for 14 weeks prior to surgery. Five months following RYGB or sham surgery, rats were sacrificed following an overnight fast. A control group fed a standard chow diet was included. CD36 mRNA levels were assessed by Real Time PCR. Sham-operated rats consuming the HFD weighed more than RGYB rats fed HFD and control rats, at the time of sacrifice. CD36 mRNA levels were increased by the consumption of the HFD in the sham-operated rats

compared to the control rats. RYGB attenuated the HFD-induced increase in CD36 mRNA expression on the circumvallate papillae. Decreased expression of CD36 on the circumvallate papillae following RYGB suggests that this mechanism may play a role in the reduced preference for dietary fat in these individuals. Analyses of the fatty acid receptors, GPR40 and GPR120 on the circumvallate papillae are underway. Supported by NIH Grants DK080899 and DC000240 to AH and LSUHSC to SDP.

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Poster

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Topic: E.07. Food Intake and Energy Balance

Support: 243335

Title: Obese rats induced by high-sucrose diet promote a stage of hypoalgesia: Antinociceptive effects of ketoprofen

Authors: *O. A. JARAMILLO-MORALES[1], J. V. ESPINOSA-JUAREZ[1], J. N. CORONA-RAMOS[2], F. J. LOPEZ-MUNOZ[1];
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Abstract: BACKGROUND.Obesity is a risk factor associated with alterations in the pain perception, however, it is unclear what mechanisms underlie this association. NSAIDs are the most commonly used drugs in the treatment of pain, for example, ketoprofen. However, there are no studies evaluating the analgesic response to ketoprofen in patients obese. The aim of this study was to analyze the nociceptive pain in Wistar rats obese hypoestrogenic with high sucrose diet and to compare the antinociceptive response using ketoprofen. METHODS.The hypoestrogenism was induced by bilateral ovariectomy. Animals received hypercaloric diet (30% sucrose in drinking water) or water ad libitum for 17 weeks. At the end of treatment were measured thermal nociception, body weight, tolerance to oral glucose, weight of abdominal fat and it was evaluated differents dosis of ketoprofen (10, 31.6 or 100 mg/Kg p.o) in both groups. The nociception was assessed using the “Plantar test” method. RESULTS.The hypoestrogenic Wistar obese rats with high sucrose diet had significantly higher body weight and abdominal fat weight than the control groups. Also, it was observed significantly increased in thermal latency compared to their control group. There were no differences in basal blood glucose levels, however, showed altered oral glucose tolerance. The administration of ketoprofen showed dependent effect of dose in both groups, nevertheless, the antinociceptive efficacy was not different but potency showed statistical differences. CONCLUSIONS. Our data suggests that

obesity may contribute stage of hypoalgesia, as well as changes in the antinociceptive potency of ketoprofen in Wistar rats obese hypoestrogenic.

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Poster

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Title: A role for vitamin D in diet-induced obesity and dopamine-related behaviors

Authors: *J. R. TRINKO[1], B. B. LAND[1], W. B. SOLECKI[1], R. J. WICKHAM[1], L. A. TELLEZ[1,2], J. G. MALDONADO-AVILES[1], I. E. DE ARAUJO[1,2], N. A. ADDY[1], R. J. DILEONE[1];
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Abstract: Human obesity rates have increased over the past two decades. Interestingly, vitamin D3 deficiency rates have also increased within a similar timeframe and clinical research has identified an inverse relationship between circulating vitamin D3 levels and body mass index (BMI). However, a causative role for this deficiency in the development of obesity has not been explored. Using mice, we assessed the effect of this deficiency on diet-induced obesity (DIO). A high-fat diet was modified to have reduced dietary vitamin D3 (HF-D, containing 9-11% of the control HF vitamin D3). At day 50 of ad libitum feeding, HF-D mice were found to have significantly reduced circulating levels of vitamin D3. From days 50 to 100, the HF-D mice began to display a persistent gain in body weight (BW), demonstrating that deficiency can contribute to obesity, as well as increased food intake (FI). Alternatively, we explored the effects of exogenous treatment of fully active vitamin D3 (calcitriol) on mice that were made leptin-resistant by previous ad libitum consumption of the HF diet. Calcitriol caused a reduction in FI and BW after an acute treatment with fully active exogenous D3 (calcitriol, 10 μg/kg). These effects were enhanced in mice that received both calcitriol and leptin (3 mg/kg). Chronic calcitriol (1.0 μg/kg daily) also reduced FI and BW in mice previously exposed to 1 yr of HF diet. Because similar alterations in dopamine (DA) signaling have been observed in the brains of both obese individuals and drug addicts, and since evidence exists supporting vitamin D3 effects on DA neurons in rodents, we explored the possible role for D3 in regulating the DA system and

associated behaviors. Vitamin D3 receptor (VDR) protein, which functions as a transcription factor, was detected in DA neurons of the midbrain and striatum of mice. Acute calcitriol caused upregulation of tyrosine hydroxylase and dopamine transporter in the midbrain, and upregulation of dopamine receptor type 2 in the ventral striatum. Treatment also enhanced amphetamine-induced (2.5 mg/kg) dopamine release as assessed by microdialysis and fast scan cyclic voltammetry, suggesting a role in DA signaling. In the deficient state, HF-D mice showed attenuated locomotor responses to acute amphetamine, but compensatory increases of oral amphetamine intake consistent with a state of "reward deficiency". In contrast, naïve mice treated with calcitriol showed enhanced locomotor responses, but decreased consumption of oral amphetamine. These data suggest a causative role of dietary vitamin D3 deficiency in the development of obesity and drug consumption, and that DA centers may be therapeutic targets for exogenous calcitriol.

Disclosures: **J.R. Trinko:** None. **B.B. Land:** None. **W.B. Solecki:** None. **R.J. Wickham:** None. **L.A. Tellez:** None. **J.G. Maldonado-Aviles:** None. **I.E. de Araujo:** None. **N.A. Addy:** None. **R.J. DiLeone:** None.

Poster

249. Food Intake and Energy Balance: Integration of Peripheral Signals I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 249.13/T12

Topic: E.07. Food Intake and Energy Balance

Title: The effects of obesity and type-2 diabetes on performance in open-field and light-dark box tasks

Authors: *J. A. HICKS[1], J. A. SEGGIO[2];
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Abstract: Previous studies have shown that animal models with type-2 diabetes or obesity either through genetic modification or access to high-fat diets have shown deficits in learning and memory, anxiety, and explorative behaviors. On the other hand, voluntary exercise has been known to reduce anxiety and increase explorative behaviors and learning. This study investigated the effects of obesity and type-2 diabetes C57BL6/J (B6) mice were placed into running-wheel cages - half were locked and thus no wheel-running could occur, while the other half were unlocked, and given either 60% high-fat diet or regular chow. These mice were then exposed to both the open field and light-dark box tests to test for anxiety- and explorative-like behaviors, using the AfaSci(™) system. Additionally, TallyHo/JngJ (TH) mice were also assessed in order to allow us to assess the behavioral capabilities of a mouse selectively bred for type-2 diabetes and obesity without the need of a high-fat diet. In an open-field test, obese mice exhibited reduced rearing, indicating a possible reduction in explorative behaviors. However, obese mice

did not show deficits in other parameters of an open field task (total distance traveled, total active time, or time spent in the center). In the light-dark box test, the amount of time spent in the darkened area or the latency of entry to the darkened area were not different, however, obese mice exhibited an increase in the number of light-to-dark area transitions. As previously shown, wheel-running did produce a slight decrease in high-fat diet preference, but did not produce differences in body weight. Access to a running-wheel did not significantly reduce the adverse effects of high-fat diet on these behavioral tasks. These results indicate that exercise can only slightly restore the behavioral problems caused by obesity and type-2 diabetes, indicating an overall healthy lifestyle which includes good diet and exercise are both necessary to overcome these deficits. This study contributes to the body of evidence that diabetes and obesity are linked with neurobehavioral deficits.

Disclosures: J.A. Hicks: None. **J.A. Seggio:** None.

Poster

249. Food Intake and Energy Balance: Integration of Peripheral Signals I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 249.14/T13

Topic: E.07. Food Intake and Energy Balance

Support: CIHR MOP-136776

CIHR Frederick Banting and Charles Best Canada Graduate Scholarship

Title: Brain self-control networks and weight loss in humans

Authors: *S. NESELILER[1,2], W. HU[3], M. ZACCHIA[2], K. LARCHER[1], S. SCALA[2], M. LAMARCHE[3], S. STOTLAND[4], M. LAROQUE[4], E. MARLISS[3], A. DAGHER[1,2];
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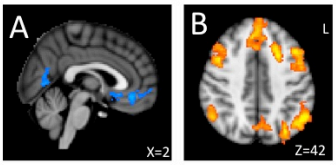
Abstract: While for most adults, dieting does not result in sustainable weight-loss, successful weight losers can be predicted by the initial weight loss at 1 month. We used fMRI to investigate how the neural response to food cues changes as weight-loss progresses over three months. 24 adults enrolled in a three month weight loss program based on calorie restriction (mean BMI: 30.1±3.2, range: 25-37). The average BMI of the participants decreased over the 3-month period from 30.1 to 28.6 ($F(1.2, 21.4)=32.7$, $p<0.001$). Subjects underwent fMRI prior to dieting, at 1 month, and at 3 months, while viewing and rating food and scenery images. fMRI response for food cues was contrasted to scenery cues. Compared to the baseline, at 1 month, participants showed reduced activation in the ventromedial prefrontal cortex, a brain region that has been implicated in encoding stimulus value. In addition, there was a correlation between weight lost

and increased activation in a fronto-parietal network that has been implicated in cognitive control. Despite continuous weight loss, at three months the activity of the brain regions associated both with the reward value and with cognitive control returned to baseline. Behavioral results followed a similar pattern: at 1 month participants rated food cues, but not scenery cues, as less wanted. At 3 months, the rating for food cues returned to baseline ($F(2,42)=6.9$, $p=0.002$). The initial weight-loss is associated with greater activity in regions implicated in cognitive control and reduced activity in regions associated with stimulus value. This response returns to baseline at 3 months despite continuous weight-loss. This change may contribute to reduced control over food intake and to subsequent weight regain in long-term.



Brain activation measured by fMRI to food pictures at 1 month during weight-loss.
A) Reduced activation in regions associated with reward value.
B) Increased activation in the fronto-parietal network is correlated with increased weight loss.

Disclosures: **S. Neseliler:** None. **W. Hu:** None. **M. Zacchia:** None. **K. Larcher:** None. **S. Scala:** None. **M. Lamarche:** None. **S. Stotland:** None. **M. Laroque:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Motivation Weight-loss Clinic. **E. Marliss:** None. **A. Dagher:** None.

Poster

250. Brain Blood Flow

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 250.01/T14

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Title: Short photoperiods reduce hippocampal blood vessel density in white-footed mice (Peromyscus leucopus)

Authors: *J. C. BORNIGER[1], S. TEPLITSKY[2], R. J. NELSON[1], C. RINK[2];
[1]Neurosci., [2]Surgery, The Ohio State Univ. Wexner Med. Ctr., Columbus, OH

Abstract: Organisms living outside the tropics need to adjust their behavioral and physiological repertoires throughout the year to adapt to the changing seasons. Winter and summer provide vastly different environmental challenges, and animals must predict and alter their phenotypes to survive and reproduce. Specifically, white-footed mice (Peromyscus leucopus) display reduced hippocampal volume, hippocampal-dependent spatial memory function, long-term potentiation (LTP), and altered neurogenesis in response to short (winter-like) photoperiods. During winter, these mice putatively shunt energy away from the brain to maximize peripheral thermogenesis and immune capacity. Whether these reductions in hippocampal function are accompanied by alterations in brain vasculature remains unknown. We maintained white-footed mice in short (8 h light:16 h dark) or long (16:8) photoperiods for 8 wks. Mice were then perfused with fluorescein isothiocyanate (FITC)-conjugated tomato (Lycopersicon esculentum) lectin to visualize endothelial cells in the brain. Specific FITC+ hippocampal blood vessels were isolated via laser capture to assess gene expression. Mice exposed to short days reduced hippocampal and cortex blood vessel density (FITC+ area) and vessels isolated from short-day exposed mice expressed high levels of the gelatinase MMP2, which may contribute to the vascular remodeling we observed. These changes occurred prior to gonadal regression, indicating that changes in brain vascularity precede measureable reproductive regression in P. leucopus.

Disclosures: J.C. Borniger: None. **S. Teplitsky:** None. **R.J. Nelson:** None. **C. Rink:** None.

Poster

250. Brain Blood Flow

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 250.02/T15

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: Departmental funding

Psi Chi National Undergraduate Research Award

Title: Effects of ambient temperature and hypothalamic temperature manipulations on thermoregulatory yawning in rats

Authors: *M. L. SHOUP-KNOX, K. GAFFNEY, L. GAMBRILL, K. PONDER, R. PRUETT; Psychology, James Madison Univ., Harrisonburg, VA

Abstract: Recent evidence suggests that yawning functions to cool the brain during times of mild central nervous system hyperthermia. According to this thermoregulatory hypothesis a yawn could only effectively cool the brain if air temperatures are low enough to assist in evaporative and convective cooling of blood and the mucosal surfaces of the nasal cavity. At higher ambient temperatures yawning would be replaced by more effective methods of thermoregulation. The current study explores this thermal window prediction by recording

yawning in rats under four different air temperature conditions: 27oC, 29 oC, 32 oC, and a continuously decreasing phase. During testing we also directly measured brain temperature in the pre-optic area (POA) of the hypothalamus and the prelimbic cortex (PRL). We found a strong relationship between air temperatures and brain temperature for both brain areas (rPOA = 0.61, $p < 0.01$; rPRL = 0.64, $p < 0.01$). In a second study we directly manipulated POA temperature between 33 and 38 oC to examine its effects on yawning frequency. Preliminary evidence suggests that yawning occurs most frequently during the cooling phase following heat stress, but less during the highest hypothalamic and ambient temperature conditions.

Disclosures: M.L. Shoup-Knox: None. **K. Gaffney:** None. **L. Gambrill:** None. **K. Ponder:** None. **R. Pruett:** None.

Poster

250. Brain Blood Flow

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 250.03/T16

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: MOST103-2320-B-303-001-MY3

Tzu Chi foundation

Tzu Chi university

Title: Huperzine-A in clinical concentrations does not block sympathetic nAChR-mediated nitrergic neurogenic dilation of porcine basilar arteries

Authors: *T. J.-F. LEE[1,2,3], M.-F. CHEN[2,4], C.-C. SHIH[5], P.-Y. CHEN[2,5];
[1]Southern Illinois Univ. Med. Sch., Springfield, IL; [2]Dept of Med. Res, Buddhist Tzu Chi Gen. Hosp, Hualien, Taiwan; [3]Dept of Life Sci, Coll of Life Sci, Tzu Chi Univ., Hualien, Taiwan; [4]Tzu Chi Coll of Technol., Hualien, Taiwan; [5]Dept of Pharmacol & Toxicol, Coll of Med, Tzu Chi Univ., Hualien, Taiwan

Abstract: Several cholinesterase inhibitors (ChEIs) are known to block nicotinic acetylcholine receptors (nAChRs) located on perivascular sympathetic nerves innervating the basilar arteries, leading to diminished vasodilation in the brainstem. This vascular side-effect may decrease brainstem blood flow and their efficacy in clinical therapy. Huperzine A (Hup-A), a naturally-occurring ChEI, is indicated for treating Alzheimer's disease (AD). We examined if Hup-A inhibited nAChR-mediated nitrergic vasodilation in porcine isolated basilar arteries, using blood-vessel myography technique. Transmural nerve stimulation (TNS) of perivascular parasympathetic nerves and activation of sympathetic nAChRs by nicotine (100 μM) induced nitrergic dilation of basilar arteries. Nicotine-induced vasodilation was inhibited by Hup-A (30-

300 μM) and donepezil (1-30 μM) concentration-dependently with the former being 20 folds less potent than the latter. Hup-A, but not donepezil, in low concentrations (3-10 μM) significantly increased nicotine-induced vasodilation. Both ChEIs, however, equally decreased TNS-elicited neurogenic nitrergic vasodilations which, unlike those induced by nicotine, were reversed by atropine. Similarly, donepezil was significantly more potent than Hup-A in inhibiting nicotine-elicited calcium influx in rat superior cervical ganglion (SCG) neurons and inward currents in α7- and α3β2-nAChR- expressing *Xenopus* oocytes. Although both ChEIs in high concentrations inhibit sympathetic α7- and α3β2-nAChRs leading to blockade of parasympathetic nitrergic vasodilation, Hup-A in clinical concentrations increases neurogenic nitrergic vasodilation. Hup-A may have advantages over donepezil and other ChEIs in maintaining brainstem blood flow during AD therapy.

Disclosures: **T.J. Lee:** None. **M. Chen:** None. **C. Shih:** None. **P. Chen:** None.

Poster

250. Brain Blood Flow

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 250.04/T17

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: Royal Society University Research Fellowship

Title: The effects of anodal tDCS on neurovascular coupling in rat somatosensory cortex

Authors: ***C. THOMAS**[1], T. F. D. FARROW[2], C. MARTIN[3];
[1]Psychology, [2]Academic Clin. Neurol., [3]Psychology, Neurovascular and Neuroimaging Res. Group, Univ. of Sheffield, Sheffield, United Kingdom

Abstract: Background: Transcranial direct current stimulation (tDCS) is a non-invasive stimulation technique that holds great promise in the modulation of cortical excitability and behavior in clinical and experimental applications. The polarity-specific effects on neuronal firing thresholds are most clearly seen towards the end of stimulation and shortly afterwards. This alludes to a long-term change resulting from the low-level DC stimulation. It is likely that this type of stimulation mechanistically modifies both spontaneous firing rates and responsiveness to afferent synaptic inputs (Stagg & Nitsche 2011). However, the actual mechanism by which these effects arise is unknown. Neuronal activity can be indirectly measured by hemodynamic changes (cerebral blood flow, CBF). We aim to gain a mathematical and physiological handle on the effects of anodal tDCS on neurovascular coupling by examining the relationship between somatosensory neural activity and CBF responses, following whisker pad stimulation. We hypothesise that anodal tDCS will modulate neuronal responses and that changes will also be observed in cerebral blood flow in the rodent somatosensory cortex

(whisker barrel). These changes are anticipated to occur both during and after a short period of DC stimulation concurrent with whisker stimulation, peaking at the end of stimulation then declining overtime. Methods: Anesthetized Hooded Lister rats will be prepared with a thin cranial window over somatosensory cortex to enable laser speckle imaging of cortical blood flow and optical imaging of cerebral blood volume and oxygenation. Neuronal responses will be measured from barrel cortex using a multichannel electrode probe. 15 minutes of anodal tDCS (400μA, anode 7mm2) will be applied through a circular electrode positioned rostral to the cranial window (~15mm2 surface area), with the cathode positioned on the animals back. Before, during and after tDCS, the contralateral whisker pad will be electrically stimulated to evoke neuronal and hemodynamic responses. In accordance with our hypotheses, initial data indicates tDCS increases baseline and stimulus-evoked CBF compared with pre-tDCS measures.

Disclosures: C. Thomas: None. **T.F.D. Farrow:** None. **C. Martin:** None.

Poster

250. Brain Blood Flow

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 250.05/T18

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: Royal Society University Research Fellowship (CM)

Sir Henry Dale Wellcome Trust Royal Society Fellowship (CH)

Wellcome Trust Research Project Grant (WT093223MA , CM/AS)

Title: Cortical and subcortical modulations of neurovascular coupling by serotonin

Authors: A. SPAIN[1], C. HOWARTH[1], J. BERWICK[1], *C. J. MARTIN[2];
[1]Neurovascular and Neuroimaging Res. Group, Dept. of Psychology, [2]The Univ. of Sheffield, Sheffield, United Kingdom

Abstract: The rapid and local adjustment of brain hemodynamics in relation to changing neuronal activity is termed neurovascular coupling. Our understanding of the physiological mechanisms underpinning neurovascular coupling has advanced substantially in recent years, including the roles played by the cells that comprise the neurovascular unit and associated inter- and intra-cellular signalling mechanisms. Whilst empirical work to date has focussed on the role for excitatory, glutamate-mediated neurotransmission in neurovascular coupling, little research has addressed whether and how important modulatory neurotransmitters such as serotonin (5-HT), which are known to have (a) a range of vasoactive properties and (b) altered functionality under many pathological conditions, might affect neurovascular function. To address this, we investigated the cortical and subcortical effects of pharmacological manipulation of the 5-HT

system upon neurovascular coupling *in vivo*, by applying a range of concurrent measurement techniques including fMRI, tissue oxygen fluorescence, laser speckle and laser Doppler blood flow measurement, optical imaging spectroscopy and electrophysiological recording. Under anaesthesia, the skull overlying somatosensory cortex was thinned to translucency for cortical imaging whilst burr holes were made to allow for the insertion of tissue oxygen or laser Doppler probes and recording or stimulating electrodes. Hemodynamic and neuronal data were acquired in cortical (barrel cortex) and subcortical (striatum) structures under baseline and stimulation conditions (via whisker stimulation or trans-callosal cortical stimulation). Data were acquired both before and after the administration of 5-HT modulating drugs (or control substances) including the 5-HT releasing agent fenfluramine (10mg/kg), the 5-HT 2A receptor agonist (and metabolite of the hallucinogen psilocybin), psilocin (2mg/kg), and the 5-HT 1B/1D receptor agonist (and anti-migraine drug) sumatriptan (2mg/kg). Initial data indicate alterations in the relationship between stimulus evoked neuronal activity and hemodynamic responses attributable to pharmacological manipulation of the 5-HT system. For example in the case of psilocin and sumatriptan, hemodynamic responses were augmented by the drug whereas neuronal responses were reduced (psilocin) or unaltered (sumatriptan). Alterations in neurovascular coupling by changes in the function of the 5-HT system may have important implications for the application of functional MRI to investigate drug action or disease mechanisms that involve 5-HT systems in both humans and animal research models.

Disclosures: **A. Spain:** None. **C. Howarth:** None. **J. Berwick:** None. **C.J. Martin:** None.

Poster

250. Brain Blood Flow

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 250.06/T19

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Title: Does vasomotion alter functional connectivity; a multi-modal study using optical imaging spectroscopy, multi-channel electrophysiology and bold fmri

Authors: ***P. PATEL**, A. KENNERLEY, L. BOORMAN, M. JONES, J. BERWICK;
Dept. of Psychology, Univ. of Sheffield, Sheffield, United Kingdom

Abstract: Perturbations in brain connectivity is often inferred by examining correlations in the spontaneous fluctuations in Blood Oxygenation Level Dependent (BOLD) functional magnetic resonance imaging (fMRI) signals in the absence of stimuli or tasks. However, systemic parameters and neurovascular coupling are known to affect cerebral hemodynamics which may also be altered in disease states; therefore, inferring connectivity changes from the BOLD fMRI and the underlying hemodynamics may be problematic. To investigate this issue, we employed the following techniques: two-dimensional optical imaging spectroscopy (2D-OIS) for the

underlying changes in blood volume and oxygenation and multi-channel electrophysiology recording to measure the cortical neural activity in the somatosensory and motor cortex of anaesthetized rodents. A low frequency cerebral hemodynamic oscillation (0.1Hz) was examined following the manipulation of systemic blood pressure (BP) in order to part emulate physiological conditions in disease states. This 0.1Hz oscillation known as vasomotion is a spontaneous oscillation in the small vessel lumens observed in many microvascular beds and is unrelated to cardiac rhythm (Hudetz et al, JCBFM, 1992). Despite a significant amount of literature on the vasomotion signal, its physiological function and pathophysiology are not yet clear. One hypothesis is that vasomotion rhythms are significant for its ability to increase optimal delivery of oxygen to the tissue (Hudetz et al, Advn Exp Med Bio, 1998). A series of experiments to manipulate the size of vasomotion by altering the levels of BP and the level of inspired oxygen were conducted. Data suggested that when there is low BP there is a presence of vasomotion and when BP is increased to normal physiological levels the magnitude of vasomotion is significantly reduced; suggesting that this signal is present when blood flow in the brain becomes compromised. To further understand if the vasomotion signal is dependent on the amount of tissue oxygen tension in the brain, we increased levels of oxygen in the inspired air during the periods of lowered BP. This resulted in an increase in the oxygen saturation levels in the blood which were associated with reduced magnitude of vasomotion. A further experiment making direct measurements of tissue oxygen tension confirmed that this manipulation increased tissue PO2. To investigate the spatial extent of vasomotion in these physiological perturbations, similar whole brain measurements were made with BOLD fMRI.

Disclosures: P. Patel: None. **A. Kennerley:** None. **L. Boorman:** None. **M. Jones:** None. **J. Berwick:** None.

Poster

250. Brain Blood Flow

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 250.07/T20

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: Royal Society University Research Fellowship (CM)

Wellcome Trust Research Project Grant WT093223MA (AS)

Title: Cholinergic modulation of neurovascular coupling and neuroimaging signals

Authors: *G. BREZZO, A. SPAIN, J. BERWICK, C. MARTIN;
Univ. of Sheffield, Sheffield, United Kingdom

Abstract: In recent years a number of brain diseases, such as Alzheimer's disease (AD), have become associated with pathological changes in blood flow regulation and the neurovascular

apparatus that supports it, the neurovascular unit. This has led to research into how interventions to improve neurovascular function could provide a degree of neuroprotection from neurodegenerative diseases. Several central nervous system (CNS) penetrating cholinergic drugs such as donepezil have been developed for the treatment of AD and have been shown to reduce disease symptoms. A recent study (Kocsis et al., JCBFM, 2014) suggested that cognitive enhancement effects of CNS cholinergic drugs were mediated by vascular and not neuronal actions. An important question is whether and how these vascular effects are manifested in the brain's neurovascular coupling response, in terms of the rapid and local adjustment of cerebral blood flow (CBF) to changing neuronal and metabolic demands. To investigate this, we used a rodent model in which CBF and neuronal activity were measured across a range of sensory stimulation parameters, in order to identify the effects of pharmacological manipulations of brain acetylcholine on neuronal, hemodynamic and neurovascular coupling measurements. In anaesthetised animals, a thin cranial window was prepared over the somatosensory barrel cortex to enable recording of CBF using laser speckle contrast imaging. A burr hole was made in the thin cranial window for insertion of a multichannel electrode for electrophysiological recording of neuronal activity. Whisker stimulation protocols included a mixed frequency design (1-40Hz range, 2s duration) and long duration stimulation (5Hz, 16s). Data were acquired at baseline and following the administration of the anticholinergic drug scopolamine (2mg in 1ml, IV). Results indicate that scopolamine decreased baseline blood flow and stimulus-evoked hemodynamic response magnitude, although electrophysiological data show little alteration in concurrently measured neuronal responses. The study is currently being extended to include the non-CNS penetrating anticholinergic drug neostigmine and the current AD drug, donepezil. Initial findings indicate a cholinergic modulation of neurovascular coupling which may have important implications for (i) our understanding of how drugs which modulate vascular function can impact upon neurovascular coupling (ii) the interpretation of functional brain imaging (fMRI) signals acquired in the context of cholinergic manipulations, (iii) our understanding of the physiological mechanism underpinning the effectiveness of cholinergic drugs for the treatment of AD.

Disclosures: **G. Brezzo:** None. **A. Spain:** None. **J. Berwick:** None. **C. Martin:** None.

Poster

250. Brain Blood Flow

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 250.08/U1

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant NS37853

Title: NMDA-dependent neurovascular dysregulation in mice overexpressing mutated human tau

Authors: *L. PARK, K. KOIZUMI, P. ZHOU, C. IADECOLA;
Feil Family Brain and Mind Res. Inst., Weill Cornell Med. Col., New York, NY

Abstract: The microtubule-associated protein tau is present in neurons, wherein stabilizes microtubules at the synapses. In Alzheimer's disease and in brain diseases associated with tau mutations, such as frontotemporal dementia (FTD), tau becomes hyperphosphorylated (p-tau), detaches from microtubules, and aggregates in paired helical or straight filaments disrupting synaptic function (Neuron, 82:756-71, 2014). Although its deleterious neuronal effects are well established, it is not known if p-tau also disrupts neurovascular regulation, which, in turn, could contribute to brain dysfunction in tauopathies. To address this question, we examined neurovascular regulation in transgenic mice expressing mutated human tau. Cerebral blood flow (CBF) was assessed using laser-Doppler flowmetry in anesthetized rTg4510 and PS19 mice (n=5/group) equipped with a cranial window and with controlled blood pressure and blood gases. The increase in somatosensory cortex CBF evoked by neural activity (whisker stimulation, WS), a critical response coupling energy supply and demand in the active brain, was suppressed in both strains (WT: 23±2%; rTg4510: 11±2%; PS19: 14±1-%; $p<0.05$; mean±SE), which was not related to reductions in the field potential evoked by electrical whisker pad stimulation (WT: -5.1±1.8; PS19: -4.4±2.1 mV; $p>0.05$). Furthermore, the increase in CBF produced by neocortical application of acetylcholine, a response mediated by the endothelial NO, was not attenuated (WT: 24±2%; rTg4510: 24±1%; PS19: 22±1%; $p>0.05$). Since glutamatergic synaptic activity is a key contributor to the increase in CBF evoked by WS, we examined the CBF increase induced by glutamate receptor activation. The CBF response to neocortical application of NMDA (40 μM) was attenuated (WT: 26±3%; rTg4510: 13±2%; $p<0.05$), whereas the response to AMPA (10μM) was not (WT: 31±4%; rTg4510: 36±3%; $p>0.05$). To determine whether the attenuation in the CBF increase produced by WS in p-tau mice was a consequence of failure of the NMDA-dependent component of the vasodilatation, we examined neurovascular coupling in the presence of the NMDA receptor inhibitor MK801, superfused on the neocortex. MK801 (10 μM) attenuated the CBF increase evoked by WS in WT mice (-51±1%; $p<0.05$), but did not reduce the response in rTg4510 mice ($p>0.05$). The data provide the first demonstration that p-tau impairs neurovascular regulation. The effect is specific for the increase in CBF evoked by neural activity and reflects a deficit in NMDA receptor-mediated vasodilatation. Overall, the findings unveil a previously unrecognized aspect of tau pathobiology with potential diagnostic and therapeutic implications.

Disclosures: L. Park: None. **K. Koizumi:** None. **P. Zhou:** None. **C. Iadecola:** None.

Poster

250. Brain Blood Flow

Location: Hall A

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Program#/Poster#: 250.09/U2

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

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AHA grant SDG7600037

Title: Pericyte regulation of neurovascular coupling

Authors: *K. KISLER[1], S. V. REGE[1], A. R. NELSON[1], A. RAMANATHAN[1], A. AHUJA[1], P. S. TSAI[2], D. A. BOAS[3], S. SAKADŽIĆ[3], B. V. ZLOKOVIC[1];
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Abstract: Neurovascular coupling, the regulation of cerebral blood flow (CBF) and oxygen supply to match neuronal functional activity, is regulated by synchronous action of different cell types comprising the neurovascular unit - neurons, vascular cells such as vascular smooth muscle cells and endothelium, and glia. Pericytes, first described by Rouget in 1873 as mural cells of the capillary vessel wall, are critical for the stabilization of the capillary wall, maintenance of the blood-brain barrier and have recently been implicated in the regulation of capillary diameter. However, their role in regulation of neurovascular coupling remains debatable. Using pericyte-deficient mice, we show that pericyte reduction *in vivo* leads to delayed capillary dilation in response to neuronal stimulus, and reduced red blood cell flow velocity in capillaries carrying oxygen to activated brain sites. Furthermore, we show diminished local oxygen supply to cortex in response to stimulus in pericyte-deficient mice in spite of an intact arteriolar response and vasoactivity. Together, these data imply that pericytes play an important role in neurovascular coupling and oxygen supply to the brain, and that pericyte degeneration contributes to neurovascular uncoupling. Degeneration of pericytes, as is seen in neurological disorders including Alzheimer's, likely contributes to neurovascular dysregulation, leading to neurovascular dysfunction and eventually neurodegeneration.

Disclosures: K. Kisler: None. **S.V. Rege:** None. **A.R. Nelson:** None. **A. Ramanathan:** None. **A. Ahuja:** None. **P.S. Tsai:** None. **D.A. Boas:** None. **S. Sakadžić:** None. **B.V. Zlokovic:** None.

Poster

250. Brain Blood Flow

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 250.10/U3

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: IBS-R015-D1

Title: *In vivo* longitudinal imaging and probing of brain system through a clear and soft cranial window

Authors: *C. HEO[1,2], H. PARK[3], T. KIM[4], T.-I. KIM[4,5], S.-G. KIM[2,5], M. SUH[3,2,5];
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Abstract: In order to investigate living brain functions, we need a chronic cranial window.. Here, we introduce a transparent and flexible silicone based material for chronic animal cranial window. This soft material is used as a substitute for the skull for longitudinal study of rat and mouse brains. This cranial window ensures good physiological states because it can maintain intracranial pressure and cerebrospinal fluid flow within a normal range demonstrated by a computer simulation. This cranial window was utilized for several chronic *in vivo* experiments such as laser-speckle imaging, optical recording of intrinsic signal of cortex, and multi-photon microscopic imaging. Also, this soft cranial window allows repeated penetration of electrodes for drug delivery and neuronal signal recording without any cerebrospinal fluid leakage and inflammation. Thus, our soft cranial window technique might facilitate effective longitudinal studies of living brain function in conjunction with electrophysiological recording, drug delivery, and imaging.

Disclosures: C. Heo: None. **H. Park:** None. **T. Kim:** None. **T. Kim:** None. **S. Kim:** None. **M. Suh:** None.

Poster

250. Brain Blood Flow

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 250.11/U4

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Title: The local cerebral blood flow in the dorsal hippocampus of rats during initial and deep stages of informational pathology of behavior

Authors: *M. NEBIERIDZE[1], I. KVACHAKIDZE[2], L. GUMBERIDZE[2], L. DAVLIANIDZE-GOBECHIA[2], M. MANTSKAVA[2], N. MOMTSELIDZE[2], N. SIKHARULIDZE[2];
[1]I. Beritashvili Ctr. of Exptl. Biomedicine, Tbilisi, Georgia; [2]Cerebral Blood flow and Metabolism, Beritashvili Ctr. of Exptl. Biomedicine, Tbilisi, Georgia

Abstract: Introduction, materials and methods. The local cerebral blood flow (LCBF) was studied by the hydrogen clearance technique in the dorsal hippocampus (DH) of rats with initial and deep stages of informational pathology of behavior (IPB). The initial stage of IPB was induced by the negative emotional stress developed during the short period of delayed testing (indirect version, 2-3s delay), in I group of animals, while the deep stage of IPB was induced by the chronic negative emotional stress developed during the long period of testing delayed reactions (indirect version, 2-3s delay) in II group of animals, under conditions of the time shotage between the signals (30s) and the existence of high level of motivation. Results. A significant increase in the LCBF level was observed in I experimental group in comparison with the control one, while in II experimental group a significant decrease in the LCBF level in comparison with the control group was demonstrated. Conclusion. It is supposed, that this alteration of LCBF in I group may be caused by the strengthening of DH functional activity as result of short time negative emotional stress, thus being a form of the cerebral self-regulation activity. In II group 1) the decrease in the LCBF may have a secondary character as a result of suppression of functional activity of the DH by exposure to the chronic negative emotional stress; 2) It is not inconceivable that the LCBF decrease is of a primary character and may account for dysfunction of this structure facilitating the emotional stress and its acquisition of pathogenic properties, thus being an important factor of the IPB formation.

Disclosures: M. Nebieridze: None. **I. Kvachakidze:** None. **L. Gumberidze:** None. **L. Davlianidze-Gobechia:** None. **M. Mantskava:** None. **N. Momtselidze:** None. **N. Sikharulidze:** None.

Poster

250. Brain Blood Flow

Location: Hall A

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Program#/Poster#: 250.12/U5

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH grant OD006831

NIH grant EB003832

MH108503

AHA grant 14GRNT2O48O366

Title: Evidence that resting-state functional connectivity is mediated via arteriole vasomotion

Authors: *C. MATEO[1], P. KNUTSEN[1], P. S. TSAI[1], A. Y. SHIH[3], D. KLEINFELD[1,2]; [1]Physics Dept, [2]Neurobio., UCSD, La Jolla, CA; [3]Neurosciences, Med. Uni. of South Carolina, Charleston, SC

Abstract: Neural activity leads to changes in metabolism that are reflected in a blood oxygen level dependent (BOLD) signal in functional magnetic resonance imaging (fMRI) and an intrinsic optical signal (IOS) in reflection imaging. In the absence of stimulation or a cognitive task, infra-slow oscillations (~ 0.1 Hz) of the BOLD fMRI signal correlate between regions with mirrored functionality across hemispheres (Biswal et al. MRM 1995), as well as with spontaneous fluctuations in the envelope of gamma-band (~ 30-80 Hz) neural activity (Schölvinck et al. PNAS 2010). Correlations in the infra-slow BOLD fMRI signal between brain regions form the basis of functional connectivity studies in human and nonhuman primate. We hypothesize that entrainment of the infra-slow vasomotor oscillations in cortical arterioles could provide a bridge between spontaneous fluctuations in neural activity and the BOLD signal. To address this hypothesis, we employed imaging, electrophysiology, and optogenetic manipulation of neural activity and hemodynamics in awake, head-fixed mice, in which arterioles exhibit vasomotion near ~ 0.1 Hz (Drew et al. PNAS 2011). First, we observe strong spectral coherence between the envelope of the gamma rhythm of neural activity and changes in the diameter of nearby arterioles (n = 20) measured with two-photon microscopy. Changes in the envelope of the gamma rhythm lead the dilation of arterioles by about 2 s. Next, optogenetic activation of cortical neurons (PV-Cre x Ai32 and Thy-1:Chr2) reveal that induced infra-slow oscillations in the gamma-envelope cause concurrent oscillations in arteriole diameter (n = 10). In contrast, optogenetic-induced dilation of cortical arterioles at these frequencies fails to change field activity. Lastly, concurrent IOS imaging and measures of arteriole diameter show that arteriole dilation leads the local increase in pO2 in the parenchyma (n = 6). These data establish a sequence: gamma-band fluctuations drive arteriole dilations that in turn drive an increase in tissue oxygenation. As a means to directly relate the entrainment of vasomotion to functional connectivity, we performed simultaneous bilateral measurements of arteriole diameter with ultra-wide scale two-photon microscopy (Tsai et al. OE 2015). We find that infra-slow changes in diameter are strongly coherent between mirrored regions across hemispheres, but less so between functionally distinct regions (n = 4). The correlations are consistent with axonal connections via the corpus callosum and support the notion that entrainment of vasomotion by neural activity is responsible for correlations in blood oxygenation that underlie functional connectivity.

Disclosures: C. Mateo: None. **P. Knutsen:** None. **P.S. Tsai:** None. **A.Y. Shih:** None. **D. Kleinfeld:** None.

Poster

250. Brain Blood Flow

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Program#/Poster#: 250.13/U6

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant R01NS078168

NIH Grant R01NS079737

Scholar Award, McKnight Endowment Fund for Neuroscience

National Scientist Development Grant, AHA

Title: Coupling of spontaneous and sensory evoked hemodynamic signals to neural activity in the barrel cortex of awake mice

Authors: *A. WINDER, P. J. DREW;
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Abstract: Spontaneous hemodynamic signals are used as indicators of neural activity in resting-state MRI experiments. However, it is not clear whether spontaneous hemodynamic signals reflect ongoing neural activity in the same way that sensory evoked hemodynamic signals do. To determine if the coupling of neural activity and hemodynamic signals is the same for both sensory-evoked and spontaneous activity, we examined neurovascular coupling in the barrel cortex of awake, head-fixed mice during several behaviors. We simultaneously measured local neural activity, CBV, and behavior in awake, head-fixed mice. Blood volume changes were measured using intrinsic optical signal imaging in the vibrissae cortex. The local field potential (LFP) and multiunit activity (MUA) were measured from the same location. Whisker position was tracked to determine when volitional motions occurred. The vibrissae were also stimulated with brief puffs of air. The data were categorized into three behaviors: sensory evoked, volitional whisking, and rest. Neural and CBV increases to whisker stimulation were substantially stronger than during volitional whisking. During rest, neural activity and CBV were weakly correlated. Neurovascular correlations during spontaneous behavior were not disrupted by transection of the facial nerve, indicating that they were not due to sensory input during whisker movement. We calculated the hemodynamic response function (HRF), the linear kernel that relates the measured CBV to the local neural activity for each behavior. We found the HRFs obtained for each behavior condition to be similar, indicating that neurovascular coupling was conserved across behavioral state. We then used these HRFs to determine what fraction of the spontaneous and sensory evoked changes in CBV could be explained by local neural activity. Here there were substantial differences among behaviors in the variance of the hemodynamic signal captured by the HRFs. Sensory evoked hemodynamics, on average and during individual trials, were better predicted by local neural activity than those measured during volitional whisking or resting behaviors. The prediction error, for all behaviors, could be accounted for by the presence of ongoing CBV fluctuations that were not associated with any measured neural activity. This uncorrelated signal obscures neurally-evoked hemodynamic changes to a greater degree during

volitional whisking and resting behaviors since these behaviors drive smaller increases in neural activity and CBV. These results highlight the need to investigate additional sources of spontaneous hemodynamic fluctuations in alert subjects in order to better interpret fMRI.

Disclosures: A. Winder: None. **P.J. Drew:** None.

Poster

250. Brain Blood Flow

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 250.14/U7

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

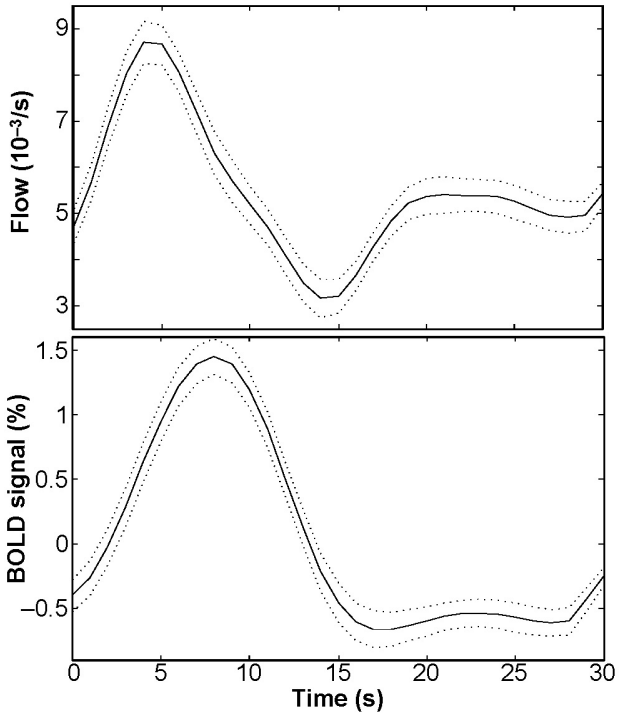
Support: NIH R21HL108143

Title: Strong blood flow undershoot induced by brief stimulation in the human brain

Authors: *D. RESS, J. KIM;
Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Brief neural activation creates a hemodynamic response function (HRF), a stereotypic manifestation of neurovascular coupling. A recent theory (Kim et al., JCBFM, 2013) predicts that the flow response underlying the HRF has an underdamped oscillatory behavior. This hypothesis was tested and confirmed by arterial spin-labeling MRI experiments, which also produce simultaneous BOLD HRF measurements. **Methods**: Subjects ($N = 6$) view a 2-s duration full field of flickering black & white dots on a gray background. These impulses were separated by a 32-s inter-stimulus interval, and repeated 16 times per run for 5—6 runs to create a total of 80—96 measurements per session. Combined flow and BOLD HRF data were obtained using the standard (PICORE) sequence on a Siemens 3T scanner with 2-mm voxels and TR = 4 s, using 2× GRAPPA acceleration on 14 quasi-axial slices. Stimulus presentations were jittered relative to the TR to increase temporal sampling to 1 s. The flow and HRF data were transformed into a high-resolution (0.7-mm) reference anatomy so that they could be averaged within visual areas V1—3, predefined for each subject. **Results**: All subjects show a significant flow undershoot (Figure), and some show a more complex oscillatory return to baseline. Standard errors across the many measurements, shown by dotted lines, demonstrate the high quality of the quantification. Flow response peaks ~4 s earlier than the BOLD HRF. BOLD signals also show a significant undershoot that is always later than the flow undershoot, and can also persist substantially longer. **Discussion**: The flow induced by brief stimulation has a strong undershoot and complex late time behavior consistent with underdamped oscillation. HRF responses are substantially delayed from the flow response, consistent with convective time delays as the flow propagates into downstream venous microvasculature that dominates the BOLD signal. The

ability to perform these combined measurements, coupled with the theoretical model, will enable the quantification of oxygen metabolism ($CMRO_2$) consequent to brief neural stimulation.



Disclosures: D. Ress: None. **J. Kim:** None.

Poster

250. Brain Blood Flow

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 250.15/U8

Topic: E.09. Brain Blood Flow, Metabolism, and Homeostasis

Support: Mayday Fund New York

NIH K24-NS064050

Title: Regional cerebral blood flow in pediatric post-traumatic headache

Authors: *D. J. HODKINSON[1], P. SERRANO[1], M. O'BRIEN[2], A. LEBEL[1], L. BECERRA[1], D. BORSOOK[1];
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Abstract: Introduction: Post-traumatic headache (PTH) is a common outcome of mild traumatic brain injury (mTBI) and concussion. The consequences of PTH in children can be severe, and may result in the interruption of normal healthy development [1] and increased risk of pain chronification [2]. **Aims & Objectives:** To determine whether PTH onset and progression is related to the dysregulation of cerebral blood flow (CBF) in brain regions involved in autonomic function that may occur as a result of closed head injury. **Methods:** *Participants:* 12 PTH patients and 22 healthy controls were recruited for the study (age range 14-18 years). PTH patients were identified at Boston Children's Hospital (Emergency Medicine Department, Division of Sports Medicine, and Chronic Headache Clinic) and scanned on average 133.2/4.4 days/months post-concussion/mTBI. *Image acquisition:* Participants were scanned on a 3T MRI scanner. High-resolution T1-weighted anatomical scans were acquired for image registration. Quantitative regional cerebral blood flow (rCBF) measurements were performed using pseudo-continuous arterial spin labeling (pCASL) [3] [PLD=1.3sec, LD=1.5sec, GE-EPI, TR/TE= 3870/12, FOV=220mm, matrix=64x64, 26 slices, slice thickness = 5mm]. *Image processing:* Data were pre-processed using SPM8. Group-wise changes in regional CBF (rCBF) were calculated using a random effects two-sample t-test. **Results:** Quantitative CBF maps were calculated for controls and PTH patients. Ratio between the CBF in the controls and PTH groups shows an approximate 10-20% global CBF increase in the PTH group. Statistical comparisons between healthy controls and PTH patients revealed significant regional CBF increases in the forebrain, including basal ganglia and limbic system. **Discussion:** Regional CBF is closely coupled with glucose utilization, oxygen consumption, and aerobic glycolysis in the resting human brain [4,5]. Increased CBF in PTH patients may potentially reflect disruptions in autonomic function [6]. This experimental technique may be used for reliable identification of successful brain recovery or potential long-term neurological impairments, and could help to determine whether and when it is safe for patients to return to activity. **References:** [1] Kirk et al., (2008). *Dev Med Child Neurol*, **50**(6): p. 422-5 [2] Gavett et al., (2011). *Clin Sports Med*, **30**(1): p. 179-88 [3] Dai et al., (2008)., *Magn Reson Med*; **60**: 1488–1497 [4] Raichle et al., (2001). *PNAS*. **98**: 676–682 [5] Vaishnavi et al ., (2010). *PNAS*. **107**: 17757–17762 [6] Cambron et al., (2014). *Headache.* **54**(4): p. 655-62.

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Poster

250. Brain Blood Flow

Location: Hall A

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Program#/Poster#: 250.16/U9

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant HL36279

NIH Grant DK104184

NIH Grant GM104357

Title: Role of 20-HETE in the development of hypertension-related cerebral vascular disease

Authors: *F. FAN[1], L. RICK[2], M. R. PABBIDI[1], E. GOMEZ-SANCHEZ[1], R. J. ROMAN[1]; [1]Pharmacol., [2]Neurobio. and Anatom. Sci., The Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract: We have reported that a reduction in the expression of CYP4A and the production of 20-HETE contributes to development of impaired myogenic response and autoregulation of cerebral blood flow (CBF) in Dahl salt sensitive (SS) rats. The present study examined whether this impaired myogenic response in the cerebral circulation promotes the development of cerebral vascular disease after the induction of hypertension. 20-HETE production was 6-fold higher in cerebral arteries of CYP4A1 transgenic and SS. 5BN consomic rats (in which chromosome 5 from Brown Norway was transferred into SS background) than in SS rats. The diameter of the middle cerebral artery (MCA) decreased to 70 ± 3 % and 65 ± 6% in CYP4A1 and SS. 5BN rats when pressure was increased from 40 to 140 mmHg. In contrast, the myogenic response of the MCA isolated from SS rats did not constrict. Administration of a 20-HETE synthesis inhibitor, HET0016, abolished the myogenic response of MCA in CYP4A1 and SS. 5BN rats, but had no effect in SS rats. Autoregulation of CBF was impaired in SS rats in comparison to CYP4A1 and SS. 5BN rats. Blood brain barrier leakage was 5-fold higher in the brain of the SS rat than in SS. 5BN and SS.CYP4A1 rats. Neurodegeneration and vascular remodeling was observed in neocortex and CA3 area of hippocampus in hypertensive SS rats in comparison to CYP4A1 and SS. 5BN rats. The SS rats exhibited a cognitive impairment and took 4 times longer time to escape from an eight-arm water maze in comparison with SS.CYP4A1 rats after induction of hypertension with a high salt diet for 4 weeks. These findings indicate that a genetic deficiency in the formation of 20-HETE contributes to an impaired myogenic response in MCA and autoregulation of CBF in SS rats and this may contribute to cerebral vascular disease and a vascular cognitive impairment following the onset of hypertension.

Disclosures: F. Fan: None. **L. Rick:** None. **M.R. Pabbidi:** None. **E. Gomez-Sanchez:** None. **R.J. Roman:** None.

Poster

250. Brain Blood Flow

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 250.17/U10

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant HL36279

NIH Grant DK104184

NIH Grant GM104357

Title: Role of gamma-Adducin in cerebral vascular disease

Authors: *R. J. ROMAN[1], A. M. GEURTS[4], R. LIN[2], M. R. PABBIDI[1], E. GOMEZ-SANCHEZ[1], G. RAJKOWSKA[3], D. R. HARDER[5], F. FAN[1];
[1]Pharmacol., [2]Neurobio. and Anatom. Sci., [3]Psychiatry, The Univ. of Mississippi Med. Ctr., Jackson, MS; [4]Human and Mol. Genet. Ctr., [5]Physiol. and Cardiovasc. research Ctr., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: We recently identified a K572Q mutation in Add3 in FHH rats in comparison with FHH.1BN congenic rats in which chr. 1 from the BN rats containing 15 genes including Add3 was transferred into FHH genetic background. The present study examined the contribution of this mutation to cerebral vascular function. Expression of Add3 is lower in FHH than in FHH.1BN rats. The diameter of the middle cerebral artery (MCA) decreased to 80 ± 2 % and 71 ± 4% in FHH.1BN and in our newly generated FHH.Add3 transgenic rats, respectively, when perfusion pressure was increased from 40 to 140 mmHg. In contrast, the MCA isolated from FHH and SD. Add3 knockout rats did not constrict. Moreover, we observed that the myogenic response is also impaired in MNS rats that share the same mutation in Add3 as the FHH rat and this phenotype complemented in a F1 cross of FHH and MNS rats. CBF rose by 99 ± 7%, 64 ± 5% and 42 ± 4% in FHH, FHH.1BN and FHH.Add3 rats, respectively, when MAP was increased from 100 to 190 mmHg, demonstrating that the impaired autoregulation of CBF in FHH rats was rescued when replacement with wildtype Add3. BBB leakage was greater in FHH rats than in FHH.1BN and FHH.Add3 rats after induction of hypertension with DOCA/salt. In contract to FHH.1BN and FHH.Add3 rats, hypertensive FHH rats exhibited marked neurodegeneration and vascular remodeling of the neocortex and hippocampus with a 33% reduction of both neuronal density and the size of neuronal cell bodies. Gap junctions between endothelial cells were larger in hypertensive FHH rats with neuronal, mitochondrial and myelin degeneration in the vacuolated area around the leaky capillaries. The hypertensive FHH rats took 2.5 times longer time to escape from an eight-arm water maze in comparison with FHH.1BN rats. These findings suggest that the K572Q mutation of Add3 contributes to the impaired myogenic response in the MCA and autoregulation of CBF in both FHH and MNS rats and that this may contribute to cerebral vascular disease and vascular cognitive impairments following the onset of hypertension.

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Poster

251. Higher Cognition

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 251.01/U11

Topic: F.01. Human Cognition and Behavior

Support: National Institute of Neurological Disorders and Stroke (R01NS078396)

US National Science Foundation (BCS1358907) to J.P.

Title: Exploring the neuronal population responses in the human intraparietal sulcus during real life speech processing

Authors: *X. YANG[1,2], A. DAITCH[2], J. SCHROUFF[2], S. GATTAS[2], S. DEHAENE[3], H. KOOPMAN[4], J. PARVIZI[2];
[1]Dept. of Neurol. and Neurolog. Sci., Stanford Univ., Stanford, CA; [2]Stanford Human Intracranial Cognitive Electrophysiology Program (SHICEP), Stanford Univ., Lab. of Behavioral and Cognitive Neurosci. (LBCN), Stanford, CA; [3]INSERM-CEA Cognitive Neuroimaging Unit, Collège de France, Paris, France; [4]Dept. of Linguistics, Univ. of California at Los Angeles, Los Angeles, CA

Abstract: We recorded electrocorticography (ECoG) signals in three human subjects implanted with intracranial electrodes in the lateral parietal lobe. We analyzed high-frequency broadband (HFB) or high gamma activity during an experiment, which required retrieval of autobiographical and arithmetic knowledge, and employed ROC curves to select optimal amplitude and duration parameters to identify the suprathreshold events as 'HFB peaks'. We then applied the same parameters to capture HFB peaks in the ECoG data during a condition in which participants listened to natural narrative stories and recalled the story facts with as many details as possible. Behavioral events of interest were determined as numerical words (numerals, ordinals, and quantifiers) in the stories heard or spoken by the participants. Electrodes that had shown significant increase of HFB activity during the experimental arithmetic condition exhibited only infrequent HFB peaks in the natural condition, most of which occurred at the times when the participants were processing numerical words in natural speech. Our analyses revealed that the IPS neuronal populations activated during the experimental arithmetic condition were also activated when participants were speaking or listening to numerical words in natural settings. Our findings also suggested that during speech comprehension the HFB responses in the IPS occurred after hearing numerical words, while most of the HFB peaks appeared early before the expression of numerical words during speech production. Our findings are in line with and confirm our previously reported findings in three other subject during spontaneous speech. Real life experience has a rich and constantly changing context that is shaped by the natural environment and human interactions, making it challenging to study the underlying brain dynamics during natural conditions. This study provides a means for both exploring the activity of a specific neuronal population in the human brain during a controlled experimental condition and the activity of the same population of neurons during real life settings.

Disclosures: X. Yang: None. **A. Daitch:** None. **J. Schrouff:** None. **S. Gattas:** None. **S. Dehaene:** None. **H. Koopman:** None. **J. Parvizi:** None.

Poster

251. Higher Cognition

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 251.02/U12

Topic: F.01. Human Cognition and Behavior

Support: NINDS Grant R01NS078396

NSF Grant BCS1358907

Title: Dynamics within and between ventral temporal and parietal cortices during numerical processing

Authors: *A. L. DAITCH[1,2,3], I. KASIKCI[1,2,3], B. L. FOSTER[1,2,3], S. GATTAS[1,2,3], V. RANGARAJAN[1,2,3], J. PARVIZI[1,2,3];
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Abstract: In every culture, arbitrary symbols are assigned specific meanings; for example numerals are associated with numerical quantities. While the ability to approximate and compare quantities is innate in infants and many other species, the ability to manipulate numerals to perform a computation is learned. Such computations likely engage a network of brain regions, from those processing visual features of each symbol, to those with more abstract numerical representations. Recent work from our lab has revealed an anatomical region within the human ventral temporal cortex (VTC) selective for visual numerals, compared to other morphologically, phonologically, or semantically similar stimuli. Additionally, human neuroimaging and animal electrophysiological studies have shown the involvement of the intraparietal sulcus (IPS) region in coding for a more abstract, amodal numerosity representation (i.e. “4” vs. “four” vs. “::”). To date, the fast temporal dynamics by which these regions interact with each other to interpret and manipulate visual numerals is not understood. In this study, we used direct cortical recordings in six human subjects (four with simultaneous VTC and IPS coverage), to track the fast temporal dynamics at and between VTC and IPS, as subjects read and manipulated visual numerals across several tasks, ranging from simple recognition to more complicated arithmetic computations, as well as during rest. We investigated the selectivity of each recording site to various stimuli (e.g. numerals vs. letters vs. false fonts), and their relative levels of engagement during different levels of numerical processing (e.g. reading versus active arithmetic). We computed the precise timing of activations at VTC and IPS to assess feed-forward versus feedback interactions. Finally, we measured resting state correlations between the VTC and IPS to see whether these regions are functionally correlated. We found that within VTC, there exist at least two distinct neuronal populations, both of which are engaged during active computations. One, more posterior region, exhibited an earlier latency response to numerals (~50-100 ms), but was less

selective for numerals relative to other similar visual stimuli. A slightly more anterior VTC region exhibited a later response to numerals (~100-200 ms), but was more selective for numerals relative to other stimuli, and exhibited higher activity during active manipulation of numerals than passive viewing. The activity at the anterior VTC region was also selectively correlated with that at IPS, both during task and rest conditions, highlighting in part the architecture of network interactions for numerical processing.

Disclosures: **A.L. Daitch:** None. **I. Kasikci:** None. **B.L. Foster:** None. **S. Gattas:** None. **V. Rangarajan:** None. **J. Parvizi:** None.

Poster

251. Higher Cognition

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 251.03/U13

Topic: F.01. Human Cognition and Behavior

Title: How the visual system constructs perceptions of two- and three-dimensional manifolds (flat, corrugated and curved surfaces and volumes) from zero, one and two dimensional building blocks

Authors: *E. L. ALTSCHULER[1], H. J. KIM[2], S. RONCATO[3];
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Abstract: The principles of the emergence of planar figures from dots, lines and two-dimensional figures is much better understand than how dots, lines and planes are perceptually organized by the visual system into two-dimensional flat and curved surfaces (manifolds) and three dimensional volumes (manifolds). In particular we have been studying when assemblies of line and planar shapes are perceived as being flat vs. a corrugated surface or a shape with volume. We have found that when the Gestalt principles of good continuity and closure are put in conflict, e.g., by an X-junction they "conspire" to produce corrugation. Also we have found that contrast polarity preservation (Parlangeli & Roncato, 2010; McCormick et al. & Altschuler, 2012; Altschuler et al. & Roncato, 2013) influences whether a corrugated surface vs. 3-dimensional manifold is perceived. Equally spaced outlined diamonds are perceived as planes spaced in depth. But the orientation of this set of planes is ambiguous-the perception is reversible. T-junctions can be used to disambiguate the orientation of the set of planes. Another cue we find that disambiguates orientation is providing thickness to the planes (diamonds (perceived as squares)) by adding in the form of shading making the perceived flat planar regions as parallelepipeds with height small compared with their length and width. Consider an equally spaced array of such parallelepipeds: Let the plane of the page be the XY plane with the depth (small) dimension of the parallelepipeds be in the X direction and the spacing between

parallelepipeds also be in the X direction. The large dimensions of the parallelepipeds are then along the Y-axis and the perceived into the plane Z-axis. When the spacing between parallelepipeds is made unequal-so that there is alternately very small spacing and large spacing between pairs of parallelepipeds-we have made the most intriguing finding that the parallelepipeds are not perceived as being collinear with unequal spacing along the X-axis, but equally spaced now pairs of parallelepipeds with each pair consisting of two parallelepipeds aligned along the Y-axis. Interesting this is not perceived when the parallelepipeds are replaced by (two-dimensional) narrow rectangles (small dimension again along the X-axis). Our finding may be helpful in producing easily perceived higher dimension manifolds.

Disclosures: E.L. Altschuler: None. **H.J. Kim:** None. **S. Roncato:** None.

Poster

251. Higher Cognition

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 251.04/U14

Topic: F.01. Human Cognition and Behavior

Title: The influence of color and vowel context on the perception of visual speech

Authors: *W. C. HOOKS, JR.[1], L. V. DEAL[2];
[1]OtoLing, Naperville, IL; [2]Communication Sci. and Disorders, Michigan State Univ., East Lansing, MI

Abstract: A premise of studies in speech perception has been targeted attention to the lips, teeth, chin, and cheeks. Yet, the amount of visually transmitted information has not been established for critical cues or their locations. Some individuals have distinguished /p, b, m/ visemes in different vowel contexts, but an explanation of the underlying visual characteristics has not been understood (Owens & Blazek, 1985). Anatomical and psychophysical evidence indicated clear subdivisions and parallel competition between magnocellular (magno) and parvocellular (parvo) cells (Livingstone & Hubel, 1987)—that is, magno neurons and the medial temporal (MT) cortex help us in the resolution of "where" we are looking for cues, whereas parvo neurons help in the identification of "what" cues we see. We know that the dual process theory partially describes how cone receptors respond in mutually opposite directions for red-green and blue-yellow. Our study probed whether interaction of opponent colors and vowel context would influence the recognition of /p, b, m/. Colors were scaled into Munsell notations and converted to Commission Internationale de L'Eclairage (CIE 1931) *y, x, z* coordinates for approximate wavelengths (λ) in nanometers (nm). A female talker with cosmetically applied opponent-colored lips [e.g., green upper/red lower (G/R) & blue upper/yellow lower (B/Y)] was videotaped in a television studio for broadcast quality playback in two experiments (Expt.). The talker spoke visually dissimilar vowel-consonant-vowel disyllables /ipi/, /ibi/, /imi/ in Expt. 1 and /apa/, /aba/, /ama/ in Expt. 2.

Six subjects viewed a mute video playback of the talker and generated 1,800 observations (per experiment) across five counterbalanced color conditions [R/G, G/R, B/Y, Y/B, and natural (NAT)]. The subjects' recognition of /p, b, m/ was assigned binary coding for logistic regression analysis. We assumed that the R/G and G/R would be influential because green ($\lambda = 565.8 \pm 0.2$ nm) approximated the human photopic luminosity peak ($\lambda = 555$ nm). Statistical significance was indicated for positive likelihood estimates in (R/G and B/Y), interaction effects for vowel (/i/), and phoneme (/b, m/). A composite analysis indicated statistical significance for (Y/B, B/Y, R/G, & NAT) and interaction effects between /p, b, m/, and the vowel /a/. We concluded that color interacted with vowel context as a relevant predictor for speech recognition. The data analysis suggested that specific colors could be used in the design of speechreading training. However, more research is needed to characterize eye movement and gaze patterns during speech recognition with opponent lip-color conditions.

Disclosures: W.C. Hooks, Jr.: None. **L.V. Deal:** None.

Poster

251. Higher Cognition

Location: Hall A

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Program#/Poster#: 251.05/U15

Topic: F.01. Human Cognition and Behavior

Title: Multivariate decoding of numerosity-evoked fMRI activity patterns in human parietal cortex is reflective of individual differences in number discrimination performance

Authors: G. LASNE[1,2], M. PIAZZA[1,3], S. DEHAENE[1], A. KLEINSCHMIDT[1,4], *E. EGER[1]; [1]INSERM Cognitive Neuroimaging Unit CEA/NEUROSPIN, Gif/Yvette, France; [2]Univ. Pierre et Marie Curie, Paris, France; [3]Ctr. for Brain/Mind Sciences, Univ. of Trento, Trento, Italy; [4]Dept. of Clin. Neurosciences, Univ. Hosp., Univ. of Geneva, Geneva, Switzerland

Abstract: The importance of parietal cortex for numerical processing is supported by a variety of methods. Recently, we have shown that beyond overall recruitment during numerical tasks, human intraparietal cortex contains information discriminative of individual numbers which can be detected by fMRI pattern recognition, in similar subregions to those where number selectivity of individual neurons is observed in macaque monkeys. However, it remains open in how far such differences in activation patterns elicited by different numbers are behaviorally relevant, e.g., whether they can reflect the acuity with which numbers can be behaviourally discriminated. Here, we measured discrimination of non-symbolic numerosities in 12 healthy young adult subjects outside the scanner psychophysically in a delayed number comparison task, where a given sample numerosity had to be compared with a match numerosity appearing 3 or 6 s later, resulting in a measure of the precision of each subject's behavioral discrimination performance (Weber fraction) for the different sample numerosities (8, 13, 21, or 34 dots). We then scanned

the same subjects during a similar paradigm and tested for multivariate discrimination of activity evoked by the different sample numbers seen and held in mind, in ROIs within parietal and early visual cortex, as well as within parietal cortex in the human functional homologues of areas LIP and VIP, as determined by a previous study. The different sample numerosities could be decoded above chance in both early visual and parietal cortex with approximately the same average accuracy. However, in parietal but not early visual cortex fMRI decoding accuracies were negatively correlated across subjects with the psychophysically determined Weber fraction, indicating that subjects with a more precise behavioral discrimination measured prior to scanning showed a better discriminability of evoked fMRI activity patterns. Furthermore, within parietal cortex this relation was strongest in the right LIP area. Thus, we demonstrate for the first time that the discriminability of fMRI activation patterns for individual numbers in parietal cortex can be reflective of a subject's behavioural accuracy for discrimination of those numbers. Although information discriminative of numerosities was present in both early visual and parietal cortex, our results suggest a crucial role for the latter region in supporting a number representation which is explicitly accessed for behavior.

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Poster

251. Higher Cognition

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Topic: F.01. Human Cognition and Behavior

Support: AFOSR Grant FA9550-12-10388

Title: Neural networks for pulse perception in musical rhythm

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Abstract: Entrainment of cortical rhythms to acoustic rhythms has been hypothesized to be the neural correlate of pulse and meter perception in music. Dynamic attending theory first proposed synchronization of endogenous perceptual rhythms nearly forty years ago, but only recently has the pivotal role of neural synchrony been demonstrated. Significant progress has since been made in understanding the role of neural oscillations and the neural structures that support synchronized responses to musical rhythm. Synchronized neural activity has been observed in auditory and motor networks, and has been linked with attentional allocation and movement coordination. Here we describe a neurodynamic model that shows how self-organization of

oscillations in interacting sensory and motor networks could be responsible for the formation of the pulse percept in complex rhythms. We test the model's prediction that pulse can be perceived at a frequency for which no spectral energy is present in the amplitude envelope of the acoustic rhythm. This model provides a theoretical link between oscillatory neurodynamics and the induction of pulse and meter in musical rhythm.

Disclosures: **E.W. Large:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Circular Logic, LLC. **J. Herrera:** None.

Poster

251. Higher Cognition

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Program#/Poster#: 251.07/U17

Topic: F.01. Human Cognition and Behavior

Support: US ARL ARO W911NF-13-1-0121

Title: Eeg based brain computer interfaces for numerical cognition

Authors: *V. MANIAN[1], O. NIEVES[1], J. CAMACHO[1], T. P. COLEMAN[2];
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Abstract: Electroencephalogram (EEG) is an effective tool for studying the complex dynamics of brain activities. It has been used widely to identify neuro cognitive problems. The goal of this work is to identify brain centers that show varied activity during the performance of a numerical cognitive task using a Brain Computer Interface (BCI). This will be used to identify subjects with cognitive problems. Cognitive problems have various causes such as side effects from prescription medication, alcoholism, emotions and stress, aging and cannabis abuse. In this work, we will investigate alterations in the EEG data collected when the subject performs a motor imagery (MI) based numerical cognition tasks. A 32 channel EEG cap will be used to acquire data during the performance of number comparison using MI. The goal is to identify the brain regions that have a probability of reduction in cognition. Different bandwidth will be investigated to see if they keep up sustained attention during the MI task. The BCI is implemented using Python and C++. It displays two random integers, the subject imagines left or right depending on which number is greater, the signals are collected over several trials. All the 32 EEG channels will be analyzed. Frequency domain and time-domain methods are widely used to assess EEG signals from varied subjects. Here, we will use time-frequency (TF) analysis methods by transforming time domain signal to a two-dimensional signal represented in time and frequency space. The short-time Fourier transform and Morlet wavelet transform are ways for

identifying characteristics at time and frequency (scale in case of Morlet) variations, and amplitude from EEG signal. The Energy and Entropy computed from the spectra will be used as indices to identify increased and slowly active regions. A threshold will be applied to discard signals with lower strength. The active centers will then be used for improving the subjects' performance in the BCI cognitive task. This in turn can lead to the use of BCIs for computer assisted cognitive behavioral therapy.

Disclosures: V. Manian: None. **O. Nieves:** None. **J. Camacho:** None. **T.P. Coleman:** None.

Poster

251. Higher Cognition

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Topic: F.01. Human Cognition and Behavior

Support: Chinese Scholarship Council

Frankfurt Institute for Advanced Studies

Max Planck Institute for Brain Research

Title: The neural substrate of the Eureka effect

Authors: *Y. LU[1,2,3,4], W. SINGER[1,2,3];
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Abstract: For the segmentation of complex scenes and recognition of objects, sensory evidence has to be compared with Gestalt rules and acquired knowledge about objects (priors) stored in the functional architecture of sensory systems. However, it is unknown how the brain knows when the computations required for the solution of a perceptual problem have converged to a valid result. The latency of this convergence depends on the sensory evidence and its match with prior knowledge and hence is highly variable. Here we investigate the neural dynamics associated with the convergence towards the result state by analysing with high density Electroencephalography (EEG) recording the activity patterns preceding recognition and comparing them with conditions when convergence did not occur. Subjects had to detect figures in cluttered scenes while we manipulated both sensory evidence and prior knowledge in order to obtain trials that did or did not converge towards a perceptual solution and subjects had to signal the convergence with a manual response. We find that the "Eureka" effect is associated with the emergence of coherent oscillations in the alpha band. The corresponding network comprises

widely distributed regions of the cortical mantle with a marked lateralization over the right hemisphere. These results suggest that the signature of the transition towards the solution of a perceptual task is associated with a transient increase of alpha coherence within an extended network.

Disclosures: Y. Lu: None. **W. Singer:** None.

Poster

251. Higher Cognition

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Program#/Poster#: 251.09/U19

Topic: F.01. Human Cognition and Behavior

Support: Brain and Behavior Seed Grant

Title: Neural correlates of musical improvisation

Authors: *K. DHAKAL[1], M. NORGAARD[2], M. DHAMALA[3];
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Abstract: Musical improvisation offers a unique model for the investigation of brain regions related to real-time creative behavior in which revision is not possible. Prior research shows contradictory results in which either activation or deactivation in the dorsolateral prefrontal region has been reported; thus, the role of this region in creative production is poorly understood. Because of confounding motor and perceptual variables in previous studies, this study incorporates a musical imagery task. Currently we are collecting and analyzing functional magnetic resonance imaging (fMRI) data from advanced jazz improvisers during an experimental improvisatory imagery task and comparing these results to a control task in which subjects imagine a pre-learned melody. The same contrast is investigated during vocalization of both improvised and pre-learned melodies allowing for behavioral data analysis. Four jazz melodies from the bebop era are used for vocalizing and imagining, as the complexity of these melodies is comparable to expected improvisations. We hypothesize that vocalizing and imagining an improvisation will produce similar patterns of activation. Our preliminary results show changes in activity that might be linked to improvisatory behavior, including the supplementary motor area, medial prefrontal cortex, dorsolateral prefrontal cortex, primary motor cortex, cerebellum, and premotor cortex. Brain activity during improvisation is distinctly higher and involving more regions than the performance of pre-learned melodies, both in the vocalization and imagine conditions. This suggests that musical improvisation is a complex creative behavior that may recruit a large network of brain regions. A deeper understanding of

the neural underpinnings of creativity could influence instructional strategies in both music and other domains.

Disclosures: **K. Dhakal:** None. **M. Norgaard:** None. **M. Dhamala:** None.

Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIH R01-MH094480

Title: Dynamics of auditory cortex during internal speech

Authors: ***M. REGEV**[1], U. HASSON[2];
[1]Neurosci., [2]Princeton Univ., Princeton, NJ

Abstract: Does listening to and imagining auditory stimuli elicit similar activity in the brain? Auditory imagination of speech has previously been studied by looking for spatially overlapping neural responses while listening and imagining punctate stimuli such as isolated words or short sentences. In the present work, we take an alternative approach using continuous, complex linguistic stimuli. This approach allows us to explore beyond basic spatial localization of averaged signals to comparisons of the temporal response profiles of listened and imagined speech. Subjects learned to sing a 4-minute narrative song accompanied by a metronome, practicing until high reliability in content and timing across repetitions was attained. Next, using fMRI, we recorded neural responses from the trained subjects in three conditions: 1) listening to a recording of themselves performing the song ("external speech"); 2) silently imagining the song to the rhythm of the metronome ("internal speech"); and 3) listening to the metronome alone without imagining the song ("control"). We directly compared the response time courses in the three conditions within auditory cortex. Neural time courses during internal speech and during the metronome control were significantly different despite sharing the same external auditory information (the sound of the metronome). In contrast, response time courses were remarkably similar for the internal and external speech conditions ($R = 0.53$, $p < 0.0001$). These results indicate that the auditory cortex is not simply active in response to internally generated speech, but also that it shows highly similar neural dynamics during processing of external speech as when imagining the same content.

Disclosures: **M. Regev:** None. **U. Hasson:** None.

Poster

251. Higher Cognition

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Topic: F.01. Human Cognition and Behavior

Support: Psychology Department Internal Costs Recovery Funding Award

Title: Spelling with Color? An investigation into the Bidirectionality of Synesthesia

Authors: *B. HACKNEY, J. F. AWAD, J. BUENROSTRO, S. A. DREW;
California State University, Northridge, Northridge, CA

Abstract: Synesthesia is a condition in which a single stimulus elicits multisensory experiences. Many cases studying grapheme-color synesthetes have reported synesthetic perceptions as unidirectional experiences: graphemes inducing colors, but not vice versa. In what few studies have examined the phenomena of bidirectionality (that is, colors inducing a grapheme percept), the focus has been on inducing the perception of numbers from colors. We instead aimed to examine the induced perception of letter graphemes from color. Subjects for this study included three synesthetes with matched controls. Stimuli consisted of two sets of color patches; the colors corresponded with the colored photisms each synesthete associated with letter graphemes. One set of color patches was arranged to correspond with a word and the other with a non-word. As an example, for a synesthete that associates red with “U” and blue with “P”, a red square followed by a blue square would represent the word “UP” while a blue square followed by a red square would represent the nonword “PU.” Participants completed a series of forced-choice tasks, with the instruction to select the set of color patches that corresponded with a word. Two of three synesthetes performed significantly better than chance, with all 10 non-synesthetic controls scoring around chance. These preliminary results suggest possible bidirectional synesthestheic percepts associated with letter graphemes.

Disclosures: B. Hackney: None. **J.F. Awad:** None. **J. Buenrostro:** None. **S.A. Drew:** None.

Poster

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German research foundation grant Nr. EXC307

Max Planck Society Germany

Title: Parietal cortex mediates perceptual grouping of dynamic stimuli independent of stimulus size

Authors: *P. R. GRASSI[1], N. ZARETSKAYA[1,2], A. BARTELS[1];
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Abstract: The perception of objects and scenes as unified structures is crucial for a meaningful interaction with the environment. To achieve this, the brain must interpret the retinal information received and combine local visual elements into coherent percepts. This process is referred to as grouping, spatial binding, or Gestalt perception. Despite the numerous approaches to study spatial binding, the neural mechanisms underlying this process are still largely unexplained. In a recent functional magnetic resonance (fMRI) and transcranial magnetic stimulation (TMS) study we showed that human parietal cortex was causally involved in mediating the conscious percept of global Gestalt from local moving elements [1]. We used a bistable motion illusion that causes spontaneous alternations between the perception of locally moving dots and of global planar motion of two illusory squares spanning all four visual quadrants. However, the local elements used in that study spanned a large visual space (about 10 visual degrees), which would potentially only be covered by the large receptive fields of parietal neurons. Here we asked whether other cortical regions with smaller receptive fields could also mediate spatial binding of moving elements when stimuli are sufficiently small. We used fMRI to measure activity over the whole-brain and in motion selective areas (V5/MT, MST, V3A and V6) in response to the bistable stimulus described above. The stimulus was presented in two sizes (large and small) to account for differences in receptive field sizes of neurons in the areas analyzed. Group whole-brain analysis revealed only two clusters in the parietal cortex involved in Gestalt perception. The results were indistinguishable for both, large and small stimuli, with no further regions involved. The analysis of independently localized motion responsive regions showed no differential response favoring global Gestalt perception. These results suggest that the posterior parietal cortex is exclusively involved in perceptual grouping of dynamic stimuli across both, large and small distances of visual space. Grouping is thus achieved by a receptive field size-independent mechanism mediated by the posterior parietal cortex. 1. Zaretskaya N, Anstis S, Bartels A. 2013. Parietal cortex mediates conscious perception of illusory gestalt. J Neurosci. 33:523-531.

Disclosures: **P.R. Grassi:** None. **N. Zaretskaya:** None. **A. Bartels:** None.

Poster

251. Higher Cognition

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Program#/Poster#: 251.13/U23

Topic: F.01. Human Cognition and Behavior

Support: NSERC

Title: Familiar size relationships decrease size-contrast illusion

Authors: *M. MALTSEVA, K. STUBBS, M. A. GOODALE, J. C. CULHAM;
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Abstract: The image of a large dog beside a small cat is familiar to us because it is congruent with our knowledge of the real world sizes of these animals. The familiar size effect, which is characterized by slower reaction times when judging the size of objects that are incongruent with their familiar sizes, suggests that previous knowledge of an object's real world size can influence visual perception. But does familiar size also affect the perceived size? Here we examined the effect of familiar size on the classic Ebbinghaus illusion in which a central image surrounded by larger images is perceived as smaller than it actually is (and one surrounded by smaller images is perceived as larger). Participants used a keyboard to adjust the size of the target image on a computer screen so that it matched the perceived size of the central image in an Ebbinghaus illusion. The central image was identical throughout all trials (a 25-mm-wide dog), but the surrounding images (i.e., annuli) could differ according to physical size (12 mm vs. 37 mm), semantic category (animate vs. inanimate), and familiar size (cat vs. horse for the animate category; shoe vs. car for the inanimate category). Importantly, the physical size relationship between the central and surrounding images was either congruent (e.g., dog surrounded by small shoes or large cars) or incongruent (e.g., dog surrounded by large shoes or small cars) with their familiar size relationship. Illusion strength was defined by the difference in the perceived size of the central image between the conditions with physically small and physically large annuli. The strength of the illusion was significantly weaker in the congruent conditions than the incongruent conditions. For example, a dog of constant size was perceived as much smaller when surrounded by large shoes compared to small cars but the difference in perceived size was much attenuated for a dog surrounded by small shoes compared to large cars. These results show that perceived size is affected not just by retinal size but also by familiar size relationships.

Disclosures: M. Maltseva: None. **K. Stubbs:** None. **M.A. Goodale:** None. **J.C. Culham:** None.

Poster

251. Higher Cognition

Location: Hall A

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Topic: F.01. Human Cognition and Behavior

Title: Neural oscillations and illusory rotatory motion: spatiotemporal analysis of neuromagnetic activities related to unusual visual perception

Authors: *A. TAKEDA[1], T. MAEKAWA[1], T. URAKAWA[2], T. OKAMOTO[3], K. OGATA[1], N. HIRONAGA[1], S. TOBIMATSU[1];
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Abstract: “Rotating Snakes (RS)” is a static picture that makes the observers perceive rotatory illusory motion. Previous studies have reported that luminance difference in the color arrangement of the image and microsaccades contribute to the production of the illusory motion perception. fMRI studies have also shown that area hMT/V5+ is activated when the participants perceive the illusory rotation. The aim of this study was to elucidate the spatiotemporal dynamics of neural activities related to the occurrence of the subjective unique illusory perception by magnetoencephalography (MEG). The RS image and control image without illusory motion were projected onto the screen in front of the healthy participants in a dim magnetic shield room for 5 s in a random order with 2 s inter-stimulus interval. Participants were required to report the perception of rotatory motion by pushing a computer mouse button as quickly as possible. Neuromagnetic activities were recorded by a 306-channel whole-head MEG system (Elekta Co. Ltd., Neuromag). The data were analyzed by time-frequency analysis with Wavelet transformation, and source localization was performed by a DICS beamformer method. We found that robust visual responses were elicited in the occipital region at around 150 ms after both stimulus onset, but there were no significant differences in the amplitudes and latencies between the two images. Regarding the results of the time-frequency analysis in grand-averaged data, α- and β-band oscillatory activities in the bilateral temporal regions tended to desynchronize beginning from around 400 ms before the participants reported the illusory motion. Furthermore, the sources of the desynchronized α- and β-bands were estimated in the areas including hMT/V5+ in each individual brain. Therefore, our results first demonstrated that neural activities related to the subjective recognition of illusory rotatory motion could occur at around 400 ms before reporting the perception. From our results and previous reports, this illusion could be related to the activation of area hMT/V5+.

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Poster

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PIFI-PROMEP-VIEP (Elias Manjarrez)

VIEP-BUAP MEBI-EDH-15 (Ignacio Mendez-Balbuena)

Title: Effects of mechanical tactile noise on the amplitude of visual evoked potentials

Authors: *I. MENDEZ-BALBUENA[1], N. HUIDOBRO[2], M. SILVA[2], R. CORRIPIO[3], A. FLORES[2], C. TRENADO[4], L. QUINTANAR[1], O. ARIAS-CARRION[5], R. KRISTEVA[6], E. MANJARREZ[2];
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Abstract: The aim of the present study was to demonstrate the electrophysiological occurrence of multisensory stochastic resonance (SR) in the human visual pathway elicited by tactile noise. We examined whether a particular level of mechanical Gaussian noise applied to the index finger can improve the amplitude of the visual evoked potential (VEP). The VEP was elicited by checkerboard pattern reversal stimulation. The level of vibrotactile noise inducing multisensory SR varied between 30 and 200 mN. For all eight subjects, the highest amplitude of the VEP P100 occurred in occipital areas, particularly at Oz (4/8), O1 (2/8), and O2 (2/8). We compared the amplitude of the positive P100 VEP component between zero noise (ZN), optimal noise (ON) and high mechanical noise (HN). The percentage of change for the averaged P100 VEP amplitude (ON versus ZN) for all subjects was 50.7 ± 9.7 % (mean ± SE). We performed the nonparametric Friedman test to examine the statistical significance of the change in the P100 VEP amplitude, between the three conditions (ON, ZN and HN) in all subjects. The results showed significant differences between the three conditions ($p<0.002$, df 2). The post hoc Wilcoxon test revealed statistically significant differences between ZN and ON ($p<0.008$) and between ON and HN ($p<0.008$). In contrast, no statistically significant differences were found between conditions ZN and HN. We performed an identical analysis for the signals recorded from the C3 electrode placed on the region overlying the somatosensory area. In this region, we did not observe P100 evoked responses to the visual stimuli like those observed in occipital areas. For evoked potentials (EP) recorded at C3 we did not observe evidence of SR type behaviour and also no significant differences between the three conditions (ZN, ON and HN, $p>0.5$, df 2). Our electrophysiological study extends the psychophysical experiments by Lugo et al., (2012) and Mendez-Balbuena et al., 2012 in the context of an EEG paradigm. Our results

demonstrate that tactile noise could potentially modulate the amplitude of a relevant VEP component (P100) by following the principles of the multisensory SR phenomenon.

Disclosures: I. Mendez-Balbuena: None. **N. Huidobro:** None. **M. Silva:** None. **R. Corripio:** None. **A. Flores:** None. **C. Trenado:** None. **L. Quintanar:** None. **O. Arias-Carrion:** None. **R. Kristeva:** None. **E. Manjarrez:** None.

Poster

251. Higher Cognition

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 251.16/U26

Topic: F.01. Human Cognition and Behavior

Support: Ecole des Neurosciences a Paris, Graduate Program

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Title: Color categorization and color knowledge after left occipitotemporal damage: behavioral and neuroimaging evidence

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Abstract: Humans group color, a continuous perceptual attribute, into discrete categories. Evidence about the neural locus of color categorization is contradictory across different studies involving healthy participants. Here, we compare color categorization to other aspects of color perception and cognition in a patient with a focal brain lesion. RDS is a 51-year-old patient who, after a stroke in the left occipitotemporal region, presented with right-sided hemianopia, alexia without agraphia and signs of associative agnosia and color agnosia. His visual mental imagery for orthographic material and for object form and color was spared. We assessed RDS's color perception with the Ishihara and Fansworth-Munsell 100 Hue tests, and used a set of tailor-made behavioral tests to assess color naming, categorization and knowledge. RDS also underwent fMRI localizers with images of words, numbers, houses, tools, body parts, faces, colored and gray-scale Mondrians, as well as objects in gray-scale, or colored with typical vs. atypical colors

(eg a green frog vs. an orange frog). RDS had no substantial problems in tasks of color discrimination, color constancy or color contrast. His object color knowledge was virtually intact. In contrast, color categorization and naming were severely impaired. RDS was unable to name color patches, to group colors in categories and to judge colors on category membership. fMRI revealed that the lesioned left ventral visual stream did not hold any residual functional abilities. However, images of houses, tools, body parts and faces did activate the typical specialized regions in the right hemisphere. Colored Mondrians contrasted with gray-scale Mondrians activated the right hemisphere V4, which has been suggested to engage in passive color perception. Typically colored images compared to grayscale images revealed activations in the right fusiform gyrus, anatomically consistent with the localization of area V4α engaged in high-order color processing; in the left superior temporal gyrus and in the left middle frontal gyrus, areas active in language processing and recently shown to engage in color processing. Behavioural and neuroimaging results showed that the patient has spared color discrimination and object color knowledge, but disrupted color categorisation. These results suggest that color categorisation is dissociatied from those other processes of color perception, and is most probably guided by different neural mechanisms. As RDS' lesion is left-sided, we conclude that those mechanisms of color categorisation are most probably located in the left occipito-temporal areas.

Disclosures: K. Siuda-Krzywicka: None. **C. Witzel:** None. **K. Moreau:** None. **S. Ferrieux:** None. **L. Cohen:** None. **P. Bartolomeo:** None.

Poster

251. Higher Cognition

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Topic: F.01. Human Cognition and Behavior

Title: Visual cortex activity predicts subjective experience after reading books with colored letters

Authors: *O. COLIZOLI[1], J. M. J. MURRE[2], H. S. SCHOLTE[2], D. M. VAN ES[3], T. KNAPEN[3], R. ROUW[2];
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Abstract: One of the most astonishing properties of synesthesia is that the evoked concurrent experiences are perceptual. Is it possible to acquire similar effects after learning cross-modal associations that resemble synesthetic mappings? In this study, we examine whether brain activation in early visual areas can be directly related to letter-color associations acquired by training. Non-synesthetes read specially prepared books with colored letters for several weeks

and were scanned using functional magnetic resonance imaging. If the acquired letter-color associations were visual in nature, then brain activation in visual cortex while viewing the trained black letters (compared to untrained black letters) should predict the strength of the associations, the quality of the color experience, or the vividness of visual mental imagery. Results showed that training-related activation of area V4 reflected the degree to which acquired letter-color associations had localizable color experiences, measured by the projector-associator dimension of grapheme-color synesthesia. In contrast, the strength of the acquired associations (measured as the Stroop effect) was not reliably reflected in visual cortex activity. The reported vividness of visual mental imagery was related to veridical color activation in early visual cortex, but not to the acquired associations. We show for the first time that subjective experience related to a synesthesia-training paradigm was reflected in visual brain activation.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: The effects of visual scene complexity on human cortex

Authors: A. J. BIES, J. B. WEKSELBLATT, C. R. BOYDSTON, R. P. TAYLOR, *M. E. SERENO;
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Abstract: Complex natural forms (e.g., mountains, trees, clouds) exhibit fractal characteristics yet there has been little systematic, neuroscientific investigation of human responses to fractal stimuli. Fractals are patterns that repeat at increasingly fine magnifications, and have fractal dimensions (D) that fall between Euclidian dimensions (e.g., a fractal line has a D value lying between a smooth line, D = 1, and a filled plane, D = 2). The visual complexity generated by the repeating patterns increases with their D value. The horizon lines of natural landscapes have D values that fall in the low to middle portion of the range $1 < D < 2$. It has been hypothesized that humans are predisposed to prefer environments and scenes with a level of complexity near that of the landscapes in which we have evolved. In support of this idea, recent research has shown that individuals' preference for particular natural scenes depends on complexity, with greater preference for low-to-mid range complexity as characterized by a scene's fractal dimension (Taylor et al., 2011). Because natural scenes do not vary across the entire range of complexity without changing content, we produced a set of abstract scenes that sample the full range of complexity quantified by the fractal dimension (viz., D = 1.1, 1.3, 1.5, and 1.9 for each of 16

patterns). In the current study, human participants viewed these computer-generated fractal images and rated the aesthetic value of each, during functional magnetic resonance imaging. We used a subtraction paradigm to isolate brain areas that were more active when viewing a particular dimension than the others. Primary visual cortex fell within the region more activated by viewing images with D = 1.9, whereas lateral occipital cortex (LO) fell within the area more activated by viewing images with D = 1.1. We created masks for contrasts 1.1 > 1.9 and 1.9 > 1.1, and found that brain activity in early visual areas (1.9 > 1.1) shows a significant, linear increase in activity as complexity increases, whereas later visual areas (1.1 > 1.9) show a significant, linear decrease in activity in response to increased complexity. Meanwhile, preference ratings do not scale linearly with complexity. Ratings were high and exhibited little change in the low-to-mid range of D, but were considerably lower at high D values. Thus, visually appealing fractal patterns of low-to-mid range fractal D stimulate higher-level visual areas such as LO, regions known to be involved in processing objects. This activity in object-processing areas provides a putative explanation for why certain patterns naturally evoke object percepts (e.g., perceiving faces or animals in clouds, rock formations, ink blots).

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Poster

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Title: Attention and motion-induced position shifts: evidence from visual neglect

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Abstract: When we look at bars flashed against a moving background, we see them displaced in the direction of the upcoming motion (flash-grab illusion). It is still debated whether these motion-induced position shifts are low-level, reflexive consequences of stimulus motion, or

high-level compensation engaged only when the stimulus is tracked with attention. To investigate whether attention is a causal factor for this striking position illusion, we evaluated it in six patients with damaged attentional networks in the right hemisphere and signs of left visual neglect and six age-matched controls. We presented a background motion of radial sectors centered at fixation, rotating at 180° per second and reversing direction every 660 ms. Importantly, the background was only visible in one half of the visual field, and was presented in different conditions in the top, bottom, right or left visual field. On each reversal of direction, two lines appeared briefly at the same location and both aligned horizontally (or vertically, depending on the condition) with the fixation point. However, due to the alternating background motion, they typically appeared shifted away from horizontal (or vertical) in opposite directions. Under instructions from the participants, the experimenter adjusted the locations of the lines to oppose any perceived offset until they again appeared to be superimposed, as the half-disk continued to rock back and forth. The amount of shift required to make the lines appear superimposed was the measure of the illusion strength. With stimuli in the top, right, and bottom visual fields, neglect patients experienced the same amount of illusion as controls. Strikingly, patients showed no significant shift when the test was presented in their left hemifield, even though they made equally precise judgments at this location. Thus, paradoxically, neglect patients perceived the position of the flash more veridically in their neglected hemifield. These results suggest that impaired attentional processes can reduce the interaction between a moving background and a superimposed stationary flash, and indicate that attention is a critical factor in generating the illusory motion-induced shifts of location.

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Poster

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Support: NIH Grant R01-MH43454

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Title: Dorsolateral prefrontal cortex, metacognitive awareness of emotional visual stimuli, and emotion regulation: a TMS/EEG study

Authors: *R. C. LAPATE, J. SAMAHA, B. ROKERS, A. AUSTERMUEHLE, H. HAMZAH, B. R. POSTLE, R. J. DAVIDSON;
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Abstract: Prior work suggests that awareness of emotional stimuli can benefit regulation of subsequent behavioral responses. For example, in the emotion misattribution paradigm, novel neutral faces are evaluated more negatively following unaware compared to aware exposures to negative facial expressions. Awareness is associated with the engagement of the prefrontal cortex, in particular dorsolateral prefrontal cortex (DLPFC), a region implicated in metacognitive awareness of simple visual stimuli, and in emotion regulation. Accordingly, less emotion misattribution during aware emotional processing correlates with DLPFC function. Here, we tested whether DLPFC causally promotes metacognitive awareness of emotional stimuli and prevents emotion misattribution by administering a transcranial magnetic stimulation protocol previously shown to inhibit cortical activation (theta-burst; tbTMS) to DLPFC, and to a control region (somatosensory cortex; S1). Following each application of tbTMS, 19 participants completed the emotion misattribution paradigm as well as assessments of face-stimulus awareness (face orientation & expression). Order of task and tbTMS target (DLPFC vs. S1) was counter-balanced across subjects. Using the method of constant stimuli at 6 contrast levels, we assessed both 'objective' (2-alternative forced choice stimulus judgments; 2AFC) and 'subjective' awareness (i.e., confidence ratings) of emotional faces, permitting the computation of an index of metacognitive accuracy, meta-d', at each contrast level, which reflects the correctness of subjective ratings given the accuracy of objective responses. To ascertain the effectiveness of the tbTMS protocol, we recorded resting state electroencephalography (EEG) before and after each tbTMS administration, and computed changes in power in the alpha frequency band (8-12 Hz). Alpha power at DLPFC electrodes increased following tbTMS to DLPFC (compared to S1), suggesting DLPFC inhibition. Critically, a valence by region interaction revealed that tbTMS to DLPFC (relative to S1) increased the influence of emotional faces on subsequent neutral-face judgments. While tbTMS did not significantly impact objective awareness metrics (2AFC performance or threshold), tbTMS region and contrast level interacted in predicting meta-d' for face orientation judgments, suggesting a reduction of metacognitive accuracy for faces shown at low contrasts. Collectively, these findings suggest that DLPFC plays a critical and causal role in promoting subjective awareness of complex emotional face stimuli and in attenuating their influence on subsequent behavior.

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Poster

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Title: Generic decoding of seen and imagined objects using hierarchical visual features

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Abstract: Brain decoding has achieved much success in neuroimaging analysis by reading out mental contents from fMRI activity patterns. However, the conventional classification-based approach has a fundamental constraint on the number of possible outputs: the outputs are limited to the number of the classes used in the training of a decoding model. While the image identification and reconstruction approaches enabled predictions about numerous states, including those not used for training the decoding model, they are designed for decoding retinotopically organized, image-level features. Here, we propose a method (termed generic object decoding) for decoding seen and imagined object categories including those that were not used in decoder training. Assuming that an object category can be represented by a set of mid-level visual features with invariances to position and/or rotation, we trained decoders to predict the features extracted by computer vision models or a deep neural network from fMRI signals measured while participants viewed natural images (150 categories). We examined whether a seen or imagined object that was not used for decoder training could be identified by comparing the predicted feature vector and object-specific feature vectors derived from annotated images in an online image database (15,322 categories). We performed this analysis for each combination of visual features (Convolutional Neural Network, HMAX, GIST, and SIFT) and brain regions of interest (V1-V4, LOC, FFA, and PPA). The trained decoders successfully predicted the values of individual features, making it possible to identify objects from fMRI signals with most of the feature-ROI combinations. Higher-order visual features tended to be better predicted from fMRI signals in higher than lower cortical areas (and vice versa). Mid-level features predicted from higher cortical areas were most useful in identifying object categories. Furthermore, imagining about an object category was sufficient to induce brain activity that was predictive of mid-level visual features and to perform object identification at a statistically significant level. Our results demonstrate that the decoding model trained on a limited set of object categories generalizes to decode arbitrary object categories. The successful identification with brain activity during imagery suggests that feature-level representations elicited in visual perception may also be recruited during top-down visual imagery. Our approach may provide a basis for a brain-based information retrieval system by translating brain activity into words/concepts.

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Poster

251. Higher Cognition

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Title: Progressive enhancement of phase synchronization in fronto-occipital regions during visual perception

Authors: *M. HAYAKAWA, K. AKIBA, Y. MORISHITA, Y. KAKIMOTO, O. ARAKI; Dept. of Applied Physics, Tokyo Univ. of Sci., Tokyo, Japan

Abstract: We can identify objects accurately from an incomplete picture involving them. To identify objects from limited information in the object perception process, visual disambiguation process is necessary. Success in the object identification should be guided not only by visual inputs but also by much top-down information. In many previous studies, degraded visual stimuli were used as insufficient visual inputs to induce the visual disambiguation process. These results showed that the cortical and subcortical areas were relevant to the visual disambiguation process. Moreover, electroencephalography (EEG) and fMRI data indicated the enhancement of functional connections between various cortical regions at the timing of cognition. However, it is still unknown about the dynamics in functional connections before and after the object recognition. We aimed to solve this issue by recording EEG of 14 subjects during a two tone (TT) image perception task. TT image might look meaningless at first sight, but the objects in it will gradually appear. Degradedness of the TT image presented to a subject was gradually changed over 10 levels from hard to easy by adjusting the threshold value. Each image of a level was presented once for 200 ms, and mask screen was presented for 1000 ms between each image. This intermittent presentation makes it easy for us to capture the timing of object recognition. Subjects were instructed to press a button as soon as possible, if they can identify objects in the presented TT images. We analyzed EEG data and calculated Phase locking value (PLV), which was used as an index of strength of functional connections. As a result, beta and gamma band PLV enhancement (increase of phase synchronization) between the frontal and occipital areas was observed in 100 ms after the onset of the TT image to which the subject has responded. Furthermore, the PLV enhancement was also observed in the previous presentation of an image which subjects have not recognized yet. The PLV in the block (B0) which the subject responded was more enhanced than that in the previous block (B1). Additionally, the PLV in the B1 block was more enhanced than the second preceding one (B2). The results suggest that beta and gamma band phase synchronization between fronto-occipital regions is correlated with visual disambiguation process. Furthermore, the phase synchronization enhancement may reflect a higher brain function accompanied by flow of information between cortical areas.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIH R01EY022605-01

Title: The temporal sequence of cortical activity during probe-induced binocular rivalry

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Abstract: Binocular rivalry occurs when highly dissimilar images are presented to the eyes. Perception alternates between the two images such that at times one image dominates awareness while the other is suppressed from awareness. Prior research has shown that brief, yet salient probes presented to the suppressed eye can cause a rapid switch in perceptual dominance. Previously we showed that these switches (i.e. reversals) are mediated by P1 and P3b ERP components (Metzger et al., VSS 2014) (Metzger et al., CNS 2015). In particular, we showed that P1 and P3b amplitudes increased monotonically as a function of reversal latency (from the suppressed-eye probe) such that faster reversals were associated with larger ERP amplitudes. These data suggest that the speed with which perception switches is determined by the degree to which the probe is initially processed (as indexed by the P1 amplitude) as well as the degree to which the probe forces a reallocation of attention (as indexed by P3b amplitude). Here, we used fast event-related optical imaging (recorded concurrently with EEG) to ask what cortical areas differentially respond to slow- and fast-switching suppressed-eye probes. Based on the observed ERP data, we would predict an early difference in a similar time window as the P1 and a later difference in parietal attention areas, in keeping with the P3b effects we observed. The EROS data showed greater activity for fast-switching suppressed-eye probes relative to slow-switching suppressed-eye probes in early visual cortex from 127-153 ms following the onset of the probe, a time frame that is comparable to the observed P1 ERP activity. In addition, activity in right superior parietal cortex was greater for fast-switching suppressed-eye probes in two distinct time windows that fell within the P3b window: at approximately 281 and 665 ms. following probe onset. Both time windows of activity precede the perceptual transition, which for the fastest switches occurred at approximately 900 ms. Taken together, the data suggest that the speed with which switches occur depends first on the saliency of the probe (as indexed by activity in early

visual cortex), which then influences the probability the probe will reallocate attention to the image in the suppressed eye (as indexed by activity in right parietal cortex).

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Poster

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Title: Differential cortical responses to high- and low-Level visual features of naturalistic movie stimuli

Authors: *S.-C. HUNG, K.-H. LU, H. WEN, Z. LIU;
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Abstract: Previous functional magnetic resonance imaging (fMRI) studies have shown that human cortical activity can be highly reliable within and across individuals while responding to complex and dynamic naturalistic stimuli. However, to what extent the cortical synchronization is evoked by different levels of visual features embedded in the natural stimuli still remains unclear. Also, whether or not the reliability of cortical response was affected by the eye movement is unknown. Here, we (i) scrambled a black-and-white movie by perturbing the high-level features of visual representations while preserving the low-level visual features, and (ii) employed the eye-tracking technique in fMRI experiments to assess the contribution of eye movement to blood-oxygen-level-dependent (BOLD) signals. Our results show that the cortical response to the low-level features of the movie is primarily revealed in the early visual cortex, while the intact movie evokes broader cortical response into higher-order association areas. These differential cortical patterns hold both across stimuli repetition and across subjects. Critically, the results are robust to the degree of coherence of eye movement under a free-viewing condition. Taken together, our data demonstrate reliable intra- and inter-subject synchronization of cortical activity in response to hierarchical levels of visual features during naturalistic stimulation. We suggest that the human cortical areas can reliably function in a hierarchical manner under a more realistic condition.

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Poster

251. Higher Cognition

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NYU Center for Brain Imaging

Title: Visually-responsive regions around the right TPJ and attentional reorienting

Authors: *L. DUGUÉ, E. P. MERRIAM, D. J. HEEGER, M. CARRASCO;
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Abstract: Purpose: The right temporal-parietal junction (TPJ) has been proposed to play a central role in the attentional reorienting system (Corbetta et al., 2008). We tested the hypothesis that reorienting both endogenous (voluntary) and exogenous (involuntary) visual attention is mediated by visually selective regions within and around the TPJ. **Approach:** Cortical responses were measured using fMRI (8-ch phased-array surface coil, 2x2x2.5 mm, 28 slices covering temporo-parietal regions), while observers performed a two-alternative forced-choice orientation discrimination task. Two sinusoidal grating stimuli briefly appeared in the lower left and right quadrants of the visual field. A central response cue at the end of each trial indicated which of the two gratings was the target. Observers reported the orientation of the target stimulus (clockwise or counterclockwise relative to vertical) with a button press. In the endogenous attention task, a central precue instructed observers to attend to one of the two stimulus locations. In 75% of the trials the cue and target locations matched (valid), and in the remaining trials they did not match (invalid). In the exogenous attention task, a peripheral cue automatically attracted attention to one of the two stimulus locations. The cue was non-informative (valid in 50% of the trials). For both endogenous and exogenous visual attention tasks, based on the reorienting hypothesis, we predicted greater activity for invalid trials (that require reorienting at the end of each trial) than for valid trials (that do not require reorienting), in visually-responsive regions. **Results**: Three visually-responsive regions were identified within and around the right TPJ. The first matched a region called vTPJ (Horiguchi et al., 2014). The second region was located at the most caudal portion of the STS, just posterior to vTPJ and anterior to MST. The third region was located in posterior insula, just anterior to vTPJ. Each of these three regions was 0.5-1.0 cm^3 in volume, averaged across observers. Within each region, average activity was measured for valid and invalid trials, independently for endogenous and exogenous attention conditions. We found that in both the vTPJ and STS regions, activity was higher for invalid than valid trials, for both endogenous and exogenous attention tasks. In the insula region, there was no difference in activity between valid and invalid trials in either task. **Conclusion**: vTPJ and a visually-

responsive region within posterior STS participate in the reorienting of attention in both endogenous and exogenous visual attention tasks.

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Poster

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NSF 1353571

Title: Behavioral evidence that skin reflectance is used as an illumination cue for color constancy

Authors: ***R. LAFER-SOUSA**[1], K. L. HERMANN[1,2], B. R. CONWAY[1,2];
[1]BCS, MIT, Cambridge, MA; [2]Neurosci., Wellesley Col., Wellesley, MA

Abstract: The dress in #TheDress photograph is typically perceived as either blue/black (B/K), white/gold (W/G), or blue/brown (B/B) (Lafer-Sousa et al., Curr Biol, 2015). We hypothesize these percepts arise because the illumination cues in the image are ambiguous and individuals infer either a warm, cool, or neutral illuminant. By embedding the dress in a chromatically tinted scene on a model with chromatically tinted skin to make the color of the illuminant explicit (expt. 1), we found that regardless of how subjects initially perceived the dress' colors, their percepts conformed to a percept predicted by the color of the illuminant (warm illuminant: 77% of subjects report B/K; cool illuminant: 83% of subjects report W/G; N = 53). Could these results be attributed simply to chromatic contrast? We tested this hypothesis by probing percepts of the dress' color in images in which we surround the dress by a uniform field of average chromatic bias matching that used in the first experiment; we ran separate experiments in which the dress was presented on a model with skin tint or by itself. When presented without the model on a uniform chromatic background (expt. 2), less than 50% of subjects conformed to the percept predicted by the color of the background, significantly fewer than did in the first experiment (warm conditions: $p = 3*10^{-6}$, cool conditions: $p = 8*10^{-5}$; McNemar's chi-squared test). But when skin tint was the only cue (i.e. the dress and model presented on a white background, expt. 3), reports were predicted by the color of the skin tint (warm: 70% of subjects report B/K, cool: 79% of subjects report W/G). The extent to which subjects conformed to the percept predicted by the illumination did not differ between expt. 1 and 3 (the stimuli were equally effective) (warm conditions: $p = 0.6$, cool: $p = 0.1$), but did between expt. 2 and 3 (skin tint was more effective than color-contrast) (warm conditions: $p = 10^{-4}$, cool: $p = 10^{-4}$). These results suggest people use

skin chromaticity to recover the illuminant and compute object color, providing behavioral evidence to support modeling experiments showing that skin could be used as a cue to illumination for achieving color constancy— skin color may be useful for color constancy because the gamut of human skin has a distinctive profile in cone-contrast space (across skin types) that shifts predictably under varying illuminations (Crichton et al., CGIV, pp. 266, 2012; Bianco & Schettini, CVPR, pp. 65, 2012). People are likely to have a robust internal model of skin color and how it varies with illumination because we carry our skin with us wherever we go and skin color is a behaviorally relevant feature (e.g. face color signals emotional and health status).

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Title: Neural coding of navigational affordances in visual scenes

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Abstract: An essential component of visually guided navigation is the ability to perceive features of the environment that afford or constrain movement. For example, in indoor environments, walls limit one's potential routes, while passageways facilitate movement. Here we attempt to identify the cortical mechanisms that encode such navigational features. Specifically, we test the hypothesis that scene-selective regions of the human brain represent navigational affordances in visual scenes. In an fMRI experiment, subjects viewed images of artificially rendered rooms that had identical geometry as defined by their walls, but varied on the number (one to three) and position (left, right, center) of spatial passageways (i.e., open doorways) connected to them. The layout of these passageways defined the navigable space in each scene. Several versions of each layout were shown, each with the same set of passageways but different textures on the walls and floors. Furthermore, half of the rooms were empty except for the walls and passageways, while the other half included visual clutter in the form of paintings along the walls. The paintings were similar in size and shape to the passageways. Images were presented for two seconds while subjects maintained central fixation and performed an unrelated color-discrimination task on two dots overlaid on each scene. Using multivoxel

pattern analysis, we identified representations of navigational layout that were invariant to other visual properties of the images. This analysis revealed a consistent representation of navigational layout in the occipital place area (OPA), a scene-selective region near the transverse occipital sulcus. In this region, information about navigational layout could be decoded across changes in texture or visual clutter (i.e., paintings on the walls) even though the overall spatial geometry was the same for all scenes. These findings suggest a mechanism in the OPA for coding fine-grained information about the layout of navigational space.

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Poster

252. Cognitive Development

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Title: Neuroconnectivity in human fetuses subsequently born preterm

Authors: *M. E. THOMASON[1,4], D. SCHEINOST[5], J. H. MANNING[2], L. E. GROVE[2], P. K. JELLA[3], K. M. HERMEZ[2], J. L. HECT[2], S. JIRJIS[2], R. T. CONSTABLE[5], L. R. MENT[5], R. ROMERO[4];
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Abstract: Many infants born preterm demonstrate mild to severe motor abnormalities. It is difficult to ascertain whether motor impairments result from altered neurological development *in utero*, as opposed to secondary injury. Functional connectivity mapping via resting state fMRI can measure fetal brain function before birth. It is thus possible to identify alterations in neural networks that present before birth to ascertain origins of neurological anomaly associated with preterm birth. We tested the hypothesis that altered motor network development precedes preterm birth. We enrolled pregnant women, some of whom were high-risk for preterm delivery, but who were experiencing otherwise healthy pregnancies. We compared 18 fetuses born prior to 37 weeks to 18 term-delivered fetuses matched on age, gender and motion during MRI. Postmenstrual gestational age (GA) at the time of the scan and sex of fetuses subsequently born preterm (PT) were 29+5 (weeks+days; SD=3+4), 11 males; age and sex of term-born control fetuses (TC) were 30+1 (SD=3+3), 10 males; study sample range was 22-36 weeks GA. We measured brain function *in utero* using non-invasive resting-state functional MRI. Functional data were co-registered to a 32-week gestational age fetal template. Seed-based connectivity

analyses were used to compute signal covariance maps from 5 regions in the left motor cortex and cerebellum, and in one control region. All analyses were conducted at uncorrected $p \leq 0.001$ and $k \geq 10$. We observed increased functional connectivity in TC compared to PT fetuses from motor and cerebellar seeds. Between group differences were greatest in distant brain regions, suggesting that PT fetuses lack long-range connectivity. In contrast, increased short-range connectivity was observed in PT fetuses, providing some evidence for a trade-off in local over distal connectivity in the PT fetus. Differences between groups were smaller in the parietal network that served as a control, indicating specificity in these observations. Here, we shift the timeline for discoveries about functional brain maturation to a time of exceedingly rapid change, the fetal period, when foundations for brain circuitry are being laid. For the first time we report altered neural connectivity in fetuses that will be born preterm. Reduced long-range and cross-hemispheric connectivity in PT fetuses indicates that deficient neuroconnectivity in the prenatal period precedes disturbances in motor learning in infancy. Scientific data that can non-invasively operationalize functioning of the fetal brain has the potential to transform our understanding of this sensitive and defining period in human life.

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Poster

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Title: Family nurture intervention alters relationships between preterm eeg coherence and 18 month neurodevelopmental outcome

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Abstract: Background: Despite decreased mortality and morbidity, prematurely born infants remain vulnerable to a broad range of developmental disabilities, including increased risk for autism. A randomized controlled trial (RCT) of the Family Nurture Intervention (FNI) in the

NICU, designed to enhance the emotional relatedness between prematurely born infants and their mothers, demonstrated that FNI led to robust increases in EEG power and decreased coherence at term age in the frontal polar region of the brain when compared to infants receiving standard care (SC). Both findings are consistent with FNI promoting brain maturation. Objective: This analysis assessed the correlation between EEG coherence in preterm infants measured at term age and neurodevelopmental outcomes at 18 months. Design/Methods: Infants (26-34 weeks postmenstrual age) were enrolled in an RCT testing efficacy of FNI. FNI is delivered via nurture specialist-facilitated sessions between the mother and her infant. Activities include odor cloth exchange, vocal soothing, comfort touch and skin-to-skin and wrapped holding. At 18 months corrected age, Bayley's III and Modified Checklist for Autism in Toddlers (MCHAT) were used to assess cognition and language, and risk for autism, respectively. Results: In SC infants (N=17), term age EEG coherence was negatively correlated with 18 month cognition and language and positively correlated with risk for autism spectrum disorder (ASD). In FNI infants (N=34) there was no relationship between EEG coherence and outcome. Conclusion: The results from SC infants suggest that high coherence in brain regions involved in language, cognition and emotion regulation predicts poorer outcomes in these domains at 18 months. This indicates that in SC infants, measures of EEG coherence may provide an early marker to identify which preterm infants are in greatest need of intervention. Our prior studies showed that FNI infants have lower frontal coherence at term age and improved neurobehavioral outcomes at 18 months. Here we find that in contrast to SC, FNI appears to abrogates the impact of high coherence at term age on later outcomes. It may be that FNI promotes maturation of other processes that become more significant determinates of outcome than term age coherence. It may also be that the relationship between coherence and outcome in SC infants becomes less robust at later stages of development and that FNI has advanced infants into this more mature stage.

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Poster

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ITN- 2013-606901

Title: Visual short term memory capacity in preterm born adults is associated with fractional anisotropy in posterior thalamic radiations and splenium of the corpus callosum

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Abstract: Preterm birth is associated with increased risk for lasting cognitive impairments particularly in attention. It was shown recently that reduced visual short term memory (vSTM) capacity in preterm born adults was linked with changes in intrinsic functional connectivity in a way that suggested a compensatory reorganization of posterior intrinsic brain networks. However, the relationship to the severe white matter changes that are known to result from preterm birth is unknown. Thus, in the current study we related vSTM storage capacity functions in preterm and term born adults to white matter fractional anisotropy (FA) as assessed by diffusion tensor imaging. We used whole and partial report paradigms of briefly presented letter arrays based on the computationally specified Theory of Visual Attention (TVA) to assess the parameter visual short term memory capacity in 26 preterm and 21 full-term born adults. Based on the neural interpretation of TVA - the NTVA - the posterior thalamic radiations and splenium of Corpus Callosum (CC) were defined as regions of interest (ROI). Using tract-based spatial statisctics (TBSS) for these ROIs, we found that FA values in right posterior thalamic radiation were significantly reduced in preterm born adults. In both groups, vSTM capacity was significantly correlated with FA in right posterior thalamic radiation. In term born adults, the correlation was positive but in preterm born adults higher vSTM capacity was related to lower FA. In preterm born adults, furthermore, higher vSTM capacity was significantly correlated to higher FA in splenium CC. These results indicate that, in preterm individuals, an impairment of posterior thalamic radiations integrity might lead to the recruitment of splenium CC as a compensatory tract relevant for vSTM storage functions.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: Neural and behavioral markers of metacognititive sensitivity in infants

Authors: *S. KOUDIER, L. GOUPIL;
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Abstract: Responding optimally to the external world involves not only selecting among alternative options, but also reflecting upon one's own choices to evaluate their accuracy and adapt subsequent behaviour. Whether this capacity for metacognition is already present in infancy remains unknown. Past research showed a rather late development of metacognitive capacities. Yet, these studies were based on children's report about their own mental states. Here, we investigated the possibility that basic metacognitive mechanisms such as decision confidence and error detection are already present in infancy, although they would be discernible through implicit behavioural measures and neural signatures rather than explicit self-reports. Here, infants first performed a binary choice (i.e., pointing or fixating towards the correct/incorrect location of a hidden object). Next, infants were probed for differential overt behavior (e.g. persistence in their choice, change of mind) or electrophysiological responses (e.g., error-related negativity) following correct vs. incorrect decisions. A first study with 18 month-old infants (N = 29) showed that infants compute decision confidence in a manual search paradigm, by relying on post-decision search persistence as a proxy for metacognitive monitoring. A second study (N = 22) showed that they can use confidence to flexibly adapt their behavior, hence providing evidence of metacognitive control. Infants, after making a first choice, had to make a second decision which consisted either in persisting in their initial choice by asking for help, or in changing their mind towards the alternative choice. Crucially, this experiment revealed that their decision to ask for help vs. change their mind depends on the accuracy of their initial choice, revealing that they can not only evaluate their own performance, but also use this information to guide subsequent actions. In a third study relying on high-density electroencephalography (EEG), we show that 12 month-old infants (N = 55) evaluate the correctness of their anticipatory eye movements. Furthermore, we show that infants elicit the equivalent of an error-related negativity, an EEG component observed over fronto-central electrodes in human adults whenever they make an error. This demonstrates not only that infants can detect their own errors after making a decision, but also that they rely on the same mechanisms as those involved when adults detect their own errors. We propose that although explicit metacognition develops much later, it stems from implicit metacognitive abilities that are already present in infants.

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Poster

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Topic: F.01. Human Cognition and Behavior

Title: Neural dynamics of prediction and surprise in infants

Authors: ***S. V. GELSKOV**[1], B. LONG[2], L. LE STANC[1], S. CHARRON[1], L. S. BARBOSA[1], A.-C. FIEVET[1], S. KOUIDER[1];
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Abstract: Perception is not only determined by external stimulation, but also by our internal beliefs and predictions about the nature of upcoming events. In adults the neural mechanisms of predictive coding have been extensively studied. While behavioral studies have suggests that infants can also rely on probabilistic inferences, neural mechanisms underlying these computations have yet to be demonstrated during early development. To address this issue, we studied how the infant brain implements predictions. We combined high-density EEG recordings with a cross-modal cueing paradigm in which auditory cues acted as predictive signals to form expectations about upcoming visual events. Twenty-eight 12-month-old infants received one of two arbitrary sounds that were predominantly associated with one of two visual categories (i.e., faces vs. flowers). When collapsing across categories, scalp voltages over occipito-temporal sites revealed a series of well-known face-and object related components including the early P1, intermediate P400 and finally a Late Slow Wave (LSW). Consistent with Bayesian accounts of perception, we found an increased neural response for unexpected events. However, this effect of neural surprise was observed only during late processing stages. Interestingly, the LSW in infants has recently been found to share the same characteristics as neural markers of consciousness in adults. Early perceptual components, by contrast, revealed an amplification of neural responses for predicted rather than surprising events, suggesting that selective attention enhances perceptual processing for expected events. Taken together, our study shows 1) that cross-modal statistical regularities can bias sensory responses in the infant brain, revealing the neural mechanisms underlying the use of predictive signals by the end of the first year of life, 2) that the neural impact of predictive cues follows different dynamics depending on whether target stimuli were predicted or instead surprising, and 3) that there is a privileged link between infants' reaction to surprising events and conscious processing.

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Poster

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Topic: F.01. Human Cognition and Behavior

Support: NIH 5R01HD047520-10

Title: Interactive Specialization and the development of functional systems support complex cognitive skills in children

Authors: *C. J. BATTISTA[1], T. NGOON[2], T. CHEN[2], L. CHEN[2], A. BAKER[2], J. KOCHALKA[2], T. EVANS[2], V. MENON[2];
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Abstract: Functional brain development is thought to involve cascading set of interactions between brain regions as they acquire new functional roles (Johnson, 2000; 2001; 2011), and studying this process requires a longitudinal design in order to avoid confounding effects from age and ability. To provide a more accurate characterization of development, we extended the framework of interactive specialization framework to investigate age-related changes in connectivity and activity that support mental arithmetic in children. We focus on the intraparietal sulcus (IPS), a region that plays a critical role in numerical cognition (Dehaene, 2003; Menon, 2014). Using data from 33 children between the ages of 8-14 (2-4 timepoints per child), we constructed hierarchical linear models of growth of task-based activity and connectivity (gPPI) during arithmetic problem solving to identify functional circuits undergoing change across our age range. We found linear decreases in left IPS connectivity with left prefontal cortex (dorsolateral PFC, ventrolateral PFC, and insula) and increases in connectivity with right superior parietal lobule, right IPS and right fusiform gyrus (FG). Activity in FG increased over time. To investigate the interaction between the formation of intraparietal, fronto-parietal, and dorso-ventral circuits, we constructed growth models of connectivity using bilateral IPS connectivity as a covariate. This revealed a link between changes in bilateral IPS connectivity and changes in fronto-parietal, but not dorso-ventral circuits. Specifically, individuals with higher left-right IPS connectivity also had higher fronto-parietal connectivity at the age of 8, which then decreased as left-right IPS increased over development. Brain-behavior relationships were also found: (a) bilateral IPS connectivity was related to gains in visuospatial working memory; (b) initial arithmetic ability (assessed by the Numerical Operations subtest of the WIAT-III) related to change in bilateral IPS connectivity, with high performers showing greater connectivity than low performers, with this difference decreasing with age. Taken together, these results suggest that development of the neural systems supporting cognitive skills requires the dynamic reconfiguration of several functional circuits, and that these changes reflect the result of multiple, rather than a single, maturational process. Critically, these changes relate to both arithmetic and working memory performance. Our findings provide new quantitative insights into interactive specialization and the development of functional brain systems supporting complex cognitive skills in children.

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Poster

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Title: Brain activity during expectation violation triggered by magic tricks

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Abstract: Prior knowledge is essential to survive in a complex environment. Humans rely on their prior knowledge to predict the outcome of actions. This makes the case of magic so interesting: Magic tricks violate the expected action-outcome sequences. We aimed at identifying the neural correlates of such expectation violations by contrasting 24 video clips of magic tricks with 24 control clips in which the expected action-outcome relationship is upheld. Using fMRI, we measured the brain activity of 25 normal volunteers while they watched the clips in the scanner. Additionally, we measured the professional magician who had performed the magic tricks under the assumption that, in contrast to naïve observers, the magician himself would not perceive his own magic tricks as an expectation violation. As main effect of magic - control clips in the normal sample, we found higher activity for magic in the head of the caudate nucleus bilaterally, the left inferior frontal gyrus and the left anterior insula. As expected, the magician's brain activity substantially differed from these results, with mainly parietal areas (supramarginal gyrus bilaterally) activated, supporting our hypothesis that he did not experience any expectation violation. These findings are in accordance with previous research that has implicated the head of the caudate nucleus in processing changes in the contingency between action and outcome.

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Poster

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Title: Development of medial prefrontal cortex is related to statistical learning and inference

Authors: K. F. GUARINO[1], M. L. SCHLICHTING[1], A. C. SCHAPIRO[2], N. B. TURK-BROWNE[2], *A. R. PRESTON[1];
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Abstract: Recent advances highlight the importance of a hippocampal-medial prefrontal (MPFC) circuit for memory integration across related experiences. Evidence suggests that abstracted representations in MPFC guide reactivation of related memories during encoding of new events, promoting hippocampal-mediated integration and generalization. For instance, both hippocampus and MPFC have been implicated in novel inference judgments that require consideration of the relationships among distinct episodes, and in a form of statistical learning, in which participants must extract temporal regularities from the environment by associating information across time. However, the protracted developmental trajectory of prefrontal cortex suggests that while hippocampus may support associative learning early in development, MPFC-guided integration may only reach maturity in adulthood. Here, we used high-resolution structural MRI to quantify developmental differences in MPFC structure, specifically cortical thickness of MPFC, in participants aged 6-30 years, further linking these differences to the development of memory integration. We aimed to identify the role of particular MPFC subregions in memory integration, as MPFC has largely been considered as a single region in prior work. Participants also completed two behavioral tasks, involving associative inference and statistical learning, respectively. Performance on both tasks improved into adulthood, suggesting that children do not integrate across events as effectively as adults. The cortical thickness of MPFC subregions decreased across the age range, consistent with protracted thinning of this region. Moreover, cortical thickness of MPFC was related to associative inference and statistical learning performance in the adult group, suggesting that this region plays an important role in memory integration when mature. This brain-behavior relationship was unique to posterior MPFC. These results suggest a fundamental shift in memory integration over development: Adults may integrate across events through the engagement of a hippocampal-MPFC circuit, but children may have to rely on hippocampal mechanisms alone and thus may be limited to encoding separate memories of related events.

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Poster

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Title: Neural correlates of spatial navigation in young children: Linking brain volumetry and performance on a virtual Morris Water Maze task

Authors: *Q. YU, A. SHAFER, D. ANDERSON, A. HEITZER, J. PIERCY, D. BRUSH, W. ANGELL, S. RAZ, N. OFEN;
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Abstract: Navigation through space is a complex cognitive task supported by a number of brain regions including the prefrontal cortex, hippocampus and cerebellum. Although spatial navigation ability develops with age, little is known about the neural correlates of this complex task in young children. Moreover, preterm birth may influence cognitive and brain development, and has specifically been associated with poor spatial cognition and alteration in brain regions important for spatial navigation. In the current study we tested whether, in young children, individual variability in brain volumes is related to individual variability in measures of spatial navigation ability. Forty-two children born either at term (n=19, age 6.11±0.14) or preterm (n=23, age 5.99±0.13) performed 15 repeated trials of a virtual Morris Water Maze (vMWM) task. Average time, distance, and fractal dimension (FD, a measure of path complexity) were calculated. High-resolution structural MRI was conducted in a subset of the participants (15 term, age 5.97±0.16; 13 preterm, age 6.09±0.18) and a semi-automatic method (FreeSurfer) was used to compute volumes of the prefrontal cortex (PFC), hippocampus (Hc), and cerebellar gray (CbGM) and white matter (CbWM). CbWM was related to both average time ($F(1,22)=4.86$, $p=.04$) and FD ($F(1, 23)=6.65$, $p=.02$), such that larger CbWM was associated with poorer spatial navigation ability as indicated by being slower in finding the target and using a more complex path. Hc volume was marginally related to average FD ($F(1,23)=3.72$, $p=.07$), such that larger Hc was associated with the use of a more complex path. Children born prematurely were faster in finding the target compared to term born children ($F(1,38)=4.39$, $p=.04$). Children born prematurely had smaller PFC volumes ($F(1,25)=7.39$, $p=.01$), however variability in PFC volume was not related to group differences in task performance. Term status, however, marginally interacted with CbGM in accounting for average time ($F(1,23)=3.14$, $p=.089$), such that in the preterm ($r=-.58$, $p=.06$), but not the term ($r=.15$, $p=.64$) group, larger CbGM was related to faster performance. Taken together, we demonstrate the feasibility of using the vMWM task to study the neural correlates of spatial navigation in young children. Moreover, our

findings suggest that variability in the volume of the hippocampus and the cerebellum relates to spatial navigation ability in young children.

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Poster

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Title: STSp functional connectivity in adults and children during biological motion perception

Authors: ***S. DASGUPTA**[1], Z. J. MCINTIRE[1], M. A. NGUYEN[1], J. X. LI[2], K. H. JAMES[2], E. D. GROSSMAN[1];
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Abstract: Background: Humans are quite adept in recognizing actions from the movements of the joints alone ("point-light" biological motion). Neuroimaging studies have identified neural signals selective for biological motion (BM) in the posterior part of the superior temporal sulcus (STSp) in both adults and children, a region that serves as an important hub for a larger network of ROIs that implicated in social perception more generally (Grossman et al., 2000; Carter & Pelphrey, 2008). The larger network includes parts of the ventral temporal lobe (Vaina et al. 2001), the lateral occipital gyrus (Grossman & Blake 2002; Beauchamp et al. 2002), as well as some regions within the prefrontal cortex (Grezes, J., 1998). The aim of this study is to map the neural communication between the STSp and other cortical regions during biological motion perception, and to assess how the connectivity pattern in this large-scale network changes developmentally. Thus, we computed task-based functional connectivity (FC) between the STSp and other brain regions during biological motion and non-biological motion-matched control tasks in both children and adults. Methods. Fifteen adults (all 18 years of age or older) and thirteen children (aged 4-6) participated in this study. Thirty-three bilateral ROIs, including the bilateral STSp, were identified from the conjunction of the children and adults whole-brain univariate analysis of biological motion selective brain signals. The timeseries from these regions were segmented into the blocks corresponding to the biological and non-biological experimental intervals, and then subjected to FC (Pearson's r) analysis. Significance was assessed via bootstrapping. Results. STSp FC in children has a pattern distinct from that found in

adults. STSp FC In the adults' brain was strongly bilateral, with more connections between STSp and visual cortex and between STSp and frontal cortex (particularly in the left hemisphere). In the children's brain, significant STSp connections were largely right lateralized, with the STSp-frontal connections absent. Instead, the STSp was more strongly connected to parietal cortex. These results are consistent with the notion that children using biological motion to engage social orienting cues, and have fewer long-range connections overall which are believed to develop later in life.

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Title: Brain state flexibility predicts diverse cognitive functions during critical periods in neurodevelopment

Authors: J. D. MEDAGLIA, T. D. SATTERTHWAITE, M. YANG, S. GU, *Q. K. TELESFORD, R. C. GUR, R. E. GUR, D. S. BASSETT;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Adolescence is marked by several critical periods of rapid cognitive and social development, accompanied by alterations in brain dynamics across distributed cognitive circuits. Yet, exactly how these circuit dynamics relate to changes in cognitive function during normative neurodevelopment is unknown. Recent tools to quantify circuit organization have been drawn from graph theory, which traditionally ignores the time-dependent changes in the regional interactions and is therefore unable to quantify circuit dynamics. To address this gap, we develop a novel technique to assess the transition of the brain through cognitive states. We use this tool to

quantify brain state flexibility during early development and to test its relationship with effective cognitive function, as measured by a cognitive neuroscience based computerized battery which assesses accuracy and efficiency in executive function, memory, social cognition, and complex reasoning. In 780 youths aged 8-21 from the Philadelphia Neurodevelopmental Cohort, we acquired resting state functional magnetic resonance imaging (fMRI) data over the course of 6 minutes. We extracted 264 regional mean time series from functionally defined regions of interests in cortical and subcortical areas. We defined a temporal adjacency matrix A whose elements Aij give the similarity in regional BOLD magnitude across regions between time i and time j. We defined a brain state as a pattern of whole-brain activation that was frequently observed across the scanning session, and extracted such states in a data-driven fashion using community detection techniques applied to the temporal adjacency matrix. We defined brain state flexibility as the number of transitions among brain states observed in a single subject during the resting state scan. We observed that individual subjects visited approximately 15 brain states during the course of the 6-minute resting state scan, and the number of visited states remained constant over the full age range (8-21 yrs). Furthermore, we observed that brain state flexibility was positively correlated with cognitive performance in all cognitive measures during puberty and again in late adolescence. These results indicate that brain state flexibility may predict individual cognitive development over the life span during periods of rapid brain network consolidation. Effective transitions between states may drive developmental trajectories during these critical periods.

Disclosures: J.D. Medaglia: None. **T.D. Satterthwaite:** None. **M. Yang:** None. **S. Gu:** None. **Q.K. Telesford:** None. **R.C. Gur:** None. **R.E. Gur:** None. **D.S. Bassett:** None.

Poster

252. Cognitive Development

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 252.12/V7

Topic: F.01. Human Cognition and Behavior

Support: National Science and Engineering Research Council (NSERC)

Alberta Children's Hospital Foundation

Title: Age predicts functional connectivity of the intraparietal sulcus during early childhood: implications for attention development?

Authors: *C. ROHR[1,2,3], S. VINETTE[1,2,3,4], K. PARSONS[1,3,4], S. BRAY[1,2,3,4];
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Abstract: Skills like visual-spatial attention mature rapidly in early childhood and lay the foundation for acquiring more complex skills such as reading. In many neurodevelopmental disorders, visual-spatial attention is impaired, suggesting that the underlying brain networks may have several distinct vulnerabilities. One promising site for investigation is the intraparietal sulcus (IPS), which has been implicated in visual sustained and selective attention, and is connected to prefrontal regions of the dorsal attention network and occipital visual regions. Our group recently demonstrated the effect of age on IPS functional connectivity in older children and adolescents (Vinette & Bray, 2015), however, an investigation of age effects in early childhood is lacking. To address this question, we examined the relationship between age, attention skills and IPS connectivity in 22 children between 4.14-6.67 years old (mean=5.26; sd=0.86). Attention measures were adapted from the Early Childhood Attention Battery (Breckenridge et al., 2013) and comprised sustained attention, selective attention and attention control measures. Following training in an MRI simulator, children freely watched clips from the TV show 'Elmo' in a 3T GE 750w scanner while undergoing functional magnetic resonance imaging. Data preprocessing included censoring of motion-corrupted volumes, specifically those which exceeded both framewise displacement 0.2mm and 0.3% change in BOLD signal intensity (Power et al. 2014). IPS connectivity was then assessed by regressing the average time-course of volumetric masks for IPS0-5 (Wang et al., 2014) against every voxel in the brain. At the group level, effects of age were assessed while controlling for IQ (mean=112.11; sd=11.25). Inferences were drawn at a $z>2.3$ voxel-wise threshold with cluster correction using Gaussian Random Field theory at $p<0.05$. Behaviorally, age correlated significantly with composite scores for both sustained and selective attention ($r=.59$ and $r=.65$, both $p<0.01$). On a functional connectivity level, age positively predicted IPS connectivity with visual areas (V2, V4) and supplementary eye fields (SEF), while negatively predicting IPS connectivity with thalamo-striatal regions. Our results suggest that IPS connectivity with dorsal attention and early visual regions increases with age in early childhood, while connectivity with subcortical areas decreases. IPS functional connectivity changes in early childhood may underlie the rapid development of children's visual-spatial attention skills.

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Poster

252. Cognitive Development

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Topic: F.01. Human Cognition and Behavior

Support: NICHD/NIH R01 HD044073

NICHD/NIH R01 HD046130

NICHD/NIH P30 P30HD015052

NCATS/NIH UL1 TR000445

Title: Resting state connectivity at 1st grade predicts math competence at 2nd grade

Authors: *G. PRICE, E. D. WILKEY, D. J. YEO, L. E. CUTTING;
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Abstract: Mathematical competence is critical for success in modern society, predicting academic success, rates of unemployment, physical and mental illness, arrest, and incarceration. Understanding of the neural mechanisms underlying math competence can aid the development of effective educational interventions. Previous research suggests that functional activation of the intraparietal sulcus (IPS) is related to individual differences in math competence (Bugden et al , 2012) and may be impaired in children with mathematical learning disabilities (Price et al, 2007). Information on connectivity between brain regions may yield important insights beyond traditional region-based analyses (Power et al, 2010). Previous studies report that functional connectivity during numerical and mathematical processing between frontal and parietal brain regions relates to math competence (Emerson & Cantlon, 2012; Rosenberg-Lee, Barth, & Menon, 2011). However, no studies that we are aware of have related math competence to connectivity while the brain is not engaged in an explicit task (i.e. 'resting state'). The present study related resting-state connectivity at the end of 1st Grade to math competence at the end of 2nd grade. Resting-state data from 42 1st grade children (Mean age 7.4 yrs) were correlated with standard scores on the Woodcock Johnson Arithmetic Fluency and Calculation subtests were collected one year later. Seed-based functional correlation analyses were used to assess connectivity across the whole brain with left and right IPS seed regions, regressing out signal from white matter and ventricles. Connectivity with the right IPS seed region was not correlated with math competence in any region. Connectivity with the left IPS region, in contrast, was correlated with math competence in the left precentral gyrus (-44, 2, 18), the left superior temporal sulcus (-39, -50, 25), and bilateral anterior cingulate cortex (5, 19, 29; -7, 19, 26). The present results reveal that spontaneous functional connectivity of a left lateralized network related to a left IPS seed region predicts math competence a year later. Task dependent functional connectivity of similar regions has previously been related to math competence, but the present data suggest that such connectivity may be intrinsically driven, as opposed to task dependent. Furthermore, the fact that connectivity strength in this network predicted math competence a year later suggests that this network is ontogenetically important, playing an meaningful role in the acquisition of math skills over critical early school years.

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Poster

252. Cognitive Development

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 252.14/V9

Topic: F.01. Human Cognition and Behavior

Support: Ellison Medical Foundation

Title: Altered reading networks in young children with dyslexia

Authors: *J. WALTERS[1], J. MURTAGH[1], K. HALVERSON[1], A. CYR[1], T. K. PERRACHIONE[2], P. CHANG[1], P. HOOK[3], J. D. E. GABRIELI[1], J. A. CHRISTODOULOU[1,3]; [1]MIT, Cambridge, MA; [2]Boston Univ., Boston, MA; [3]MGH Inst., Boston, MA

Abstract: Despite considerable evidence for altered function of the brain's reading network in children with dyslexia, few (if any) studies have focused on children with dyslexia younger than 9. The earliest years of literacy instruction offer an important window to understand diverse reader performance. Here, we examined reading networks in early readers (ages 6-9) who are typically developing (n = 19) or who have dyslexia (n = 22). Children completed standardized behavioral assessments and a functional magnetic resonance imaging (fMRI) task using a 3.0T Tim Trio Siemens System. The fMRI task required children to view pairs of words sequentially and indicate by button push when subsequent words shared the same first sound. Activations in this first phoneme-matching task versus rest were compared between groups. Children with dyslexia exhibited reduced activation in three left-hemisphere regions associated with reading and language: frontal cortex (middle and inferior gyri; BA 44/45), fusiform cortex (BA 20/37), and temporal cortex (middle and superior gyri; BA 21/22). We provide evidence that reduced activity in the left-hemisphere reading network in children with dyslexia is apparent in early readers.

Disclosures: J. Walters: None. **J. Murtagh:** None. **K. Halverson:** None. **A. Cyr:** None. **T.K. Perrachione:** None. **P. Chang:** None. **P. Hook:** None. **J.D.E. Gabrieli:** None. **J.A. Christodoulou:** None.

Poster

252. Cognitive Development

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Topic: F.01. Human Cognition and Behavior

Support: NICHD [R01 H047520]

Children's Health Research Institute Postdoctoral Fellowship

NICHD Postdoctoral NRSA [F32 HD080367]

Title: Cortical maturation accompanying individual differences in longitudinal development of children's reading ability

Authors: *T. M. EVANS[1], M. SCHAER[2], J. KOCHALKA[2], T. J. NGOON[2], L. CHEN[2], C. BATTISTA[2], V. MENON[2];
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Abstract: Childhood is a time of extensive protracted neuroanatomical development accompanied by marked improvements in a number of cognitive domains. Behavioral research has identified core skills necessary for acquisition of reading ability, but little is known about neurobiological factors that contribute to long-term gains in children's reading ability. Previous work relating brain structure to impaired reading ability has implicated a temporoparietal and inferiotemporal network. However, these studies have, for the most part, focused on single time points. Consequently the neuroanatomical bases of individual differences in growth trajectories are unknown. This study employed longitudinal structural neuroimaging map individual differences in the development of reading skill onto corresponding maturation in brain structure. High-quality longitudinal brain imaging data were acquired from 24 children (13 females). Cognitive measures and high-resolution whole-brain structural images were obtained at 2 time points (time 1 = 8.6 ± 0.6 yrs, time 2 = 10.5 ± 1.4 yrs). Standardized scores adjusted for chronological age derived from the Word Reading subscale of the WIAT, which provides a measure of an individual's word reading abilities relative to their peers, were used to assess reading skills. Image processing and statistical analyses were carried out in Freesurfer. A GLM was used to assess the relationship between change in reading abilities and vertex-wise change in cortical thickness, with statistical thresholds of height $p < 0.01$ and cluster-corrected extent $p < 0.05$. Children showed considerable variation in the development of their reading abilities, with difference scores between two time points ranging from -9 to 30 (M = 2.1, SD = 8.6). A negative correlation between change in cortical thickness and change in reading skills was found in the right superior temporal sulcus, suggesting that increased cortical thinning in this region is associated with greater improvements in reading abilities. Our results demonstrate that growth in reading abilities in early childhood is associated with focal cortical thinning in the superior tempral sulcus, a region important for language processing, and previously shown to be abberent in struggling readers. This result is also consistent with previous longitudinal studies demonstrating an association between cortical thinning and individual performance gains in vocabulary, working memory, and executive function. This work extends previous findings by identifying a brain area where cortical thinning corresponds to individual differences in reading skills.

Disclosures: **T.M. Evans:** None. **M. Schaer:** None. **J. Kochalka:** None. **T.J. Ngoon:** None. **L. Chen:** None. **C. Battista:** None. **V. Menon:** None.

Poster

252. Cognitive Development

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Program#/Poster#: 252.16/V11

Topic: F.01. Human Cognition and Behavior

Support: Fondation de France 2012-00033701

Title: The neural development of pragmatic inference-making in discourse comprehension

Authors: *F. SCHWARTZ[1], I. A. NOVECK[2], J. PRADO[2];
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Abstract: Everyday verbal interactions often require people to make spontaneous inferences to understand implicit messages conveyed by speakers. A recent neuroimaging study suggests that such pragmatic inference-making in adults is supported by the interaction between brain regions involved in reasoning and mentalizing (i.e., processing other's intentions and emotions). To date, however, it remains unclear at what point of development this neural interaction emerges and supports pragmatic inference-making in children. The goal of the present functional Magnetic Reasonance Imaging (fMRI) study was to answer this question by investigating the neural development of pragmatic inference-making in typically developing children and adolescents from ages 8 to 14. Participants read short stories containing information (premises) that had to be integrated logically to understand the conclusion of the story. In half of these inference stories, premises were not sufficient to understand the conclusion made by the central character of the story. In these cases, participants had to make a pragmatic inference in order to understand the conclusion. We found that the ability make such inferences increases with age. We also found that such increases were associated with developmental increases of activity in regions typically associated with logical reasoning in the Rostrolateral Prefrontal Cortex (RLPFC). In contrast, stories in which conclusions required inference-making were associated with greater activity in the brain mentalizing system (i.e., right Temporo-parietal Junction and right Middle Temporal Gyrus) in both younger and older children, with no developmental increases. Therefore, our results suggest that children might detect the need for pragmatic inferences early on during development (i.e., 8-year-old). However, the ability to make these inferences might arise later in development (i.e., during adolescence) with the maturation of the RLPFC.

Disclosures: **F. Schwartz:** None. **I.A. Noveck:** None. **J. Prado:** None.

Poster

252. Cognitive Development

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Program#/Poster#: 252.17/V12

Topic: F.01. Human Cognition and Behavior

Support: NIH-NINDS P50 NS22343

Title: TITLE: Investigating social initiation behaviors with strangers in different groups of children with developmental disorders

Authors: *N. WOO-VONHOOGENSTYN[1], P. LAI[1,2,3], J. REILLY[3], U. BELLUGI[1]; [1]Salk Inst., La Jolla, CA; [2]Univ. of California at San Diego, La Jolla, CA; [3]San Diego State Univ., San Diego, CA

Abstract: Children interact with strangers as a part of social development, but how do these children initiate social interaction? For this study, we used the Salk Institute Sociability Questionnaire (SISQ), a parental questionnaire that examines various aspects of social behaviors, to investigated how school-aged children (7-14) initiate social interactions. We specifically focused on one question from the SISQ - "Describe your child's typical reaction when meeting someone for the first time" - to evaluate the social initiation behaviors of different developmental groups. The groups included children with Focal Lesion (FL; n = 15), children with High Functioning Autism (HFA; n = 22), children with Williams Syndrome (WS; n = 14), and Typically Developing (TD; n = 21) children. We had adults' blind to each group's clinical diagnoses, rate randomly sorted parental responses on a spectrum from 1 (non-social) to 7 (high-social) with 4 being a neutral response. The question, regarding meeting someone for the first time, resulted in significant difference between the groups, $f(3,68) = 4.95$, $p = 0.003$. Follow up analyzes using post-hoc Tukey-HSD showed statistically significant group differences between the WS and FL groups, and the WS and HFA groups. In both cases, the WS group was rated as more social when compared to FL and HFA groups. The TD group did not differ significantly when compared to the three groups, and there was not a significant group difference between the FL and HFA group as their means were comparable to each other. Examples of these parental responses were: "Polite, quiet, minimal conversation but will answer questions. Takes a few moments and then will jump into a conversation" which was a response by a TD parent. Whereas a parent of an HFA child response was, "He is not always the first to make contact, does not always make eye contact. Sometimes he is not paying attention as to what is going on." Similarly, a parent of a FL child response was "Shy, doesn't make eye contact, and won't start a conversation. It takes him time to get comfortable with new people." And, one of the WS parental responses was, "Very interested in their name, what they like to do, where they live, do they like (whatever her current interest is). She will then sit with them, tell them after a few minutes she loves them if they are engaged in stuff she likes." Each of the developmental groups showed atypical initiation behaviors, the FL and HFA were less likely to exhibit social interactions, whereas WS were more likely to exhibit social engagement. By studying how different developmental groups initiate social interactions, we can better understand the social and behavioral phenotypes of these groups.

Disclosures: N. Woo-Vonhoogenstyn: None. **P. Lai:** None. **J. Reilly:** None. **U. Bellugi:** None.

Poster

252. Cognitive Development

Location: Hall A

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Program#/Poster#: 252.18/V13

Topic: F.01. Human Cognition and Behavior

Support: Autism Speaks 7608

Department of Defense AR130106

Title: Implicit learning in young children with autism

Authors: *R. M. JONES[1], C. CARBERRY[1], D. DELLARCO[2], A. HAMO[1], C. LORD[1];
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Abstract: Implicit associative learning is an individual's ability to form connections between stimuli without conscious awareness. The literature is inconsistent whether individuals with Autism Spectrum Disorder (ASD) have difficulties with implicit learning compared to typically developing (TD) individuals. We hypothesize the discrepancy is not simply explained by methodological differences across studies, but reflects variability within ASD for implicit learning. To test our hypothesis, 50 children, 4-7 years of age completed the procedures, specifically 39 TD (Mean IQ = 112; 22M) and 11 with ASD (Mean IQ = 105; 10M). Building upon the implicit learning literature, we designed a child-friendly task that measures differences in reaction time (RT) behavior and accuracy to a target stimulus predicted by two cues at differing probabilities. In each trial, children were instructed to touch a target image presented on an iPad but to refrain from touching the cues and distractor image. Unbeknownst to the participants, one of the cues predicted the target image at a high probability (75%), while the other cue preceded the target at a low probability (25%); the distractor appeared when the target was not presented. Z-scored reaction times and percent accuracy were divided into thirds (early, middle and late trials) and by condition (high probability, low probability) and submitted to a 3x2 repeated measures ANOVA to assess learning. Due to an imbalance in sample sizes, TD and ASD analyses were performed separately. As expected, there were no significant differences in accuracy during the task or between groups (M = 84%; p's > 0.1), suggesting all children understood task instructions. TD children slowed in reaction times by the late trials to the target when preceded by the 25% cue compared to the 75% cue (time*condition: ($F(2,74)=6.6$, $p < 0.002$), suggesting TD children learned the contingencies with no difference in ASD (p's > 0.2). After the task, 10 of 39 TD children and 3 of 11 ASD children correctly matched the target image with the 75% cue image, with a trend of a higher IQ ($p = 0.1$) in those who made the conscious match in ASD, but not in TD ($p = 0.5$). Differences in conscious awareness suggest variability in the expression of learning. Future studies will explore the role of IQ in explicit understanding after learning in ASD. In a larger ASD sample, we will determine how variability in learning patterns may be important for predicting intervention success in children with ASD.

Behavioral findings will be the foundation for future research during functional Magnetic Resonance Imaging to understand the neural underpinnings of individual variability in implicit learning in ASD.

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Poster

252. Cognitive Development

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Program#/Poster#: 252.19/V14

Topic: F.01. Human Cognition and Behavior

Support: NSFC 31270023

Title: Neural synchronization during different types of teaching

Authors: ***F. L. ZHENG**;
Sch. of Brain and Cognitive Sci., Beijing Normal Univ., Beijing, China

Abstract: Plenty of previous studies have focused on the neural mechanisms of learning. However, only a few studies have investigated the mechanism of teaching. Thus, the neural mechanism behind teaching is largely unknown. This study used fNIRS-based hyperscanning technique to examine the neural synchronization pattern between teachers and students in different types of teaching, i.e., lecture-style teaching and interactive teaching. Nine pairs of same-gender teachers and students were recruited (18 males and 18 females, mean age 22.56 years, range from 19 to 26). The content of teaching was numerical reasoning. Students were required to complete a numerical reasoning test both before and after the experiment. Wavelet Transform Coherence(WTC) was used to assess the relationship between fNIRS signals generated by teachers and students. The results showed a significant increase of the neural synchronization in the right temporo-parietal junction (TPJ) during interactive teaching, but not during lecture-style teaching. These results demonstrated that interactions between teachers and students are associated with higher-level interpersonal neural synchronization. This result will provide important insight into the neural mechanism of teaching.

Disclosures: **F.L. Zheng:** None.

Poster

252. Cognitive Development

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Program#/Poster#: 252.20/V15

Topic: F.01. Human Cognition and Behavior

Support: MacArthur Law and Neuroscience Network

NSF Graduate Research Fellowship

Title: When does an adolescent become an adult: The influence of emotion on the development of cognitive control

Authors: *A. COHEN[1,2], K. BREINER[3], D. DELLARCO[2], A. S. HELLER[4], M. RUDOLPH[5], G. PEDERSEN[2], R. BONNIE[6], K. TAYLOR-THOMPSON[7], E. S. SCOTT[8], L. STEINBERG[9], D. A. FAIR[5], A. GALVAN[3], B. CASEY[2,10];
[1]Weill Cornell Grad. Sch. of Med. Sci., New York, NY; [2]Psychiatry, Sackler Inst. for Developmental Psychobiology, New York, NY; [3]Psychology, UCLA, Los Angeles, CA; [4]Univ. of Miami, Miami, FL; [5]Dept. of Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR; [6]Univ. of Virginia Sch. of Law, Charlottesville, VA; [7]New York Univ. Sch. of Law, New York, NY; [8]Columbia Law Sch., New York, NY; [9]Psychology, Temple Univ., Philadelphia, PA; [10]Psychiatry, Weill Cornell Med. Col., New York, NY

Abstract: For most legal purposes, an individual is considered an adult at the age of eighteen; but sometimes, that age boundary shifts. Variations in the definition of "adult" exist in many legal and social policies. These distinctions between juvenile and adult are often based on political considerations and conventional wisdom rather than empirical evidence. Although developmental neuroscience research has shown that the brain continues to develop into the early twenties, behavioral and neural distinctions between adolescents and adults continue to be delineated, particularly in the eighteen to twenty-one age range. The present study implements a novel behavioral paradigm, together with psychophysiology and fMRI, to examine impulsivity under transient and sustained states of positive and negative emotion in 110 individuals. We show that 13 to 21 year olds show similar decrements in performance as compared to adults over the age of twenty-one in response to transient cues of potential threat and under sustained positive emotion (excitement). These behavioral results are paralleled by: 1) decreased activity in dorsolateral prefrontal cortex, implicated in emotion regulation, in teens and young adults relative to adults in response to fear cues; and 2) increased activity in medial prefrontal cortex, implicated in integration of self and affective information in decision making, in teens and young adults across the sustained excite context. Our findings suggest that, under transient threat and sustained excitement, 18 to 21 year olds may behave more similarly to younger adolescents than to older adults due to continued development of prefrontal circuitry. These results may have implications for age-related legal and social policies.

Disclosures: A. Cohen: None. **K. Breiner:** None. **D. Dellarco:** None. **A.S. Heller:** A. Employment/Salary (full or part-time):; University of Miami. **M. Rudolph:** None. **G. Pedersen:**

None. **R. Bonnie:** A. Employment/Salary (full or part-time):; University of Virginia. **K. Taylor-Thompson:** A. Employment/Salary (full or part-time):; New York University. **E.S. Scott:** A. Employment/Salary (full or part-time):; Columbia University. **L. Steinberg:** A. Employment/Salary (full or part-time):; Temple University. **D.A. Fair:** A. Employment/Salary (full or part-time):; Oregon Health & Science University. **A. Galvan:** A. Employment/Salary (full or part-time):; University of California, Los Angeles. **B. Casey:** A. Employment/Salary (full or part-time):; Weill Cornell Medical College.

Poster

252. Cognitive Development

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 252.21/V16

Topic: F.01. Human Cognition and Behavior

Support: Academy of Finland grant 1265528

Title: The effects of information and communication technology use in adolescence on attention-related brain activity

Authors: *M. MOISALA[1], V. SALMELA[1,2], L. HIETAJÄRVI[1], E. SALO[1], S. CARLSON[3,4], O. SALONEN[5], K. LONKA[1], K. HAKKARAINEN[1], K. SALMELA-ARO[1,6], K. ALHO[1,2,7,8]; [1]Inst. of Behavioural Sci., Univ. of Helsinki, Helsinki, Finland; [2]Advanced Magnetic Imaging Centre, Aalto NeuroImaging, Aalto University, Finland; [3]Brain Res. Unit, Dept. of Neurosci. and Biomed. Engin., Aalto University School of Science, Finland; [4]Neurosci. Unit, Dept. of Physiology, Fac. of Med., University of Helsinki, Finland; [5]Helsinki Med. Imaging Ctr., Helsinki University Central Hospital, Finland; [6]Cicero Learning, University of Jyväskylä, Finland; [7]Helsinki Collegium for Advanced Studies, University of Helsinki, Finland; [8]Swedish Collegium for Advanced Study, Uppsala, Sweden

Abstract: As the use of modern digital technology has become an increasingly integral part of our everyday lives, concerns have been raised about whether the continuous fast-paced influx of information offered by modern media affects our ability to focus our attention. These concerns are especially relevant when it comes to the generation of “digital natives”. We investigated whether different information and communications technology (ICT) user profile groups show differences in performance or brain activity during attentionally demanding tasks. Participants were 173 healthy adolescents and young adults (aged 13-20 years) sampled from 3000 pupils and grouped according to their ICT use into three subgroups: computer gamers, social media actives, and controls. We measured brain activity with event-related functional magnetic resonance imaging (fMRI) in participants performing a sentence congruence judgment task in the auditory or visual modality (selective attention conditions), or both (divided attention condition). Performance deteriorated significantly during divided attention compared with selective

attention, and performance accuracy improved significantly with age in all conditions. There were no performance differences between the groups with different ICT profiles. fMRI analyses revealed a main effect of ICT group during all conditions in the left temporoparietal junction (which was associated with linguistic processing of the presented sentences), with the social media group showing less activity in this area than the other two groups especially during divided attention. In addition, the youngest social media users showed significantly smaller activity increases than the other two ICT profile groups within lateral and medial regions in the right frontal cortex that were activated specifically by divided attention. A correlational trend was observed between greater activity increases in these areas and better task performance. The results suggest that the type and extent of ICT use may have an impact on the functioning of brain networks involved in divided attention and language processing. More specifically, decreased activity in the left temporoparietal area was observed in the social media group, possibly reflecting insufficient recruitment of language processing areas during the experimental task. In addition, lower activation in frontal regions recruited by divided attention was observed in the youngest cohort of the social media group. This suggests that in this ICT user group, performing the experimental task placed more demands on brain networks related to attention and executive functioning.

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Poster

252. Cognitive Development

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Program#/Poster#: 252.22/V17

Topic: F.01. Human Cognition and Behavior

Support: PA-HEAL

NIH Grant MH067924

Title: Cannabis use and adolescent neurocognitive development: a prospective fmri study

Authors: ***B. C. TERVO-CLEMMENS**[1], D. SIMMONDS[1], B. LUNA[2];
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Abstract: Cannabis has been associated with impairments in working memory (WM) (Varvel et al. 2001) which continues to improve through adolescence (Luna, Garver, Urban, Lazar, & Sweeney, 2004). As such, adolescents may be particularly vulnerable to the effects of cannabis on WM. To investigate the relationship between cannabis use and adolescent brain development, we are conducting a prospective longitudinal neuroimaging study where participants were

initially assessed at age twelve, before use, and then again at age fifteen. By the second time point, approximately 25% of the sample had begun using cannabis, early-onset cannabis use (EOC). At both visits, subjects performed a Sternberg-type visuospatial working memory task during fMRI acquisition. Reaction time and accuracy were recorded. A fast event-related design was used to distinguish encoding, maintenance, and response epochs of WM. Only correct trials were included in fMRI group analysis. For behavioral analysis, we used linear-mixed-effects modeling to examine differences between users and non-users at both time points. Thus, we examined potential risk factors for (time point 1), and consequences of (time point 2), adolescent cannabis use. Prior to use (time point 1), our preliminary results suggest that those who would go onto use cannabis (pre-EOC) have faster reaction times but do not differ in accuracy, compared to healthy controls. This behavioral difference was accompanied by greater maintenance period DLPFC activation in pre-EOC participants compared to controls. After use (time point 2), the groups no longer differed in reaction time but there was a statistical trend for more commission errors in EOC users compared to healthy controls. This post-use behavioral difference was accompanied by greater maintenance period activation in canonical WM regions including the ACC, IPS, and thalamus in EOC users compared to healthy controls. Together, these results suggest that there may be neurobiological risk factors for early cannabis , which may persist and interact with cannabis use, undermining WM development.

Disclosures: **B.C. Tervo-Clemmens:** None. **D. Simmonds:** None. **B. Luna:** None.

Poster

252. Cognitive Development

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Topic: F.01. Human Cognition and Behavior

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Wellcome Trust Senior Investigator Award (Ref: 104631/Z/14/Z) to Prof. Trevor Robbins

Title: The cognitive profile of early-onset obsessive-compulsive disorder. Does age matter?

Authors: *J. GOTTWALD[1,2], A. M. APERGIS-SCHOUTE[2], S. MOREIN-ZAMIR[3], M. KASER[2], A. SULE[2], A. CONWAY MORRIS[4], W. LIMMER[2], T. W. ROBBINS[3], B. J. SAHAKIAN[2];
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Abstract: Obsessive-compulsive disorder (OCD) is a psychiatric condition characterised by recurrent distressing thoughts and acts. The majority of patients experience an onset of symptoms before the age of 18. However, most of the research has focused on adult patients, while adolescent OCD is rarely investigated. There are notable differences between juvenile and adult OCD. Teenagers generally have less insight into their disorder than adult patients and whilst there are no gender differences in adults, 2-3 times more boys than girls suffer from OCD. The aim of this study was to investigate the cognitive profile of adolescent OCD. Thirty juvenile OCD patients (22 females) without additional Axis I disorders and 30 healthy volunteers matched for age, gender, and intelligence were recruited. The Cambridge Neuropsychological Test Automated Battery (CANTAB) was used to assess visual memory (Pattern Recognition Memory), decision-making (Cambridge Gambling Task), and cognitive flexibility (Intra-Extra Dimensional Set Shift). Adolescent OCD patients took significantly longer to complete the memory task, yet their performance was worse. Patients also made less rational decisions in the Cambridge Gambling task, while they were slower and showed poor risk adjustment. Finally, cognitive flexibility was not impaired, though patients showed a general learning deficit. Follow-up analyses showed this effect to be driven by the younger subgroup. The older the patients were, the greater was their impairment of cognitive flexibility. This effect was significant when controlling for OCD, depression, and anxiety symptom severity, gender, and intelligence. An impairment in non-verbal working memory has previously been reported for adult OCD patients (Abramovitch et al., 2013); a similar impairment was observed in this sample. This suggests working memory to be a core deficit in OCD, irrespective of the patient's age. In contrast, the evidence for decision-making in adult patients is mixed (Cavedini et al., 2002, Watkins et al., 2005, Chamberlain et al., 2007), while this dataset shows a clear impairment for adolescents. Lastly, reduced cognitive flexibility is characteristic of adults with OCD (Chamberlain et al., 2006). The young patients did not show this impairment, perhaps due to a general learning deficit, which precluded formation of a stable attentional set and prepotent tendency. However, within this sample increased age correlated with a growing deficit in cognitive flexibility in OCD. Follow-up analyses will be necessary to investigate if adolescent and adult OCD should be treated as two different subtypes of the disorder, with possible implications for treatment.

Disclosures: J. Gottwald: None. **A.M. Apergis-Schoute:** None. **S. Morein-Zamir:** None. **M. Kaser:** None. **A. Sule:** None. **A. Conway Morris:** None. **W. Limmer:** None. **T.W. Robbins:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cambridge Cognition. F. Consulting Fees (e.g., advisory boards); Cambridge Cognition. **B.J. Sahakian:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cambridge Cognition. F. Consulting Fees (e.g., advisory boards); Cambridge Cognition.

Poster

253. Mechanisms of Attention I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: NSERC Grant 242252

Title: The combination of mk-801 and sch-23390 at sub-effective doses synergistically impairs attentional set-shifting in rats

Authors: *S. J. DESAI, B. L. ALLMAN, N. RAJAKUMAR;
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Abstract: Contemporary research in the pathophysiology of cognitive deficits in schizophrenia has provided a multifactorial view, in which the neurotransmitters dopamine, gamma-Aminobutyric acid (GABA) and glutamate are disturbed in the prefrontal cortex (PFC) of schizophrenic brains. This view is supported by the fact that current antipsychotic drugs, which are primarily dopamine receptor blockers, are not effective in treating the cognitive symptoms of schizophrenia. We hypothesized that abnormality in dopamine and glutamate neurotransmissions act synergistically to cause certain cognitive symptoms of schizophrenia. We tested the effect of blockade of dopamine and glutamate neurotransmitter systems on executive function using an operant conditioning-based attentional set-shifting task to assess behavioral flexibility. In a series of dose-response studies, we determined the effective doses of the specific antagonists for dopamine D1 receptors (SH-23390) and NMDA receptors (MK-801). Next, sub-effective doses were administered subcutaneously 25 min before the set-shifting task. Our results indicate that while the single antagonist treatments did not impair set-shifting, combination of sub-effective doses of SH-23390 and MK-801 severely affected the performance. Present findings will help to elucidate mechanisms underlying cognitive deficits in schizophrenia.

Disclosures: S.J. Desai: None. **B.L. Allman:** None. **N. Rajakumar:** None.

Poster

253. Mechanisms of Attention I

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Program#/Poster#: 253.02/V20

Topic: F.02. Animal Cognition and Behavior

Support: MH092438

Title: Testing sustained attention in rats in touchscreen operant chambers

Authors: *D. A. BANGASSER, B. WICKS, K. WHITE, N. DUNCAN, S. COHEN, J. BERGMANN, B. YEGLA, R. COLE, V. PARIKH, D. WAXLER;
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Abstract: Sustained attention is the ability to detect rare and unpredictable events over a prolonged period of time, and this process is central to cognitive performance. To assess attentional capacities in the laboratory, a Sustained Attention Task (SAT) has been developed for rodents, in which they are trained to detect an unpredictably occurring signal (a brief light presentation) from non-signaled events. The traditional version of this task utilizes an operant chamber with a central panel light for the signal and two retractable levers on which the rats can respond to indicate whether the signal was present or absent. However, with the rise in popularity of touchscreen operant chambers, in which one side of the chamber is a touchscreen and no levers are present, the adaptation of SAT to this style of chamber could enhance the versatility of the task, making it easier to implement and more analogues to touchscreen automated testing procedures used to assess cognitive functions in humans. Here we developed a touchscreen version of SAT where the light signal is presented in the upper part of the touchscreen and rats respond by touching their nose to one of two touchscreen response areas below the light. The remaining parameters were kept similar between the traditional and touchscreen versions of SAT. Our results indicate that rats acquired touchscreen SAT at a similar rate to the traditional version. Additionally, the presence of a distractor (a flashing houselight) disrupted performance on the touchscreen version, which was similar to effects previously observed with the traditional SAT task. Another goal of the study was to validate this task in both male and female rats, given the growing interest of including female subjects in basic research studies. Interestingly, males acquired the task more rapidly than female rats, however most females did eventually master the task. Collectively, these data suggest that the touchscreen version is comparable to the traditional version of SAT, and is an equally valid way of measuring sustained attention. Thus, many researchers with touchscreen chambers could easily implement our modifications in order to study sustained attention.

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Poster

253. Mechanisms of Attention I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 253.03/V21

Topic: F.02. Animal Cognition and Behavior

Title: The effects of different pharmacological treatments on physiological signals in rats using implanted telemetry

Authors: *R. O. PUSSINEN[1], L. TOLPPANEN[1], A. NURMI[1], R. HODGSON[2];
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Abstract: Telemetry is a method of monitoring physiological functions in awake and freely moving laboratory animals, while minimizing stress and anaesthesia-induced artefacts. It allows for real-time remote recording of biological parameters such as core body temperature, locomotor activity and electrocardiogram (ECG), electroencephalogram (EEG) and electromyogram (EMG) that is cost-effective and minimally labor intensive relative to other methods. Continually evolving refinements in telemetry methodology will undoubtedly aid researchers in acquiring high quality, physiologically relevant data and contribute to groundbreaking discoveries that may, ultimately, lead to therapeutics. In the present studies, we use telemetry to describe the effects of five pharmacological agents on multiple physiological readouts. In the present study, telemetric transmitters (F40-EET, Data Sciences International) were inserted in the abdominal sac of six adult female CD rats (200-250 g) during inhalation anaesthesia of 2% isoflurane. In order to record ECG, the negative lead was guided subcutaneously to the upper left abdomen and attached to the pectoral muscle. The positive lead was attached to the abdominal tissue below the heart and the left diaphragm. Thereafter, subcutaneous pocket was made from the abdomen through the dorsal flank of the animal such that EEG leads could be guided through the pocket and taken out towards the base of the skull. After the EEG leads were guided through the pocket, abdominal cavity muscles and skin were sutured. The rat was then turned and small incision was made to expose the skull. Two trepanations were made in the skull at coordinates AP -1 mm, ML -1 mm and at AP +1 mm, ML +1 mm. The EEG electrodes were secured with dental acrylic. ECG and EEG signals were followed during surgery to confirm the proper installation of electrodes. The transmitters were remotely activated for measurement of body temperature, locomotor activity, ECG and EEG. After 30 min baseline recording, pharmacological treatments were performed in all rats. Pharmacological treatment order was the following: nicotine (1.25 and 2.5 mg/kg), haloperidol (0.5 and 1 mg/kg), amphetamine (1 and 2.5 mg/kg), risperidone (0.01 and 0.05 mg/kg) and pregabalin (10 and 30 mg/kg). All compounds were given intraperitoneally and n=3 for a each dose. Recordings were performed over a period of 5 h after each pharmacological treatment. Washout period was 2-3 days between treatments. The telemetric results provide key reference physiological data for five compounds commonly used in CNS research and provide further validation of the use of telemetry to assess the effects of drug treatments.

Disclosures: R.O. Pussinen: None. **L. Tolppanen:** None. **A. Nurmi:** None. **R. Hodgson:** None.

Poster

253. Mechanisms of Attention I

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Topic: F.02. Animal Cognition and Behavior

Support: NSERC 341600

Title: Selective entorhinal cortex lesions influence visual attentional set-shifting performance in rats

Authors: *K. BOUAYAD-GERVAIS, Y. CHUDASAMA;
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Abstract: The entorhinal cortex provides highly processed visuospatial information from the perirhinal and postrhinal areas in the temporal lobe to the hippocampus. Whereas dorsolateral efferent connections of the entorhinal cortex synapse in the dorsal hippocampus, medioventral efferent connections of the entorhinal cortex target the ventral hippocampus. (Dolorfo and Amaral, J. Comp Neurol, 1998, 398-48; Prasad and Chudasama 2013, 33:8494-8503). This suggests that the dorsolateral and medioventral bands of the entorhinal cortex process different types of information in parallel. Consistent with this idea, behavioural studies have shown that the dorsolateral entorhinal cortex (dEC) mediates visuospatial input to the dorsal hippocampus, whereas the medioventral entorhinal cortex (vEC) relays emotional information directly to the ventral hippocampus (Steffenach et al., Neuron, 2005, 45:301-313). The entorhinal cortex has also been shown to enable attentional processing. For example, it has been shown that while control rats readily discriminate relevant stimulus dimensions at a faster rate than irrelevant stimulus dimensions, rats with entorhinal cortex lesions discriminate both relevant and irrelevant stimulus dimension at the same rate (Oswald et al., 2001, Behav Neurosci, 115:841-849). Thus, when entorhinal cortex is damaged, the animal cannot ignore incoming visual information that is irrelevant. In this study, we sought to determine the distinct contribution of the dEC and vEC in a visual attention set-shifting task using a touchscreen operant platform, a task analogous to the human and non-human primate version (Roberts et al, 1988, Quart J Exp Psycho, 40B:321-341). Rats were presented with complex visual stimuli of black lines superimposed onto white geometric shapes set on a light gray background. In the first stages, only the shape was relevant and the lines were to be ignored. Before the lesion, all rats successfully discriminated the shapes, and focused their attention to novel exemplars of shape as the relevant perceptual dimension whilst ignoring the lines (i.e. intradimensional shift). After the lesion, animals showed good retention of the same stimuli. However, when presented with novel shapes and line exemplars, both lesioned groups had difficulty in shifting attention to the new shapes and therefore committed many errors. However, when required for the first time to shift their attention from shapes to lines (i.e. extradimensional shift), only those animals with dEC lesions were highly attentive making fewer errors. These preliminary data implicate the entorhinal cortex in shifting attentional resources between different stimuli.

Disclosures: K. Bouayad-Gervais: None. **Y. Chudasama:** None.

Poster

253. Mechanisms of Attention I

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Title: Superior colliculus activity is necessary for both selectively attending and actively ignoring visual stimuli

Authors: *L. P. LOVEJOY[1], R. J. KRAUZLIS[2];
[1]Dept. of Psychiatry, Columbia Univ. / New York State Psychiatric Inst., New York, NY; [2]Lab. of Sensorimotor Res., Natl. Eye Inst. - NIH, Bethesda, MD

Abstract: Animal behavior depends on the ability to selectively attend to some sensory inputs while actively ignoring others. These two functions involve a network of cortical and subcortical structures; recent studies have shown that inactivation of neurons in the Superior Colliculus (SC) impairs selection of cued stimuli in the presence of foils. Nevertheless, there is scant evidence that any particular brain region is necessary for classic measures of selective attention such as changing sensitivity to sensory inputs based on their behavioral relevance. Here we show that activity in the primate SC is necessary for both these functions. We trained rhesus macaques to perform a motion discrimination task in which peripheral spatial cues indicated the location at which the relevant motion stimulus might appear; subjects were rewarded for indicating the direction of motion with a saccade. On some trials, the motion stimulus appeared in the absence of distractors, in which case performance and sensitivity were the highest. In the presence of distractors, sensitivity and performance decreased. Spatial cues led to recovery of performance and sensitivity when relevant stimuli appeared at the cued location, and also decreases in sensitivity and enhanced ability to ignore irrelevant stimuli at uncued locations. Reversible inactivation of the superior colliculus eliminated the ability of monkeys to use spatial cues to improve performance for stimuli placed in the affected visual field and also impaired their ability to suppress inappropriate responses based on irrelevant stimuli placed outside of the

affected visual field. This result indicates that both selectively attending and actively ignoring each depend on neural activity in the superior colliculus. In addition, we demonstrated that the combination of these two separable processes, characterized independently in these new experiments, could also accurately predict performance from published work when cued stimuli directly competed with foils. Specifically, superposition of the processes of attending and ignoring as represented by linear combination of sensitivity accurately predicted the impact of SC inactivation on selective attention as previously reported in Lovejoy and Krauzlis (2010). Thus, activity in the superior colliculus is necessary for both selectively attending and actively ignoring visual stimuli, and these simultaneous and separable processes can explain selective attention in the case of competition between cued stimuli and foils via a simple linear model.

Disclosures: L.P. Lovejoy: None. **R.J. Krauzlis:** None.

Poster

253. Mechanisms of Attention I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 253.06/V24

Topic: F.02. Animal Cognition and Behavior

Title: On the magnitude of signal correlations versus noise correlations

Authors: *C. M. LEWIS[1], A. LAZAR[2], B. LIMA[3], M. VINCK[4], S. NEUENSCHWANDER[5], W. SINGER[2], P. FRIES[1];
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Abstract: Cortical responses to repeated trials of identical sensory stimulation exhibit a large degree of variability, despite the physical constancy of the stimulus. This variability is thought to arise from activity intrinsic to the cortex itself. This intrinsically arising variability is often correlated across neurons, and these correlations may affect the fidelity of sensory responses and the effective coding capacity of neural populations. A classical approach to perceptual processing attempts to decompose sensory responses into two distinct components: an extrinsic component, arising from the physical characteristics of a stimulus, and an intrinsic component, arising from uncontrolled internal brain activity or measurement noise. The extrinsic component is most often estimated by computing the mean response across identical trials, representing the most reliable portion of the stimulus-related activity. The correlation between different neurons of their mean response to different stimuli is termed the 'signal correlation' and is a measure of the similarity in selectivity or tuning of the neurons under consideration. The intrinsic component is the residual response after removing the mean activity, and the correlation of this residual

between two neurons is termed the 'noise correlation'. Although noise correlations in a wide range (r = 0.005-0.35) have been published, recent work has stressed the small magnitude of noise correlations when spike isolation and behavioral state are properly controlled. At the same time, signal correlations are often found to be considerably larger than noise correlations (r = ~0.6), suggesting that sensory responses are dominated by extrinsic factors. Here, we point out that differences in the manner in which signal and noise correlations are computed lead naturally to different magnitudes. The fact that the values in question are averages leads to smaller variances in the samples used to compute signal correlation, as opposed to noise correlations. We present a modified measure of the signal correlation that accounts for the reproducible portion of the sensory stimulus, but avoids exaggerated values. We demonstrate that the magnitudes of the signal correlations computed in this manner are on the order of the noise correlations in a population of cells recorded from multiple electrodes in v1 of three awake, behaving macaques. This fact suggests that extrinsic and intrinsic factors contribute equally to neuronal spiking activity. We relate the relative magnitude of the extrinsic and intrinsic response components to synaptic connectivity strengths in a recurrent neural network.

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Poster

253. Mechanisms of Attention I

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Title: Two-photon imaging of state-dependent modulation in primary and higher-order visual areas

Authors: ***R. N. RAMESH**[1,2], C. R. BURGESS[2], S. SUBRAMANIAN[3], G. J. GOLDEY[2], M. L. ANDERMANN[2,1];

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Abstract: Changes in motivational state can affect the relevance of sensory stimuli and modulate neural responses to these stimuli. Our lab has developed a mouse model for understanding how different motivational states affect cortical processing. We train head-fixed, food-restricted mice in a go/no-go task to test how motivational state selectively biases behavioral and neural responses to food-associated cues. Using two-photon calcium imaging in a behaving animal, we can record from hundreds of neurons simultaneously across many days, and thus across motivational states. In this study, we imaged populations of neurons in both primary visual cortex (V1) and postrhinal cortex (POR), a higher order visual area thought to be important for object recognition. Postrhinal neurons are reciprocally connected with the hippocampus, entorhinal cortex, and amygdala - structures important for learning, memory, and encoding of motivational salience. Preliminary results suggest that POR neurons show modulation of responses to food-associated visual stimuli in hungry vs. sated states, an effect that was less common in area V1. These data suggest that POR may be part of a brain network for selective processing of motivationally-relevant sensory cues.

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Poster

253. Mechanisms of Attention I

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Title: The dependence of inter-areal functional connectivity through rhythmic synchronization on anatomical connection strength

Authors: *J. VEZOLI[1], A. M. BASTOS[1,2], C. LEWIS[1], C. A. BOSMAN[3,4], H. KENNEDY[5], P. FRIES[1,3];
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Abstract: The relationship between the strength of rhythmic neuronal synchronization and sensory stimulation, attention, or other cognitive factors is well established. We understand less well how rhythmic synchronization depends on anatomical connectivity. Synchronization has been shown to decline with distance (Leopold et al., Cereb.Cortex, 2003). Also, in primary visual cortex, synchronization between monocular neurons in strabismic animals, which largely lack lateral connections (Löwel and Singer, Science, 1992), is strongly reduced (König et al., Eur.J.Neurosci., 1993). Yet, the degree to which neuronal synchronization between brain areas depends on the strength of their anatomical connectivity has not been studied systematically. We obtained quantitative measures of anatomical connection strength for many pairs of cortical areas in the macaque (www.core-nets.org). In two macaque monkeys, we recorded with 252 channel electrocorticographic grids from the left hemisphere, while animals performed a visual attention task. We determined frequency-resolved coherence, Granger causality and power-power correlations for a large number of area pairs. This shows that across pairs of cortical areas, inter-areal functional connectivity is correlated with inter-areal anatomical connection strength. This correlation holds when it is partialized for distance, either physical distance, distance along the dural surface (relevant for potential volume conduction) or distance through the white matter. The functional-anatomical correlation was highly significant for theta, beta and gamma bands, which were prominent in this dataset. When the beta and gamma networks were investigated separately, this revealed distinct topographical differences. Consistent with the recent insight that gamma predominates in feedforward and beta in feedback signaling, we found the gamma network to be strongest among visual areas, and the beta network to be strongest among fronto-parietal areas. This is likely related to laminar differences in the origin of the respective anatomical projections. In summary, while inter-areal rhythmic neuronal synchronization can be strongly modulated by stimuli and task parameters, we have shown that it nevertheless relies on an anatomical backbone.

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Poster

253. Mechanisms of Attention I

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Topic: F.02. Animal Cognition and Behavior

Support: HFSP Fellowship LT000904/2011-L

Title: Inter-areal synchronization in visual cortex during a divided attention task

Authors: *M. L. SCHOLVINCK[1], J. R. DOWDALL[1], E. FIEDLER[2], T. STIEGLITZ[2], P. FRIES[1];
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Abstract: When processing a visual stimulus, neuronal ensembles in different brain areas along the visual hierarchy interact with each other. Attention is known to influence these interactions; attending to a visual stimulus increases the coherence between neuronal populations in V1 and V4 representing that stimulus (Bosman et al., Neuron 2011). It is unknown how dividing one's attention over several visual stimuli impacts these interactions. Behavioral evidence in humans suggest attention might switch rhythmically between the stimuli (Landau & Fries, Current Biology 2012), but attention could also be truly divided between the stimuli. We recorded local field potential (LFP) from areas V1 and V4 in one monkey (Macaca mulatta) while the monkey was engaged in a divided attention task. Two elliptical, colored drifting gratings were presented, both of which had an equal probability of transiently changing their drift orientation slightly, after which the monkey was required to make an eye movement to this particular grating to obtain a fluid reward. LFP was recorded with a 252-contact electrocorticography (ECoG) grid covering the superficial parts of V1 and V4 that correspond to the central 8 degrees of visual angle. The two gratings were placed such that they activated separate neuronal populations in V1, but fell within the same receptive field of a chosen V4 site. Therefore, the coherence between these separate V1 sites and the V4 site could be studied as both stimuli were attended to. The V1 sites that were selectively activated by one or the other grating showed stimulus induced enhancements in power in the gamma-frequency range, peaking at around 62 Hz (mean gamma-power increase of 9300 %). When the stimuli were shown separately, a large proportion of these sites also showed coherence to the V4 site exclusively for one or the other stimulus. Peaks in the coherence spectrum were around 58 Hz (mean coherence value of 0.09). When both stimuli were shown simultaneously, peak coherence values dropped to about half of what they were during single stimulus presentations. More detailed analyses into this divided attention condition focused on the relationship between the coherence between V1 and V4 just before the orientation change and the reaction time of the monkey towards that orientation change. Together, the analyses will shed more light on the neural implementation of divided attention.

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Poster

253. Mechanisms of Attention I

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Program#/Poster#: 253.10/V28

Topic: F.02. Animal Cognition and Behavior

Title: The role of theta oscillations during spatial attention in monkey visual cortex

Authors: *G. SPYROPOULOS[1], C. BOSMAN[2], P. FRIES[1];
[1]Ernst Struengmann Inst., Frankfurt Am Main, Germany; [2]Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: The presence of theta oscillations in the primate visual cortex has been mostly linked to the successful maintenance of tokens in working memory. Their relation to attention remains, however, unknown. To investigate that relation, we used dense subdural electrocorticography grids (ECoG) in two awake rhesus macaques. In particular, we recorded local field potentials from early (V1) and intermediate (V4) visual areas of the subjects' left hemisphere while they were performing a cued spatial visual attention task. Attention away from the stimulus activating the ECoG recording sites was accompanied by a theta (3-5 Hz) peak in the power spectrum. This low-theta rhythm exhibited a similar topography as stimulus induced gamma band activity and was therefore restricted to the cortical population activated by the stimulus. Moreover, both within and between areas V1 and V4, sites exhibited phase locking in the low theta range, that was stronger in the attend-away condition. Further analysis revealed that the phase of the theta oscillation modulated the amplitude of local narrow-band gamma oscillations. This cross-frequency interaction was stronger in the attend-away condition. These findings were independent of the rate of microsaccades in the two attentional conditions. Our results are consistent with the view that attentional allocation is subserved by the transition of the underlying cortical circuit, from a low-frequency dominated to a higher-frequency dominated state, mimicking increased arousal. Theta oscillations, in the part of cortex representing the ignored stimulus, shape the temporal structure of local gamma oscillations. This would result in the rhythmic breaking of local processing and inter areal communication, which may mechanistically support the filtering-out of the distracter stimulus.

Disclosures: G. Spyropoulos: None. **C. Bosman:** None. **P. Fries:** None.

Poster

253. Mechanisms of Attention I

Location: Hall A

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Topic: F.02. Animal Cognition and Behavior

Support: American Federation for Aging Research

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Title: Cholinergic contributions to PASA and functional compensation in rats

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Abstract: Neuroimaging studies have indicated increased recruitment of prefrontal regions coupled to reduced activation of posterior regions in task-performing older adults. This shift of activity in cortical networks is described as posterior-anterior shift in aging (PASA). What cellular mechanisms contribute to PASA and how it provides functional compensation for age-related decline in cognitive capacities remains unknown? Cortically-projecting forebrain cholinergic neurons modulate cortical networks and facilitate attentional processes. Here we examined whether cortical cholinergic inputs contribute to PASA expression and maintenance of attentional capacities in aging. Young (3 months) and aged (24 months) Wistar rats were trained in a sustained attention task (SAT) that requires them to distinguish between signal and non-signal events. After attaining criterion performance (≥70% correct responses for 3 consecutive sessions), rats received bilateral infusions of cholinoselective immunotoxin 192-IgG SAP either into the prefrontal cortex (PFC) or posterior parietal cortex (PPC) to produce partial cholinergic deafferentation. Control animals were infused with saline. Following behavioral testing 4 weeks post-surgery, animals were perfused 45-min after the last session to examine changes in neuronal activity in the PFC and PPC using c-fos immunohistochemistry. Partial prefrontal cholinergic deafferentation in aged rats produced robust deficits in response accuracy on signal trials as compared to aged sham ($p=0.04$) and young lesion ($p=0.03$) rats. In general, c-fos expressing neurons were higher in the PFC of aged rats as compared to young rats. Although prefrontal neuronal activity did not differ between the aged sham and PFC lesion group, there was a trend for a higher neuronal activity in the PPC of the latter. Surprisingly, attentional performance displayed a negative correlation with the prefrontal activity. Neuronal activity in the PPC did not correlate with performance. PPC-infused aged rats displayed no lesion effect on SAT and performed better than aged rats infused with 192 IgG-SAP into the PFC ($p=0.04$). Moreover, partial loss of cholinergic inputs into the PPC reduced PFC recruitment as compared to PFC lesioned aged rats. Collectively, these data suggest that reduced cortical activity in young rats compared to aged rats may represent better neural capacity, or the efficient utilization of normal brain regions, for task performance. Moreover, PASA is not triggered by prefrontal cholinergic inputs, but these inputs may regulate the reciprocal interactions between the PFC and PPC networks to maintain optimal performance in aging.

Disclosures: B. Yegla: None. **J.A. Francesconi:** None. **J.C. Forde:** None. **V. Parikh:** None.

Poster

253. Mechanisms of Attention I

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Support: NIH Grant F32 EY023465

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Title: Neural correlates of rhythmic selective attention

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Abstract: Classic attention theories characterize spatial selection as a sustained, indivisible spotlight that continuously scans the visual scene, pausing to illuminate particularly relevant stimuli. But recent evidence necessitates that we update this metaphor. For example, the attentional spotlight, rather than being sustained, flashes rhythmically, sampling the visual environment at frequencies in the theta band (4 to 8 Hz). We recently used human behavioral data to demonstrate such attention-related rhythmic sampling both (1) within a cued object and (2) alternating between a cued object and a second, less relevant (uncued) object. These data thus demonstrate an important consequence of rhythmic sampling: there are interdigitated windows when events at less relevant locations have a relatively increased likelihood of being selected for further processing. These windows persist under conditions of sustained spatial selection at a cued location. After replicating the behavioral findings in two monkeys, we used neurophysiological recordings to investigate neural correlates of rhythmic selective attention. We simultaneously recorded both SUA and LFP data from visual cortex and three hubs of the attention network (i.e., FEF, LIP, and the dorsomedial pulvinar). Our results link oscillatory patterns in neural data at both local and network levels to behavioral outcomes (i.e., hit rates). We characterize neural responses in these ROIs under three condition of selective attention: when receptive fields and response fields overlapped with either a cued location (i.e. under conditions of space-based selection), an uncued location within the same object as the cued location (i.e., under conditions of object-based selection), or an uncued location on a second object (i.e., in the absence of space- and object-based selection).

Disclosures: I.C. Fiebelkorn: None. **M.A. Pinsk:** None. **S. Kastner:** None.

Poster

253. Mechanisms of Attention I

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Simons Foundation

Title: Distinct roles of mouse visual and parietal cortex during perceptual decisions

Authors: *G. N. PHO, M. J. GOARD, B. CRAWFORD, M. SUR;
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Abstract: The posterior parietal cortex (PPC) has been implicated in perceptual decisions, but its specific role at the interface between sensation and action remains unresolved. Here, we provide evidence that mouse PPC, in functional analogy to primate parietal cortex, is neither a pure sensory area, nor directly involved in control of motor output, but rather is important for the mapping of sensory inputs to motor commands. Mice were trained on a visual discrimination task with distinct stimulus and motor epochs. We first tested the necessity of PPC and the primary visual cortex (V1) during the different task epochs using VGAT-ChR2 transgenic mice, which express ChR2 in inhibitory neurons. Optogenetic inactivation revealed that both V1 and PPC were necessary during the stimulus period, but not for execution of the motor response. We then used two-photon calcium imaging to measure population activity in V1 and PPC during engagement in the task and during passive viewing of the same stimuli. Whereas V1 responses were driven by visual stimuli alone and only weakly modulated by task engagement, PPC responses were strongly gated by engagement and signaled the impending response. PPC responses exhibited signatures of classical decision neurons: they reflected both the animal's choice on error trials, as well as the degree of sensory evidence, which was manipulated using stimuli of varying contrasts. Lastly, to test whether PPC primarily encoded information about the stimulus or the choice, we re-trained mice with a reversed stimulus-reward contingency, and imaged the same neurons before and after the switch. We found that stimulus selectivity in PPC, but not V1, was dramatically reversed after retraining on the new contingency. Our results are consistent with a role of the mouse posterior parietal cortex in transforming sensory information to motor commands during perceptual decisions.

Disclosures: G.N. Pho: None. **M.J. Goard:** None. **B. Crawford:** None. **M. Sur:** None.

Poster

253. Mechanisms of Attention I

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Topic: F.02. Animal Cognition and Behavior

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Title: Multiple, temporally-ordered gain mechanisms of attention in macaque area V4

Authors: *I. SANI[1], M. C. MORRONE[2], L. CHELAZZI[3], E. SANTANDREA[3];
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Abstract: Visual attention and perceptual saliency govern selection of relevant input and the ensuing behavioral output. Yet, we still lack a universally accepted account of the interplay in visual cortex between attention and luminance contrast - a classical dimension of saliency. Neither single-neuron recordings nor fMRI and psychophysical data have so far allowed to converge on a single model to account for the attentional modulation of Contrast Response Functions (CRF). We measured the effect exerted by top-down spatial attention on V4 neurons CRFs, and applied a new approach which emphasized the temporal dynamics of cell responses, while taking in proper consideration the heterogeneity of contrast coding in are V4. Subjects (two Macaca mulatta) performed an orientation discrimination task on stimuli of varying contrast and attention was cued to different spatial positions (either inside or outside the cell receptive field) in different blocks of trials. We found that attention modulates CRFs via the temporally-ordered engagement of distinct gain mechanisms: an early contrast-gain - strongly dependent on pre-stimulus activity changes (baseline shift), a time-limited stimulus-dependent multiplicative modulation, and a late resurgence of contrast-gain modulation of CRFs. Attention produced comparable time-dependent attentional gain modulations on cells heterogeneously coding contrast, i.e. those displaying traditional sigmoidal as well as those displaying selective CRFs (Sani et al., J. Neuroscience, 2013). However, for a considerable fraction of the total cells, attention was capable of inducing radical transformations in CRF shape: from sigmoidal (monotonic) to tuned, or vice versa. These findings help clarify divergent results in the literature and offer important insights regarding the mechanisms which underlie coding of contrast and attention in the primate visual cortex.

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Poster

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Title: Response gain is modulated by the gamma cycle

Authors: *J. NI[1,2], T. WUNDERLE[1], C. LEWIS[1], R. DESIMONE[3], I. DIESTER[1,4], P. FRIES[1,5]; [1]Ernst Strüngmann Inst. (ESI) for Neurosci., Frankfurt, Germany; [2]Intl. Max Planck Res. Sch. for Neural Circuits, Frankfurt, Germany; [3]McGovern Inst. for Brain Research, Massachusetts Inst. of Technol., Cambridge, MA; [4]BrainLinks-BrainTools Cluter of Excellenz, Univ. of Freiburg, Freiburg, Germany; [5]Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: Effective neuronal connectivity along a given anatomical projection is likely modulated by rhythmic synchronization among the connected neuronal groups. The Communication-Through-Coherence (CTC) hypothesis posits that rhythmic neuronal synchronization within the receiving group modulates the response gain of synaptic inputs, a proposal that has so far received equivocal support. Here we test whether local gamma-band synchronization in extrastriate visual cortex modulates the response to visually evoked synaptic inputs. In a first experiment, we recorded multi-unit activity (MUA) and local field potential (LFP) in awake monkey area V4 (N=2 subjects), while neuronal gamma-band synchronization was induced for several seconds by a moving grating stimulus. A sudden color change in the grating evoked a MUA response. We found this response to be modulated by the phase of ongoing gamma-band oscillations before the stimulus change. The response modulation was not an addition of rhythmic activity onto the average response but a rhythmic modulation of multiplicative response gain. The gain modulation might occur at the input to V4, or alternatively at the output of earlier areas sending input to V4 and oscillating coherently with V4, like V1 (see e.g. Bosman et al., Neuron, 2012). While both effects are consistent with CTC, we aimed at specifically demonstrating an effect emerging at the input to a higher area. Therefore, in a second experiment, we used optogenetics to induce a local, "isolated" gamma rhythm in an extrastriate area, and subsequently probed responses to visual stimulus onsets. These experiments were performed in anesthetized cats (N=2 subjects) injected with recombinant adeno-associated viral vectors (AAV9-CamKIIa-hChR2-eYFP) into area 21a, the cat homologue of monkey V4. Four to six weeks after injection, MUA and LFP were recorded both in extrastriate area 21a and primary visual area 17. Illumination of area 21a with 1.25 s constant LASER light (473 nm, 5-10 mW) induced gamma-band synchronization with peak frequencies between 40 and 80 Hz. While this optogenetically induced gamma rhythm was strong in area 21a, it could not be detected in area 17. A visual stimulus, presented from 1-1.25 s after LASER onset evoked a visual response, which showed a multiplicative modulation by the phase of the preceding gamma rhythm. As area 17 showed no gamma, the isolated gamma in area 21a likely resulted in a rhythmic modulation

of input gain. These results show that a gamma rhythm in a receiving neuronal group modulates input gain. Effective connectivity is therefore maximal between sending and receiving neurons oscillating coherently, in line with the CTC hypothesis.

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Poster

253. Mechanisms of Attention I

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Title: Rodent model of sensory prioritization: behavioral performance and neural correlates

Authors: *C. LEE[1], M. E. DIAMOND[2], E. ARABZADEH[1];
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Abstract: In a natural environment, animals need to assess when to initiate actions based on uncertain sensory evidence. This is evident when dealing with weak sensory inputs, such as small changes in luminance, sounds or vibrations induced by predators. In such scenario, animals benefit from prioritizing sensitivity in the modality that is more likely to provide the key information. To investigate the neuronal and behavioral correlates of sensory prioritization, we trained rats in two detection paradigms: a go/no-go detection (GNG) (n=4) and a two-alternative-forced-choice (2AFC) (n=4) where they detected the stimulus position (left/ right). In GNG, either a vibration (3-8μm; 40Hz) was applied to whiskers or a visual flicker (4-12% luminance change) was presented on each trial. In 2AFC, either a vibration (100μm; 40Hz) or a series of auditory pulses (50dB; 40Hz) was presented. Stimuli were adjusted in difficulty for each rat to exhibit 20% miss rate. We manipulated attention by controlling the likelihood with which the stimulus was presented in each modality. In a *whisker session*, 80% of trials were whisker vibration and the remaining 20% were visual flicker (GNG) or auditory pulse (2AFC). Likelihoods were reversed in a *visual session* (GNG) and *auditory session* (2AFC). As rats performed the task, we recorded single-cell activity from primary somatosensory cortex (n = 31) along with local field potential (LFP). Across rats, the earliest behavioral manifestation of detection (when *d'* deviated from chance) was remarkably fast in GNG task (vibration:

M=47.5ms; flicker: M=56ms) compared to 2AFC task (vibration: M = 141ms; auditory: M= 193ms). High-likelihood trials (e.g. vibration during *whisker session*) resulted in lower miss rates (vibration: 18% in high-likelihood vs. 34% in low-likelihood, $p<0.01$; flicker: 19% vs. 32%, $p<0.01$), higher performance (vibration: 66 % vs. 35%, $p<0.01$; auditory: 71% vs. 46 %, $p<0.01$), and faster decisions (vibration $_{\text{low-high likelihood}}$: 187.5ms, $p<0.01$ [GNG]; vibration $_{\text{low-high likelihood}}$: 153ms, $p<0.01$ [2AFC]; flicker $_{\text{low-high likelihood}}$: 131.3ms, $p<0.01$; auditory $_{\text{low-high likelihood}}$: 68ms, $p<0.01$) than on low-likelihood trials (e.g. flicker during *whisker session*). During entrance and exit from the stimulus presentation port, increases in single-unit activity and modulations of LFP activity reflected changes in whisking behavior. Spectral analysis of low frequency LFP (<14Hz) and changes in LFP voltage variability during nose-poke was predictive of the modality rats prioritized and was also predictive of performance. Our study provides both behavioral and neuronal evidence for sensory prioritization in rodents.

Disclosures: C. Lee: None. **M.E. Diamond:** None. **E. Arabzadeh:** None.

Poster

253. Mechanisms of Attention I

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Support: IPM

DPZ

Bernstein

Title: Principal component model of neural activities in macaque area MT

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Abstract: The modulation of neuronal activities underlying spatial attention has been shown in many areas of human and primate cortex including area MT. In this study, we have investigated the common neuronal mechanism underlying attention and direction of motion processing at the level of neuronal population. Therefore we examined the responses of 254 isolated direction-selective neurons in area MT of a male macaque monkey while the animal was performing a spatial attention task. First, the strength of the attentional modulation across the neuronal population was estimated by fitting a line with a free slope and base using the method of non-linear least squares. The estimated attention modulation is 17.1% and significant in the neuronal population (P-Value<0.001; Mann-Whiteney U test). In addition, the transformed divergence of

separability index is 0.2234 (Confidence Interval (CI): 0.2174 - 0.2295) for encoding attentional state, and 9.4307×〖10〗^(-4) (CI: 8.0×〖10〗^(-4) - 1.0×〖10〗^(-3)) for encoding motion directions. Second, the significance of information existing in the space of dissimilarities across neuronal subpopulations was elucidated using Principle Component Analysis (PCA) method applied to dissimilarity maps. Consequently, the neural distances were estimated based on the dissimilarity measurements utilizing correlation operation applied to the patterns of neuronal activities. The transformed divergence separability index is 5.0981×〖10〗^(-4) (CI: 4.697×〖10〗^(-4) - 5.5×〖10〗^(-4)) for encoding of the attentional state and it is 1.0251 (CI: 0.9904 - 1.0597) for encoding the stimulus motion direction. Moreover, in the space of neural activity, the strengths of separability between the motion directions is 3.1616×〖10〗^(-5). Our results suggest that the neural mechanism of visual attention and visual motion processing belong to different mathematical spaces. The motion directions were encoded in the space of neural distances, which did not contain any significant information about the attentional state. Reciprocally, attentional states were significantly separable in the space of neural activities, contrary to the non-severable clusters of motion directions.

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Poster

253. Mechanisms of Attention I

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Title: Attention operates uniformly throughout the Classical Receptive Field and the Surround

Authors: ***B. E. VERHOEF**, J. H. R. MAUNSELL;
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Abstract: Our eyes are constantly bombarded by a multitude of visual stimuli, yet not all stimuli can be processed thoroughly. Spatial attention sifts through the plethora of stimuli, enhancing perception at behaviorally-relevant locations, but the underlying neural principles of this process are not fully understood. During natural vision, large regions of the classical receptive field and the surround of visual-cortical neurons are stimulated. Feedforward-, feedback- and intracortical circuitries are thought to contribute differentially to the suppressive and excitatory inputs associated with stimuli in either the classical receptive field or the surround. Furthermore, the

classical receptive field and the surround presumably serve different functional roles, and the role of the surround in spatial attention is still unclear. Hence, how attention operates within the classical receptive field compared to the surround remains a fundamental open question. We trained two rhesus monkeys to perform a visual-detection task in which spatial attention was controlled and measured. Using chronically implanted microelectrode arrays we probed how attention affects neuronal responses to various stimulus configurations both inside and outside the classical receptive field of V4 neurons. We find that stimulus selectivity alone, or stimulus-related suppression (normalization or surround suppression) alone, cannot explain response modulations by attention. Instead, attention-related modulations rely on a non-additive combination of stimulus selectivity and stimulus-related suppression. A spatially-tuned normalization model, in which suppression varies across receptive-field locations, captured these non-additive relationships in all stimulus configurations. This model relates stimulus selectivity, normalization, surround suppression and attention-related modulation to each other, and unifies spatial summation and attention-related modulation across different regions of the receptive field.

Disclosures: **B.E. Verhoef:** None. **J.H.R. Maunsell:** None.

Poster

253. Mechanisms of Attention I

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Title: Prone to addiction as well as to falls: Poor attention in sign-tracking rats extends to complex movement control and is associated with regression of choline transporter capacity

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Abstract: Some animals (sign-trackers; STs) are especially prone to attribute incentive motivational value ("incentive salience") to reward cues, relative to others (goal-trackers; GTs),

and this, along with other factors, may influence the propensity to relapse in addiction (e.g., Robinson et al., 2014). One other such factor concerns poor attentional control in the presence of drug cues. Indeed, STs exhibit low and unstable levels of attentional performance and these impairments are associated with relatively low levels of cortical cholinergic neuromodulatory activity (Paolone et al., 2013). Here we asked whether the relatively poor attentional control of STs extends to their ability to deploy attentional resources for supporting complex movement, which can contribute to falls in aged and Parkinsonian subjects, and has been associated with declining cholinergic function. STs and GTs were tested on the Michigan Complex Motor Control Task (MCMCT) that assesses gait, posture control and complex movement control by measuring the ability to traverse dynamic surfaces (Kucinski et al., 2013). Compared to GTs, STs committed more slips and fell more frequently while traversing rotating rods, particularly when the rotating direction was changed to a less familiar (reversed) direction. Furthermore, the presentation of a distractor caused more falls in STs. Secondly, we asked whether the relatively low cholinergic activity in STs performing attention-demanding tasks is associated with attenuated choline transporter (CHT) function, as CHT function is a main determinant of cholinergic activity. Brains were harvested immediately following the animals' last MCMCT trial. Interestingly, frontal cortical CHT-mediated choline uptake in STs not only failed to be mobilized by MCMCT performance but regressed below levels obtained from non-performing STs. To validate this finding, we determined the effects of basal forebrain electrical stimulation (Parikh et al., 2013). Once again, rather than exhibiting enhanced choline uptake, in STs, stimulation resulted in uptake levels below those seen in nonstimulated controls. In GTs, performance on the MCMCT did not alter choline uptake, however uptake in GTs was significantly increased with stimulation. Immunoblotting experiments indicated that performance increased surface expression of CHTs in both STs and GTs and thus changes in CHT densities do not account for the functional CHT regression in STs. Together, these results indicate that the attentional vulnerabilities of STs extend to complex movement control and they are associated with regulatory silencing of CHT regulation.

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Poster

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Title: Locus coeruleus firing modes differentially regulate neural processing of salience in somatosensory cortex

Authors: *E. M. VAZEY[1], D. E. MOORMAN[2], G. ASTON-JONES[3];
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Abstract: The noradrenergic nucleus locus coeruleus (LC) projects broadly throughout the central nervous system and is the near exclusive source of norepinephrine (NE) to cortex. Among its targets, LC sends strong projections to primary sensory regions and has long been posited to have an important role in modulating sensory processing. LC neurons fire in two distinct modes, tonically, as characterized by irregular baseline activity (1-6Hz, physiological range) and phasically during short bursts of stimulus evoked activity (10-15Hz). Under normal conditions tonic LC activity changes with arousal and/or stress whereas phasic LC activity is evoked after salient sensory stimuli that engage behavioral responses. To causally probe LC influence on cortical sensory processing, we selectively expressed ChR2 in LC-NE neurons and drove LC activity during non-salient somatosensory stimulation. This paradigm allowed us to dissociate cortical representations of somatosensation from those that have been modulated through sensory evoked LC activity. We show that selective optogenetic stimulation of LC-NE in association with non-salient sensory signals changes the population dynamics within sensory cortex and differentially alters the sensory response (n= 135 single units). Both tonic and phasic LC activation modulated basal activity of sensory neurons and repeated LC stimulation sensitized short-latency somatosensory responses. In addition, phasic LC activation recruited a distinct subset of gated somatosensory neurons that generated an additional long-latency sensory response. This long-latency signal from gated neurons evoked by sensory locked phasic LC activity was also driven by increases in stimulus intensity/saliency. Collectively these results indicate a major role of LC activity on somatosensory cortical neurons is to regulate sensory processing by providing discrete saliency signals to enhance processing in cortical targets.

Disclosures: **E.M. Vazey:** None. **D.E. Moorman:** None. **G. Aston-Jones:** None.

Poster

253. Mechanisms of Attention I

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Title: Methylphenidate enhances early sensory signal processing in the rat visual thalamus through noradrenergic signaling

Authors: *R. L. NAVARRA[1], A. T. GARGIULO[2], B. D. CLARK[2], B. D. WATERHOUSE[2]; [1]Pharmacol. and Physiol., [2]Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Abnormal processing of sensory information is a core feature of many neuropsychiatric disorders, including attention deficit hyperactivity disorder (ADHD). The psychostimulant, methylphenidate (MPH), is used clinically to treat ADHD as well as off-label as a performance enhancing drug (PED) by healthy individuals. MPH enhances catecholamine transmission via blockade of norepinephrine (NE) and dopamine (DA) reuptake transporters, (NET and DAT, respectively). However, it is not clear how blockade of catecholamine reuptake impacts neural circuits responsible for sensory signal processing. We reported previously that MPH increases both speed and strength of responses to visual stimuli within the dorsal lateral geniculate nucleus (dLGN) of the anesthetized rat, suggesting that enhanced sensory signal transmission may be a significant component of the action of PEDs. To investigate the relevance of these findings to the waking state, we extended these studies to behaving animals performing a signal detection task. During a 1.5-sec nose-poke response, a light positioned above the head may or may not flash. Rats must indicate by lever response if a stimulus was detected. Consistent with findings in the anesthetized rat, latencies of multi-unit light evoked responses in the dLGN were decreased following systemic administration of MPH. Furthermore, in animals highly trained in this task, MPH administration did not improve accuracy of signal detection but did improve the speed of responding. Given the locus coeruleus (LC)-NE system's long established role in the regulation of behavioral state and state-dependent modulation of sensory processing, we propose the sensory facilitating properties of MPH depend on enhanced NE transmission within sensory circuits. Although the LC sends dense projections to the rat dLGN, it has been reported that dopaminergic innervation is sparse or non-existent. To further demonstrate that MPH-induced modulation of light-evoked activity within the dLGN is dependent on NE signaling, we immunostained sections through the dLGN for tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH), enzymes responsible for synthesis of DA and NE, respectively. The results showed that all TH-positive axons within the dLGN were also DBH-positive, indicating they were noradrenergic and not dopaminergic fibers. This work suggests that MPH, acting via noradrenergic mechanisms, can substantially impact early stage sensory signal processing, an effect that could positively influence responses to salient stimuli in ADHD patients and healthy individuals seeking performance enhancement.

Disclosures: R.L. Navarra: None. **A.T. Gargiulo:** None. **B.D. Clark:** None. **B.D. Waterhouse:** None.

Poster

253. Mechanisms of Attention I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 253.22/V40

Topic: F.02. Animal Cognition and Behavior

Support: NSF GRFP

NEI Grant R01EY017699

Title: Communication between the pulvinar and the layers of Area V4 during selective visual attention

Authors: *R. LY, S. KASTNER;
Princeton Univ., Princeton, NJ

Abstract: A fundamental question in neuroscience is how large-scale networks of neurons interact to give rise to cognitive function. A recent study (Saalmann et al., 2012) suggests that the pulvinar nucleus of the thalamus influences cortical visual areas during selective visual attention in the alpha (8-15 Hz) frequency band. The laminar profile of this influence could inform network-level models of visual processing and attention, but is as yet unknown. We simultaneously recorded laminar activity in area V4 using a linear multielectrode array, and spiking activity and local field potentials in the pulvinar using single-unit microelectrodes in a macaque monkey while the animal performed a spatial attention task. Receptive fields (RF) overlapped across V4 layers and the pulvinar, suggesting that we recorded from an interconnected network. We computed oscillatory power within area/layer, coherence within area/layer and across areas, and the Granger-causal influences between the pulvinar and laminar currents in V4, at gamma and alpha/beta frequencies. We compared these measures when the animal attended to the shared RF versus when the animal attended away. These effects are discussed in the context of recent evidence demonstrating alpha/beta coherence across areas as a marker of top-down processing.

Disclosures: **R. Ly:** None. **S. Kastner:** None.

Poster

253. Mechanisms of Attention I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 253.23/V41

Topic: F.02. Animal Cognition and Behavior

Title: Musically trained mice selectively attend to their own music

Authors: *U. LIVNEH, A. ZADOR;
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Although it is clear that sensory feedback is required for guiding movement, it is unknown how and to what extent such feedback is integrated into the control mechanisms of movement. Here we present a sensorimotor learning task in head-fixed mice that enables the study of feedback control. Mice were trained to play a computer-controlled musical instrument operated by a horizontally moveable rod. To obtain a reward, mice were required to produce a specific auditory stimulus by pulling the rod to a particular location. Importantly, the target location of the rod was varied frequently, to encourage the use of the auditory feedback as opposed to proprioceptive memory to solve the task. Corruption of the auditory feedback signal impaired performance, suggesting that trained mice indeed used the auditory feedback to guide their movement. To further establish the utility of the self-produced auditory feedback, we simultaneously presented the mice with distracting auditory stimuli that were not coupled to their movement. Performance on the task was not affected by the presence of the uncoupled feedback, suggesting that mice selectively attend the feedback coupled to their movements. A wide range of behaviors requires a tight sensorimotor control mechanism to enable proficient integration of information across different brain systems. Our task provides an experimental framework that is tailored for the study of this controlling mechanism in head-fixed mice, and is well-suited to electrophysiology, imaging and optogenetic investigations.

Disclosures: U. Livneh: None. **A. Zador:** None.

Poster

253. Mechanisms of Attention I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 253.24/V42

Topic: F.02. Animal Cognition and Behavior

Support: Johns Hopkins University

Title: A behavioral paradigm for visuospatial selection in freely-behaving mice

Authors: *W.-K. YOU, S. P. MYSORE;
Psychology and Brain Sci., The Johns Hopkins Univ., Baltimore, MD

Abstract: Attention, the ability to select and preferentially process the most important stimulus in the environment at any instant, is a hallmark of adaptive behavior. Nevertheless, the neural mechanisms underlying attention control are poorly understood. A genetically tractable model system can offer powerful advantages for investigating the underlying mechanisms. Here, we develop a behavioral paradigm for spatial attention in the freely-behaving mouse using a touchscreen-based operant platform and visual stimuli. Mice are trained to selectively respond to

the higher priority target stimulus while ignoring a simultaneously presented, task-relevant distracter. We explore the effects of systematically titrating the relative contrasts of the competing stimuli on behavioral performance.

Disclosures: **W. You:** Other; Johns Hopkins University. **S.P. Mysore:** None.

Poster

253. Mechanisms of Attention I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 253.25/V43

Topic: F.02. Animal Cognition and Behavior

Support: Dept. of Comparative Biosciences, UIUC

Title: An animal model of "shift work": the interaction of sex, scheduled food, and sustained attention on daily activity rhythms

Authors: *M. M. MAHONEY[1], R. BALACHANDRAN[2], P. EUBIG[2];
[1]Vet Biosci., Univ. Illinois, Urbana, IL; [2]Comparative Biosci., Univ. of Illinois at Urbana Champaign, Urbana, IL

Abstract: Circadian rhythms are critical for homeostasis and health as they regulate numerous biological processes. These endogenously generated rhythms are regulated by daily signals; the most salient are the light:dark cycle and timed meals. Disruptions to biological rhythms as occurs with shift work results in significant health consequences including an increased risk for cardiovascular, metabolic and reproductive dysfunction. Relatively few studies however have determined the impact of "shift-work" on attention processes. Furthermore, almost no research has examined the impact of disrupted rhythms on males compared to females. Here, we tested male and female rats on a well-established behavioral measure of attention, the 5-choice serial reaction time task (5CSRTT, n= 6-8/group). Task difficulty was modified by altering cue light duration (0.1, 0.5 and 1 sec) and the delay between cues (0, 2, 4 sec). Animals were food restricted so they were motivated to complete the task to receive sucrose pellets. Experimental rats (EXP) were tested during the light or dark (4 hrs after lights-on or off, respectively). Controls (CON) were moved to the testing room and only received sucrose rewards. We analyzed activity patterns and task performance. Within all "day" rats we observed two phenotypes; a predominantly nocturnal rat that was also active at the time of the task/food pellets, and 2) a predominantly diurnal rat that began daily activity at lights-on and remained active until lights-off. As expected, in CON rats, scheduled food rewards resulted in animals becoming diurnal (42% of females and 71% of males). When animals had food rewards coupled with the attention task (EXP), there was an increase in the number of diurnal rats (66% of females and 83% of males) thus suggesting attention tasks can also alter daily activity rhythms.

Furthermore, entraining cues may alter activity rhythmicity in a sex specific manner. Within day-tested EXP rats we found diurnal rats made significantly less incorrect choices than those that remained nocturnal, indicating that cognitive function is improved if the animals align their rhythms to the time of the attention task. This performance difference, however, was only detected at the easiest task condition of 1 sec cue duration and 0 sec cue delay. Finally, regardless of activity phenotype, day-tested females made fewer incorrect responses than day-tested males indicating there a sex difference in the impact of time of day on attention. These data are among the first to use a 5CSRTT as a model for shift work and will enable us to identify the mechanisms by which vigilance can modulate biological rhythms.

Disclosures: **M.M. Mahoney:** None. **R. Balachandran:** None. **P. Eubig:** None.

Poster

253. Mechanisms of Attention I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 253.26/V44

Topic: F.02. Animal Cognition and Behavior

Support: NIH/NIDA DA017960

Title: Cellular correlates of state dependent and methylphenidate-induced changes in prefrontal cortical activity during a sustained attention task

Authors: *B. D. CLARK[1], K. L. AGSTER[2], B. D. WATERHOUSE[2];
[1]Drexel Univ. Coll Med., Philadelphia, PA; [2]Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: The prefrontal cortex (PFC) is responsible for multiple executive functions including the ability to attend to salient events. Catecholamine projections to the PFC play a prominent role in maintaining or shifting attention as dictated by changing behavioral contingencies. The psychostimulant drug methylphenidate (MPH-Ritalin®) blocks reuptake of catecholamines and is used to treat attention disorders as well as to enhance attention and cognitive function in otherwise healthy individuals. Prior work from this laboratory has shown that MPH improves rodent performance in a sustained attention task. The goal of the present study was to identify the cellular correlates of drug-induced and state dependent changes in sustained attention. Fixed or drivable bundles of microwires were used to record the spike train activity of individual medial PFC neurons in rats performing a modified version of the McGaughy & Sarter (1995) sustained attention task. 104 single units from 11 rats were identified, displaying a bimodal distribution of spike widths. 27 (26%) were classified as putative interneurons (trough-peak duration < 250 μsec), 71 (68%) with widths > 400 μsec were classified as putative pyramidal neurons, and 6 were unclassified. 41 % of putative interneurons and 58 % of putative pyramidal neurons

exhibited brief increases or decreases in firing rate associated with either sensory or motor events in the task. Changes that were initially excitatory were more commonly observed in the anterior cingulate and prelimbic cortex, while initially inhibitory responses were more common in the infralimbic cortex. Neurons that displayed sensory responses (to signal light or lever extension) showed generally stronger responses in correct trials than in incorrect trials or omissions, consistent with the hypothesis that performance in this task is affected by the strength of representation of sensory information in the PFC. Average neuronal firing rates during task performance were not systematically altered by MPH. In contrast, MPH did have a consistent effect on sensory-responsive cells, causing a shift to shorter latencies and longer-duration responses, without having a marked effect on peak response magnitudes. The results of this study suggest that sustained attention task performance engages a subset of neurons distributed across the medial PFC, and that MPH effects on attention in the PFC are mediated not via generalized excitability changes across the region, but rather by altered activity in cells showing task-related activity.

Disclosures: **B.D. Clark:** None. **K.L. Agster:** None. **B.D. Waterhouse:** None.

Poster

253. Mechanisms of Attention I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 253.27/V45

Topic: F.02. Animal Cognition and Behavior

Support: Johns Hopkins University

Title: Inhibition and stimulus competition within a GABAergic nucleus in the midbrain attention network

Authors: ***M. STRAKA**, S. MYSORE;
Johns Hopkins Univ., Baltimore, MD

Abstract: In complex environments containing numerous stimuli that are all competing for attention, the brain selects the most important stimulus and processes it preferentially to guide behavior. Recent work has revealed that the superior colliculus (called optic tectum, or OT, in avians) is essential for the flexible, competitive selection of a target among distracters. Furthermore, our past work has shown that competitive interactions within the OT are mediated by an inhibitory nucleus in the midbrain tegmentum, called the Imc. Here, we explore the mechanisms by which the Imc contributes to stimulus competition. Our experiments involve extracellular recordings and focal, iontophoretic blockade of GABA receptors in the midbrain of head-fixed barn owls. We show that inhibition within the Imc plays a critical role in generating the representations of competing stimuli in the Imc and the OT: specifically, that information

regarding competing stimuli is communicated almost entirely by inhibitory inputs to the Imc (rather than by weakened excitatory inputs from the OT). These results shed new light on the neural implementation of stimulus selection for attention.

Disclosures: M. Straka: None. **S. Mysore:** None.

Poster

253. Mechanisms of Attention I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 253.28/V46

Topic: F.02. Animal Cognition and Behavior

Support: Johns Hopkins University

Title: Atypical encoding of sensory space by an inhibitory nucleus in the midbrain attention network

Authors: *N. R. MAHAJAN, S. P. MYSORE;
Johns Hopkins Univ., Baltimore, MD

Abstract: Selecting the most important stimulus among multiple stimuli in a complex environment is critical for the control of attention and for adaptive behavior. Previous studies have shown a key role for a midbrain network in competitive stimulus selection. This network includes the optic tectum (OT; avian analog of the superior colliculus), and a GABAergic tegmental nucleus, the isthmi pars magnocellularis (Imc). The Imc is known to receive focal excitatory input from the topographically organized OT and to send back widespread inhibition, thereby suppressing OT responses to competing stimuli and aiding selection. Here, we investigate a fundamental functional property of the Imc: how it encodes sensory space. With extracellular recordings in head fixed barn owls, we demonstrate that spatial receptive fields of Imc neurons contain multiple, discrete excitatory hotspots. With computational modeling, we explore how groups of Imc neurons might work together to generate spatially-specific competitive suppression in the OT. This work provides new insights into midbrain mechanisms of stimulus competition and selection.

Disclosures: N.R. Mahajan: None. **S.P. Mysore:** None.

Poster

253. Mechanisms of Attention I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 253.29/V47

Topic: F.02. Animal Cognition and Behavior

Support: NIH/NIDA Grant 017960

Title: Methylphenidate-induced modulation of visual evoked potentials and oscillatory states in the rat visual thalamus

Authors: *L. R. MITCHELL, R. L. NAVARRA, B. D. CLARK, B. D. WATERHOUSE; Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Low dose psychostimulants have been used for decades to treat patients with attention deficits and have more recently gained popularity as performance enhancers in otherwise healthy individuals, yet the neural circuits underlying psychostimulant-induced performance enhancement have not been elucidated. Recent studies from our laboratory have investigated the effects of the psychostimulant, methylphenidate (MPH) on early sensory-signal processing. In the rat visual thalamus, low dose MPH decreases the latency and increases the magnitude of single unit responses to light stimuli by increasing noradrenergic transmission. However, the effect of MPH on stimulus-evoked local potentials and oscillatory states in the visual thalamus has not been studied. In the present study, single unit activity and local field potentials (LFPs) were recorded from the dorsal lateral geniculate nucleus of isoflurane-anesthetized Sprague-Dawley rats using eight-electrode arrays. Light flash stimuli of varying intensities were presented unilaterally before and following MPH (2 mg/kg, IP) or saline administration. Analysis of LFPs revealed a decrease in both response latency and peak-to-peak amplitude of light-evoked potentials at 15 min post-MPH administration. As shown in previous studies, MPH administration caused a decrease in mean latency of single-unit light-evoked responses. Furthermore, cross correlations between multiple LFP channels within individual animals showed an increase in the strength of correlation following MPH administration. This increase in the correlation between locally summed potentials from multiple electrodes may be consistent with an increase in local synchrony of neural discharges. Lastly, segmented auto power spectral density analyses computed for progressive 5-minute intervals following injection of MPH revealed a shift from the prevalence of low frequency oscillations of 0-4 Hz to the clear emergence of two dominant bands at 4-6 Hz and 10-14 Hz. Between the decrease in light-evoked potential response latency and the indicated facilitation of local synchrony in the 4-6 Hz and 10-14 Hz ranges, these results suggest a combination of single unit and circuit mechanisms through which MPH can increase the efficacy of sub-cortical visual processing. Such effects likely contribute to the performance-enhancing effects of MPH.

Disclosures: L.R. Mitchell: None. **R.L. Navarra:** None. **B.D. Clark:** None. **B.D. Waterhouse:** None.

Poster

253. Mechanisms of Attention I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: B.07. Synaptic Transmission

Support: NIDA T32 Training Grant

Title: A physiological role for locus coeruleus dopamine

Authors: *A. SONNEBORN[1], C. HAMILTON[2], R. W. GREENE[1];
[1]Neurosci., [2]Psychiatry, Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: For many years the locus coeruleus (LC) has been considered the major source of cortical, cerebellar and hippocampal norepinephrine (NE) in the mammalian brain. Recently it was suggested that LC neurons can also release significant amounts of dopamine (DA), the biosynthetic precursor of NE, from their projections. These projections are known to innervate many brain regions, including area CA1 of the hippocampus. It was previously supposed that the main source of CA1 DA was the ventral tegmental area (VTA), but pharmacological studies using amphetamine (AMPH) and selective knockdown of tyrosine hydroxylase have established that the majority of CA1 DA actually originates from LC fibers. However, whether or not LC DA serves a neuromodulatory function under more physiologically relevant conditions remains unclear. In the following experiments, we use an optogenetic approach to study the co-release and electrophysiological effects of LC DA on CA1 function. We provide chemical evidence of the co-release of DA from LC terminals, and show that optogenetically evoked LC DA release is sufficient to modulate synaptic strength at hippocampal Schaffer Collateral synapses by acting on CA1 pyramidal neuron D1/D5 receptors (D1/5R). D1/5R activation can convert transient LTP into long lasting LTP, a potentially necessary step for consolidation of learning. The LC is thought to be involved in selective attention and arousal and CA1 is known to be important for the storage, retrieval, and comparison of episodic or associative memories with current sensory information. Therefore, these and future experiments provide new insight into the effects of attention and arousal states on learning and memory.

Disclosures: A. Sonneborn: None. **C. Hamilton:** None. **R.W. Greene:** None.

Poster

254. Anatomy of Stress, Anxiety, and Fear

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: E.05. Stress and the Brain

Support: NEXT Program LS070

MEXT/JSPS KAKENHI 26118508

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Title: Medial prefrontal cortex provides psychological stress signals to the dorsomedial hypothalamus to drive brown adipose tissue thermogenesis and hyperthermia

Authors: N. KATAOKA[1], *K. NAKAMURA[1,2];
[1]Dept. of Physiol. 2, Nagoya Univ. Grad. Sch. of Med., Nagoya, Japan; [2]PRESTO, JST, Kawaguchi, Japan

Abstract: Psychological stress induces hyperthermia in many mammals including humans. Recently, we have reported that stress activates a direct neural pathway from the dorsomedial hypothalamus (DMH) to the rostral medullary raphe (rMR) to drive sympathetic heat production (thermogenesis) in brown adipose tissue (BAT), hyperthermia and tachycardia (Kataoka *et al. Cell Metabolism*, 2014). To activate this hypothalamo-medullary neural pathway, DMH neurons must receive stress signals. However, the upper brain sites that provide the DMH with psychological stress signals to drive the sympathetic responses have not been determined. To identify such upper sites, we injected cholera toxin b-subunit (CTb), a retrograde neural tracer, into rat DMH. Many CTb-labeled neuronal cell bodies were distributed in the medial prefrontal cortex (mPFC) and the lateral septal nucleus (LS). Our *in vivo* physiological experiments using anesthetized rats showed that stimulation of neurons in either mPFC or LS with a nanoinjection of bicuculline, a $GABA_A$ receptor antagonist, increased BAT sympathetic nerve activity, BAT temperature and heart rate. These bicuculline-evoked sympathetic responses were all diminished by inactivation of bilateral DMH neurons with muscimol nanoinjections. Free-moving rats exposed to social defeat stress, a sociopsychological stress model, exhibited immediate increases in BAT and abdominal temperatures, as detected with a telemetry system. These stress responses were significantly reduced by inhibition of neurons in the ventral part of the mPFC (vmPFC) with bilateral nanoinjections of muscimol. In contrast, inhibition of neurons in the infralimbic cortex, a dorsal part of the mPFC, or in the LS only weakly inhibited these stress responses. Supporting the functional contribution of the vmPFC-DMH monosynaptic pathway to driving the stress-induced BAT thermogenesis, *in vivo* optogenetic stimulation of the vmPFC-derived nerve endings in the DMH increased BAT sympathetic nerve activity. These results indicate that the vmPFC-DMH monosynaptic pathway provides the DMH with stress signals to activate the DMH-rMR sympathoexcitatory pathway driving BAT thermogenesis for the development of stress-induced hyperthermia.

Disclosures: **N. Kataoka:** None. **K. Nakamura:** None.

Poster

254. Anatomy of Stress, Anxiety, and Fear

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 254.02/W2

Topic: E.05. Stress and the Brain

Support: DK096983

ODP51011132

Title: Changes in D2R binding potential within the striatum and PFC are modulated by psychosocial stress exposure and E2 treatment in female rhesus monkeys

Authors: ***M. PEREZ DIAZ**[1], V. MICHOPOULOS[1,2], L. L. HOWELL[1,2], M. WILSON[1,2]; [1]Yerkes Natl. Primate Res. Ctr., Atlanta, GA; [2]Dept. of Psychiatry and Behavioral Sci., Emory Univ., Atlanta, GA

Abstract: Chronic exposure to psychosocial stress produces a myriad of negative, often long-lasting, neurochemical and behavioral changes. Emotional feeding of calorically dense foods can result from chronic exposure to psychosocial stressors and likely contributes to excess food intake. This change is exacerbated in women, who consistently report more stress-induced eating and higher rates of obesity, suggesting that estrogen plays a modulatory role in the behavioral consequences of chronic stress exposure. Moreover, chronic exposure to psychosocial stress also leads to dysregulation of the reward pathway, which may increase susceptibility to addiction. Previous studies demonstrate that exposure to chronic stress reduces the availability of DA 2 receptors (D2R) in the brain, which results in a hypodopaminergic state characterized by reduced DA signaling. Although estrogen has been shown to affect behaviors associated with stress exposure, no one has evaluated its effect on the downregulation of D2R that results from this exposure. We employed a non-human primate model of female social subordination stress to test the hypotheses that 1) chronic psychosocial stress decreases D2R binding potential (BP) in the striatum and prefrontal cortex (PFC) and 2) estradiol (E2) treatment exacerbates this. Twenty-four socially housed adult female rhesus monkeys were studied during the species typical non-breeding season when endogenous estradiol levels are suppressed. Females received PET scans using [18F]-fallypride under the control condition (no E2 treatment) and after 1 week of E2 treatment (surgically implanted capsule) to mimic mid-follicular phase levels (~100 pg/ml). We found a significant effect ($p < .05$) of both social status and E2 treatment within the caudate and putamen, with dominant females and females under E2 treatment having higher D2R BP than subordinates and females under control conditions, respectively. Moreover, D2R BP in the medial PFC was also higher in females under the E2 treatment condition. Finally, we found a

significant interaction between E2 treatment and social status, with subordinate females having higher BP than dominants under the control condition and dominants having higher BP than subordinates under the E2 treatment condition. Overall, our results suggest that estradiol indeed modulates the effects of chronic stress exposure on D2R BP within the reward pathway, which may represent one of the mechanisms by which estrogen exacerbates the behavioral consequences of stress exposure in women. Support by DK096983 and ODP51011132.

Disclosures: **M. Perez diaz:** None. **V. Michopoulos:** None. **L.L. Howell:** None. **M. Wilson:** None.

Poster

254. Anatomy of Stress, Anxiety, and Fear

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Topic: F.02. Animal Cognition and Behavior

Support: RCMI BRAIN and MAGIC Grant 8G12MD007579

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Title: Fkbp5 in the infralimbic cortex modulates fear conditioning and extinction

Authors: ***M. CRIADO MARRERO**[1], R. MORALES-SILVA[2], B. VELAZQUEZ[1], J. PORTER[1];
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Abstract: Patients with PTSD have difficulty learning to extinguish fear memories. Current studies associate FK binding protein 5 (Fkbp5) variants with PTSD. Since Fkbp5 decreases the activity of the glucocorticoid receptor (GR), excessive Fkbp5 expression could reduce GR activity and impair extinction memory. In this study, we hypothesized that Fkbp5 regulates fear extinction by modulating GR activity in the infralimbic cortex (IL) a key structure of the fear extinction circuitry. To test this, adult male rats were exposed to fear conditioning and extinction and Fkbp5 mRNA and protein levels were determined in IL tissue punches. Both Fkbp5 mRNA and protein expression increased after fear conditioning and decreased after extinction. Interestingly, similar changes were not observed in the adjacent prelimbic cortex (PL). To determine whether changes in IL Fkbp5 affect fear conditioning or extinction, we reduced Fkbp5 expression in IL by infusing Fkbp5-shRNA plasmids into IL prior to fear conditioning and extinction. Unexpectedly, rats infused with Fkbp5-shRNA showed less fear during conditioning than rats infused with scramble-shRNA. Both groups showed similar levels of fear during

conditioning recall and at the end of extinction, however, the Fkbp5-shRNA group showed reduced fear during extinction recall. Conversely, infusion of Fkbp5-shRNA into PL did not affect fear conditioning or extinction. Our findings suggest that people with higher expression of Fkbp5 in the ventral medial prefrontal cortex may be predisposed to facilitated fear learning and impaired extinction.

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Poster

254. Anatomy of Stress, Anxiety, and Fear

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 254.04/W4

Topic: E.05. Stress and the Brain

Support: NIH R01-MH095972

Title: Prolonged elevations in corticosterone induces regressive dendritic spine alterations in the medial prefrontal cortex

Authors: *R. M. ANDERSON, S. JOHNSON, M. MILLER, R. GLANZ, S. 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 neuronal cognate receptors throughout limbic cortical cell groups 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) following chronic stress exposure. Nevertheless, information regarding the effects of prolonged glucocorticoid exposure on dendritic spine plasticity in these cell types has not been carefully investigated. Here we examined dendritic spine alterations in the prelimbic region (PL) of mPFC in rats that were adrenalectomized (ADX) and replaced with corticosterone (B) to plasma levels that approximate the circadian mean (75 ng/ml; B75 group) or peak PM (150 ng/ml; B150 group) concentrations over a 14-day period, relative to sham-ADX controls. On day 15, animals were perfused and pyramidal neurons throughout all layers of PL were selected for intracellular fluorescent dye-filling, followed by high-resolution 3D imaging and analysis of dendritic arborization and spine morphometry. B150 animals showed decreases in apical dendritic length and spine density (by 13% for each; $p < 0.05$ for each), and these

decrements were exaggerated at distal aspects (by 16%; $p < 0.01$). Thin spine subtypes showed the greatest degree of attrition throughout apical dendrites and more distally (by 12% and 17% respectively, $p < 0.05$ for each), whereas mushroom and stubby subtypes were largely preserved. Follow-up population analyses of spine characteristics in B150 rats revealed increases in spine volume across subtypes, which is consistent with the idea that smaller volume spines are more vulnerable to pruning following higher levels of B exposure. By contrast, analyses in B75 animals failed to display any reliable decrements in dendritic morphology, spine, or subtype densities, however, population analyses carried out in these animals revealed a robust decrement in spine volume relative to B150 and sham groups. These results suggest that prolonged alterations in adrenocortical activity may be sufficient to differentially induce regressive structural changes in excitatory synaptic morphology in mPFC neurons.

Disclosures: **R.M. Anderson:** None. **S. Johnson:** None. **M. Miller:** None. **R. Glanz:** None. **S. Romig- Martin:** None. **J.J. Radley:** None.

Poster

254. Anatomy of Stress, Anxiety, and Fear

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Topic: E.05. Stress and the Brain

Support: NICHD Grant HD21350

Title: Baboon fetal frontal cortex response to moderate maternal nutrition reduction (MNR) in late gestation

Authors: S. YANG[1], T. NURMAMAT[2], P. W. NATHANIELSZ[1], *C. LI[1];
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Abstract: Introduction: Moderate maternal nutrient restriction (MNR) during pregnancy is common in both developing and developed countries. Our group previously reported that MNR induced subtle cerebral developmental changes in frontal cortex (FC) at mid gestation (0.5 G) [1]; however, the impact of MNR at late gestation (0.9 G) has not been determined. The FC of the brain plays an important role in memory, learning and complex cognition, and proper fetal FC development is critical for adult cognitive function. We hypothesized that MNR programs frontal cortex by increasing cellular proliferation at the cost of decreased neuronal cell maturation in late gestation. Methods: Morphometrically homogeneous adult female baboons were randomly selected for ad libitum feed control (CTR; n = 25) or 70% CTR global diet (MNR, n = 16) from 0.16 G through 0.9 G. Fetal FC samples were collected at Cesarean section. Gene arrays were performed for whole genome profiling of the fetal FC. Ingenuity pathway

analysis (IPA) software was used for genomic analysis. Immunohistochemistry (IHC) was conducted for determining the protein expression of B-cell lymphoma 2 (BCL-2), transforming growth factor beta (TGF-ß) and glucocorticoid receptor (GR). Image J was used for quantification of stained fraction, and Student's t-test was performed for data analysis and the significance was set at $P < 0.05$. Results: Gene array results showed that gene expression of 666 genes were altered in CTR vs. MNR fetal FC. Among those differentially expressed genes, 569 genes were up regulated and 97 genes were down regulated in MNR fetuses. IPA analysis revealed that signaling pathway involved in neuronal cell proliferation and differentiation were upregulated, while signaling pathways that are important in maturation and apoptosis signaling were down regulated in MNR group. Gene expression of BCL-2, TGF-ß and GR were all up regulated ($P < 0.05$). In keeping with gene expression, % area stained for BCL-2 and GR proteins were increased ($P < 0.05$) in gray matter of MNR fetal FC, and the protein expression of TGF-ß tended to increase ($P < 0.1$), but did not reach the significance level. Increase in BCL-2 and TGF-ß expression may mediate increased cell proliferation, while increased GR expression can reduce neuronal maturation. Conclusions: Moderate MNR leads to significant changes in fetal frontal cortex gene expression in late gestation. We also found some evidence for catch up growth in the FC in late gestation due to MNR, which may predispose the offspring to deficits in cognitive function in later life. 1. Antonow-Schlorke I et al. Proc Natl Acad Sci USA. 2011 Feb 15;108(7):3011-6.

Disclosures: **S. Yang:** None. **T. Nurmamat:** None. **P.W. Nathanielsz:** None. **C. Li:** None.

Poster

254. Anatomy of Stress, Anxiety, and Fear

Location: Hall A

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Topic: F.02. Animal Cognition and Behavior

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the Intelligent Synthetic Biology Center of the Global Frontier Project Grant 2011-0031955

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Title: Selective control of fear expression by manipulation of infralimbic cortex during extinction retrieval

Authors: H.-S. KIM[1,2], H.-Y. CHO[1], G. J. AUGUSTINE[2,3,4], *J.-H. HAN[1];
[1]KAIST, Daejeon, Korea, Republic of; [2]KIST, Seoul, Korea, Republic of; [3]Nanyang Technological Univ., Singapore, Singapore; [4]Inst. of Mol. and Cell Biol., Singapore, Singapore

Abstract: Evidence from rodent and human studies has implicated the ventromedial prefrontal cortex (vmPFC), specifically the infralimbic cortex (IL), as a critical brain structure in the extinction of conditioned fear. However, lesions or pharmacological inhibition of vmPFC before extinction reportedly has no effect on extinction or even facilitates extinction. Thus, the role of the IL activity in expression of extinction memory remains unclear. To address this issue, we used an optogenetic approach in mice. By precisely manipulating the activity of defined cells, we examined the real-time contribution of IL activity to expression of auditory conditioned fear extinction. Our results reveal that inactivation of infralimbic, but not prelimbic, cortex impaired extinction retrieval. Conversely, activation of infralimbic excitatory neurons enhanced expression of fear extinction but had no significant effect on expression of unextinguished conditioned fear. Furthermore, inhibition of fear expression by IL photoactivation was specific to the conditioned stimulus (CS), such that IL photoactivation had no effect on fear expression to the context after the auditory fear extinction. Thus, artificial IL activation produced no significant effect on expression of non-extinguished conditioned fear. Therefore, our data provide convincing evidence supporting that IL activity is critical for expression of fear extinction and establish a causal role for IL activity in controlling fear expression in a CS-specific manner after extinction.

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Poster

254. Anatomy of Stress, Anxiety, and Fear

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Topic: F.02. Animal Cognition and Behavior

Support: MH093412

Brain and Behavior Research Foundation

Title: Posterior insular cortex is required for acquiring a conditioned fear inhibitor and supports fear recall

Authors: *A. R. FOILB, J. P. CHRISTIANSON;
Psychology, Boston Col., Chestnut Hill, MA

Abstract: Veridical detection of safe versus dangerous cues is critical to survival. We reported that the posterior, but not anterior or medial, insular cortex (IC) is critical for the stress-mitigating effect of safety signals on later anxiety behavior. Here, rats received “A+/B-“ fear discrimination training in a standard fear-conditioning box over 5 days. 24 h after training rats were given a summation test in the training context in which freezing, an index of fear, was

assessed during exposure to the context alone, A, A and B in compound (summation) and B alone. We then show that NMDA-receptor antagonist D-AP-5 (6μg/side) in posterior IC (Bregma -1.8) before training completely prevented conditioned inhibition learning, while intra-D-AP-5 to anterior (+2.7 Bregma) and medial (+0.5 Bregma) IC targets before training had no effect on later conditioned inhibition. Ongoing work in the lab is using optogenetic techniques to establish temporal specificity. To determine the role of IC in recall, intra-posterior IC injections of the GABAA agonist muscimol (1μg/side) were made before a summation test. Muscimol significantly reduced conditioned freezing to all cues. These data implicate a role of posterior IC in the recall of fear. This new finding may be a key to understanding why the posterior IC is a site of plasticity that leads to conditioned inhibition.

Disclosures: A.R. Foilb: None. **J.P. Christianson:** None.

Poster

254. Anatomy of Stress, Anxiety, and Fear

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Topic: E.05. Stress and the Brain

Support: NIH grants MH053851

NIH grants MH072672

Title: Chronic unpredictable stress dysregulates glutamate transmission in the rat medial prefrontal cortex: a potential role for noradrenergic modulation

Authors: *J. D. JETT, L. EVANS, M. PATTON, D. MORILAK;
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Abstract: Deficits in cognitive flexibility are associated with the onset and maintenance of stress-related psychiatric illnesses, such as depression. Cognitive flexibility, the ability to modify behavior in response to changes in the environment, depends on medial prefrontal cortical (mPFC) function. We previously showed that rats exposed to chronic unpredictable stress (CUS) exhibit deficits in mPFC-mediated cognitive flexibility, as measured by the extradimentional set-shifting task (ED) on the attentional set-shifting test. Further, acute enhancement of norepinephrine (NE) transmission in the mPFC facilitated cognitive flexibility. However, local blockade of NE receptors during CUS prevented stress-induced deficits. This evidence suggested that NE facilitation may compromise mPFC function when repeatedly evoked by CUS. We subsequently found that CUS-induced cognitive deficits are associated with attenuated *fos* induction in the mPFC following stimulation of the mediodorsal thalamus, a glutamatergic (GLU) afferent to the mPFC. NE modulates GLU in the mPFC, thus repeated NE activity during CUS may compromise cognitive function by dysregulating GLU transmission. To test this

hypothesis, we first assessed the effect of blocking NE activity during CUS on markers of plasticity associated with glutamatergic signaling and long-term potentiation, namely phosphorylation of the AMPA receptor GluR1 subunit at the S845 and 831 sites. Male Sprague-Dawley rats received injections of vehicle (40% DMSO, 2ml/kg, i.p.) or an α_1- / $\beta_{1/2}$-adrenergic antagonist cocktail (prazosin, 1.25mg/kg; propranolol, 10mg/kg) 30 min prior to each CUS or control session. Rats were then tested on AST drug free, and the mPFC collected 30 min after ED. Antagonist treatment attenuated CUS-induced cognitive deficits on ED. Further, drug treatment during CUS significantly enhanced phosphorylation of GluR1(S831) ($p<0.03$), but not S845 ($p=0.2$). S831-phosphorylation is associated with AMPA receptor translocation to the synapse. Thus, antagonist treatment may facilitate cognitive flexibility in CUS rats by enhancing GLU synaptic transmission in mPFC. For the second experiment, rats were implanted with a microdialysis cannula targeting the mPFC. After 2 weeks of control or CUS treatment, rats underwent GLU microdialysis. Following baseline sampling, rats were exposed to a novel acute immobilization stress (20 min). CUS attenuated the acute stress-evoked GLU response in mPFC compared to non-stress controls ($p<0.001$). Studies are ongoing to assess if antagonist treatment prevents CUS from compromising stress-evoked GLU response in mPFC. Funding: NIH grants MH053851 and MH072672

Disclosures: **J.D. Jett:** None. **L. Evans:** None. **M. Patton:** None. **D. Morilak:** 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.; Lundbeck A/S. F. Consulting Fees (e.g., advisory boards); Lundbeck A/S.

Poster

254. Anatomy of Stress, Anxiety, and Fear

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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SFB F4410-B19

Title: Prefrontal inputs to the amygdala instruct fear extinction memory formation

Authors: *O. BUKALO[1,3], C. R. PINARD[1], S. SILVERSTEIN[1,3], O. GUNDUZ-CINAR[1], R. CINAR[2], C. C. BREHM[4], N. D. HARTLEY[5], N. WHITTLE[4], G. COLACICCO[1], E. F. BUSCH[1], S. PATEL[5], N. SINGEWALD[4], G. KUNOS[2], A. HOLMES[1];
[1]Lab. of Behavioral and Genomic Neurosci., [2]Lab. of Physiologic Studies, NIH/NIAAA, Rockville, MD; [3]Ctr. for Neurosci. and Regenerative Med. at the Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD; [4]Dept. of Pharmacol. & Toxicology, Univ. of Innsbruck, Innsbruck, Austria; [5]Dept. of Psychiatry and Mol. Physiol. and Biophysics, Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract: Persistent anxiety following a psychological trauma is a hallmark of many anxiety disorders. However, the neural circuits mediating the extinction of fear remain incompletely understood. Here we tested for alterations in fear extinction following pathway-specific manipulation of glutamatergic projections from the ventromedial prefrontal cortex (vmPFC) to the amygdala. Selective, *in vivo* optogenetic stimulation of vmPFC-amygdala pathway facilitated extinction memory formation, but not retrieval. Conversely, silencing the vmPFC-amygdala pathway impaired extinction formation and suppressed amygdala neuronal activity. Collectively, our results provide compelling evidence that this discrete neural circuit is both necessary and sufficient for the formation of an extinction memory. Our data are consistent with a model in which vmPFC inputs instructs plastic changes in the amygdala necessary for extinction by either decreasing excitatory input or increasing inhibitory input in amygdala. To further delineate the functional contribution of vmPFC-amygdala pathway to fear extinction we test the role of amygdala local circuits in optogenetically-induced facilitation of extinction memory. One line of evidence suggests that extinction training results in increased endocannabinoids levels in the amygdala. At a cellular level, endocannabinoids cause a long-term depression of GABAergic synaptic transmission via activation of cannabinoid type 1 (CB1) receptor . Anandamide levels in the amygdala were increased by stimulation of vmPFC inputs extinction training. Since, CB1 is particularly enriched in axon terminals of cholecystokinin-positive GABAergic interneurons; we tested if these interneurons population is driven by vmPFC projection to amygdala. Our findings suggest that selectively elevating amygdala anandamide may underlie plastic changes induced by activation of vmPFC-amygdala pathway extinction memory. Taken together, these findings could help advance our understanding of the neural circuits mediating fear extinction, with possible implications for elucidating the neuropathology of impaired extinction in anxiety disorders.

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Poster

254. Anatomy of Stress, Anxiety, and Fear

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Topic: F.02. Animal Cognition and Behavior

Support: California State University, Sacramento SSIS/UEI Funding

Title: Perirhinal cortex involvement in fear extinction using a discontinuous light stimulus

Authors: *C. A. CALUB, N. M. POTTER, A. G. BECKNER, A. C. SARDO, S. C. FURTAK; California State University, Sacramento, Sacramento, CA

Abstract: The perirhinal cortex (PER) is known to have a mnemonic as well as a perceptual role in the processing of high level sensory information. One hypothesis is that PER functions to unitize stimuli across time or across sensory modalities (Kent & Brown, 2012; Graham et al., 2006). While many findings have supported the role of PER in fear acquisition to multi-feature and multi-modal stimuli (Bucci, Phillips, & Burwell, 2000; Bucci, Saddoris, & Burwell, 2002; Corodimas & LeDoux, 1995; Kholodar-Smith, Allen, & Brown, 2008; Kholodar-Smith, Boguszewski, & Brown, 2008), to date no study has evaluated PER involvement in processing such stimuli during fear extinction. To evaluate this, Sprague-Dawley derived Albino male rats were infused with muscimol, a GABA agonist, to temporarily inactivate the PER during extinction training. All subjects were surgically implanted with cannulae targeting PER bilaterally. Following recovery, all subjects were trained on a three-day fear extinction paradigm. All phases of the paradigm were conducted in the same context in order to avoid contextually dependent characteristics of extinction. On Day 1, animals received fear conditioning, which consisted of 5 presentations of a discontinuous visual light stimulus (conditioned stimulus; CS) paired with the unconditioned stimulus (US), a foot shock. On Day 2, subjects received bilateral PER infusions of either muscimol or saline 40 mins prior to extinction training, and subsequently received 20 trials of the CS alone. On Day 3, extinction recall was assessed by presenting an additional 15 trials of the CS without the US. Freezing behavior, defined as no movement except that necessary for breathing, was recorded throughout the experiment and later analyzed by an automated computer program. Results showed that bilateral muscimol infusions into the PER during extinction training impaired extinction recall to a discontinuous light CS, as indicated by significantly higher levels of freezing in muscimol infused animals than saline control animals during extinction recall. The results suggest that particular cues may engage brain regions outside what is typically considered the fear extinction neural circuit. Here, for the first time, results support a role for PER involvement in fear extinction, perhaps due to the discontinuous nature of the CS.

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Poster

254. Anatomy of Stress, Anxiety, and Fear

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R15MH093950

Title: Changes in limbic system responsiveness predict the behavioral emergence of conditioned freezing in developing rats

Authors: *M. A. BURMAN[1], A. D. DEAL[2], K. E. ERICKSON[2], J. DIONNE[2];
[1]Psychology, [2]Univ. of New England, Biddeford, ME

Abstract: Anxiety disorders have a lifetime prevalence of about 25%. Many begin during childhood or early adolescence. Despite the clear developmental link and the progress made in uncovering the neural substrates of fear and anxiety in adult organisms, our understanding of the development of these circuits lags relatively behind. One popular preparation for studying fear and anxiety in rodents is classical fear conditioning. In this task, which has a great face validity as a model of PTSD or specific phobias, rodents develop an association between a previously neutral cue and an aversive event, such that the previously neutral cue consequently elicits a conditioned fear response. Part of the difficultly in understanding the development of this task is that there are many potential areas for developmental dissociations. In addition to sensory and motor aspects, these likely include the acquisition of fear to different types of cues, the expression of fear, and the extinction of the fear response. The current work focuses on the acquisition and expression of fear acquired to both a specific auditory cue and the concomitant fear that develops to the background environment in infant (postnatal day; PD 17) and post-weanling (PD 23-24) rats. Our behavioral data show that conditioned freezing to both the context and a pure tone develops during this time. The data presented here extend this work by assessing immediate early gene (IEG) expression in various limbic system structures using 2 methodologies: immunohistochemistry and qPCR. First, examining c-FOS protein expression following fear acquisition we see three distinct patterns. First, we note that activation of basolateral amygdalar nuclei closely predicts the expression of fear, in that only older fear conditioned subjects demonstrate robust expression. Second, in the perirhinal cortex and hypothalamus, exposure to the aversive stimulus, but not eventual freezing levels, appear to predict expression. Finally, we do not see robust expression in dorsal CA1 of the hippocampus. Examining c-FOS and EGR-1 mRNA expression, activation appears to closely match levels of freezing only in the amygdala and perirhinal cortex when assessing EGR-1 expression during the auditory fear test. Activation of the hypothalamus appears to be related to aversive stimulus exposure and not levels of freezing. We also see age-related changes in in the amygdala, hypothalamus and perirhinal cortex. Overall, changes in amygdala and cortical circuitry could account for the ontogeny of fear conditioning in peri-weaning rats.

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Poster

254. Anatomy of Stress, Anxiety, and Fear

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Topic: E.05. Stress and the Brain

Support: CNRS (contract UPR3212)

Université de Strasbourg UPR3212

Agence Nationale de la Recherche (ANR-11-sv4-002)

Title: Response of the tail of the ventral tegmental area to aversive stimuli

Authors: *M. BARROT[1], M. SÁNCHEZ-CATALÁN[2], M.-A. MULLER[3], M. MAJCHRZAK[3], I. YALCIN[2];
[1]INCI - CNRS UPR3212, Strasbourg, France; [2]INCI, CNRS, Strasbourg, France; [3]LNCA, UMR 7364, Univ. de Strasbourg, CNRS, Strasbourg, France

Abstract: The GABAergic tail of the ventral tegmental area (tVTA), also named the rostromedial tegmental nucleus (RMTg), is a mesopontine structure that exerts an inhibitory electrophysiological control over dopamine cells of the VTA and substantia nigra pars compacta (SNc). The tVTA has been implicated in motor functions, responses to drugs of abuse, reward prediction error and avoidance behavior. Stimulation of lateral habenula (LHb) inputs to the tVTA, or of the tVTA itself, induces avoidance behaviors, which suggested a role for the tVTA in processing avrsive information. In rats, psychostimulant drugs share the property to induce Fos proteins in the tVTA. The aim of the present study was to test whether various aversive stimuli might similarly recruit the tVTA. We studied Fos expression in the tVTA of Sprague-Dawley rats in response to: lithium chloride, β-carboline, naloxone, lipopolysaccharide, footshocks, restraint stress, forced swimming test, fox odor, cat odor, inflammatory pain, neuropathic pain, and opioid withdrawal. Moreover, we also assessed the effect of the bilateral excitotoxic lesion of the tVTA on the LPS and LiCl-induced conditioned taste aversion. Our results confirm that foot-shocks elicit Fos induction in the tVTA, but such induction was not present with most of the other aversive stimuli. Moreover, the tVTA lesion did not impact on conditioned taste aversion. While a stimulation of the tVTA favors avoidance behaviors, the present finding suggest that the recruitment of the tVTA may not be a general outcome of exposure to aversive stimuli and that the tVTA may not be a key brain region for some avoidance behaviors.

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Poster

254. Anatomy of Stress, Anxiety, and Fear

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: F.02. Animal Cognition and Behavior

Support: MH038774

MH046516

Title: The role of norepinephrine in the expression of Pavlovian defensive reactions

Authors: *Y. GU[1], R. M. SEARS[1,2], E. M. VAZEY[3], G. S. ASTON-JONES[3], J. E. LEDOUX[1,2]; [1]Ctr. for Neural Sci., New York Univ., New York, NY; [2]Emotional Brain Inst., Nathan Kline Inst., Orangeburg, NY; [3]Dept. of Neurosciences, Med. Univ. of South Carolina, Charleston, SC

Abstract: The modulatory role of norepinephrine (NE) in the amygdala, specifically the lateral nucleus of the amygdala (LA), has been studied in great detail using aversive Pavlovian paradigms. However, the central nucleus of the amygdala (CeA) also receives dense noradrenergic innervation, the function of which is not well understood. The CeA is the major output nucleus of the amygdala and is responsible for defensive responses to aversive stimuli. We therefore hypothesized that NE acting in CeA modules the expression of defensive reactions. Here, we used a combination of intracranial drug infusions and Designer Receptors Activated by Designer Drugs (DREADDs) to uncover the role of NE modulation of CeA functions in a Pavlovian threat conditioning paradigm. Decreased and increased expression of defensive responses were observed following direct CeA infusions of the β-receptor antagonist propranolol or the β-receptor agonist isoproterenol, respectively. Next we used viral vectors expressing DREADDs and engineered to specifically target norepinephrine-expressing neurons to study the role of the locus coeruleus (LC), a major source of norepinephrine release in the brain, in CeA-mediated defensive responses. Axon terminals in CeA were directly activated or inhibited using intra-CeA Clozapine-N-oxide (CNO) infusions before memory expression tests. Consistent with our pharmacology studies, we found that hM3Dq-mediated activation and hM4Di-mediated inhibition of LC terminals in CeA increased and decreased freezing behaviors, respectively. Taken together, these studies suggest that norepinephrine from LC activates β-receptors in CeA to positively modulate defensive responses to threatening stimuli.

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Poster

254. Anatomy of Stress, Anxiety, and Fear

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant MH08690-01A1

Defense advanced research projects agency (DARPA)

Title: Vagus nerve simulation enhances extinction of conditioned fear in a rat model of PTSD

Authors: *L. J. NOBLE, I. J. GONZALEZ, K. R. RAMANATHAN, B. D. BELFORT, C. K. MCINTYRE;
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Abstract: Trauma-related disorders, such as posttraumatic stress disorder (PTSD) are typically treated with cognitive behavioral therapy. Exposure therapy is a form of cognitive behavior therapy where patients are repeatedly exposed to the cues that elicit maladaptive conditioned responses. After repeated exposures to conditioned cues in the absence of reinforcement, conditioned responses are extinguished. Because successful extinction requires new learning, studies have examined the effects of memory enhancing drugs as adjuncts to exposure therapy. However, in humans with PTSD these results have been inconsistent. One possible explanation for this discrepancy is, when the conditioned response is not fully extinguished, memory-enhancing drugs could reinforce the association between the cue and the inappropriate fear response. Optimal treatments should reduce the anxiety produced by the conditioned cues while enhancing consolidation of extinction learning. Unfortunately, most anxiety-reducing drugs impair memory consolidation and thus interfere with progress in exposure therapy. Vagus nerve stimulation (VNS) is an FDA-approved treatment for the prevention of seizures. Recent research indicates that VNS enhances memory consolidation in rats and in humans. We recently found that pairing VNS with unreinforced exposure to conditioned cues enhanced fear extinction in rats. Additionally, we found that administration of VNS prior to testing in an elevated plus maze significantly reduced anxiety and levels of plasma corticosterone in rats. Together, these findings indicate that VNS offers a rare combination of memory-enhancing and anxiety-reducing properties and thus might provide optimal benefits as an adjunct to exposure therapy for the treatment of disorders like PTSD. Impaired ability to extinguish conditioned fear is a hallmark of PTSD. The single prolonged stressor (SPS) model is an animal model of PTSD that exhibits extinction impairments. The present study was designed to determine whether VNS could enhance extinction of conditioned fear in SPS-treated animals. Following auditory fear conditioning, extinction training was paired with VNS or sham stimulation. Animals that underwent SPS showed impaired extinction of conditioned fear and VNS administration significantly enhanced extinction of the conditioned fear ($F(3,30) = 6.85$, $p = .001$ vs. sham control). Importantly, conditioned fear responding in SPS-treated rats given VNS during extinction was not significantly different from that of non-SPS control animals ($p = 0.426$).

These findings suggest that VNS may be an effective adjunct to exposure therapy in the treatment of PTSD.

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Poster

254. Anatomy of Stress, Anxiety, and Fear

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Program#/Poster#: 254.15/W15

Topic: E.05. Stress and the Brain

Title: Biallelic expression mapping of the imprinted Grb10 locus reveals novel fear-suppressive neurons in the periaqueductal gray

Authors: *E. SZELENYI[1,2], R. PALANISWAMY[1], H. SCHIFF[1], J. TUCCIARONE[1,2], J. HUANG[1], B. LI[1], P. OSTEN[1];
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Abstract: Neural ensembles participating in a behavioral circuit span both overlapping and mixed sets of cell types across several brain regions. Routine cell type-based dissections of circuits are therefore limited to deductive conclusions, being inherently unable to define and manipulate a circuit with great behavioral specificity. As an alternative to a cell type-based circuit approach, we have turned to the study of genomic imprinting the phenomenon of monoallelic expression based on parent-of-origin to explore whether the spatial profile and choice of allelic expression (e.g. paternal, maternal, biallelic) amongst an imprinted gene defines the nodes of a behavioral neural circuit. For this purpose, we have generated novel non-gene disruptive allelic-reporter/Cre mouse lines to map, trace, and manipulate the activity of neurons expressing the behaviorally relevant Grb10 imprinted locus. Single-cell, allele-specific expression was visualized in the progeny of reporter line crosses, in which nuclear-targeted fluorescent proteins with/without Cre recombinase (H2B-Venus and H2B-tdTomato-iCre) were assigned to either allele based on parent-of-origin. Dichromatic expression mapping revealed predominant and diffuse monoallelic paternal Grb10 (patGrb10) expression in subcortical neurons and monoallelic maternal (matGrb10) expression within non-neuronal cells of the vasculature. Biallelic neuronal populations, indicated by strong matGrb10 expression, were interestingly found in several subcortical nodes. Cre-dependent tracing of matGrb10+ neurons within the ventrolateral subdivision of the periaqueductal grey (vlPAG), the densest neuronal matGrb10+ node, revealed distinctive connectivity within a previously described fear circuit. Specifically, long-range ascending projections were observed most notably in midline thalamic, amygdalar, and extra-amygdalar structures, whereas inputs originated mainly from local tectal

sources including the superior colliculus and dorsolateral PAG. We therefore asked if these cells play a role in fear behavior using Pavlovian fear conditioning. Compared to Cre- animals, apoptotic ablation prior to fear conditioning unexpectedly enhanced the rate of fear memory acquisition and the level of its expression, thus suggesting a fear suppressive role for these cells. Our study therefore establishes the utility of imprinted gene expression mapping as a unique guide accommodating behavioral circuit discovery represented by our identification of novel matGrb10+ vlPAG neurons that gate fear responses.

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Poster

254. Anatomy of Stress, Anxiety, and Fear

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Topic: E.05. Stress and the Brain

Support: NIH Grant DA021801

Title: Topographic stress-induced sensitivity of the dorsal raphe following antidepressant treatment in a rat model of depression

Authors: ***J. A. BABB**[1,2], S. E. LINNROS[1,3], K. G. COMMONS[1,2];
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Abstract: The neurotransmitter serotonin (5-hydroxytryptamine; 5-HT) is strongly implicated in mood disorders such as depression. Serotonergic neurons originating in the dorsal and median raphe nuclei (DR and MR) are highly organized and provide widespread innervation to the forebrain. Yet it is poorly understood how these neurons dysfunction in depression, or if depression-related changes are topographically organized within these nuclei. Furthermore, while the effects of selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine are well studied in normal animals, it is unclear if and how they might selectively act to ameliorate dysfunction of 5-HT neurons in the depressed brain. Therefore, the goal of this study was to identify whether 5-HT neurons in the DR and MR are differentially altered in an animal model of depression, and to identify to what degree this dysfunction is reversed by antidepressant treatment. Early life stress in the form of transient maternal separation is used as a model of depression in rodents that generates many behavioral changes in adulthood. This model exhibits some construct validity for depression in humans, as it is well known that stress, particularly occurring in early life, can increase ones vulnerability to psychiatric illnesses such as depression

later in life. In this study, rat pups were subjected to 3hr of maternal separation daily from postnatal day (PND) 2 to PND 15, or were left undisturbed. After weaning, all animals were housed in same-sex groups of littermates. Then in adulthood, half of each of these groups received daily s.c. injections of the SSRI fluoxetine (10mg/kg/2ml in 0.9% saline and 1% DMSO) for 14 consecutive days while the other half of animals received daily vehicle injections. One day following the last injection, activation of 5-HT neurons in the raphe of all rats in response to an acute stress (15 min forced swim) was quantified by labeling Fos and tryptophan hydroxylase proteins using immunofluorescence. Preliminary analyses reveal that antidepressant treatment greatly attenuated activation of 5-HT neurons throughout the DR and MR, while the effects of maternal separation stress were more regionally specific. Furthermore, the capacity of fluoxetine to dampen activation of Fos in 5-HT neurons appeared larger in maternally separated rats. Taken together, these results suggest a selective dysfunction of 5-HT neurons in the DR and MR of rats in the depression model that is coupled with a more profound response to fluoxetine. Ongoing analyses are investigating the role of 5-HT1A-dependent feedback inhibition in generating these effects.

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Poster

254. Anatomy of Stress, Anxiety, and Fear

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Title: Locus coeruleus-ventral tegmental area neural circuit mediates resilience to social defeat stress

Authors: *H. ZHANG[1,2], D. CHAUDHURY[1], B. JUAREZ[1], A. K. FRIEDMAN[1], S. M. KU[1], E. S. CALIPARI[1], A. R. NECTOW[3], M. CRUMILLER[3], J. CHENG[1], H. SUN[1], S. SALTON[1], J. M. FRIEDMAN[3], J.-L. CAO[2], M.-H. HAN[1];
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Abstract: The hyperactivity of ventral tegmental area (VTA) dopamine (DA) neurons plays a key role in determining behavioral susceptibility versus resilience to stress in the chronic social defeat paradigm, a well-established model of depression that offers unique opportunities to perform beneficial comparison of these two behavioral phenotypes. It is known that the activity of VTA DA neurons can be regulated by intrinsic ionic mechanisms and extrinsic synaptic inputs from other neural substrates. Locus coeruleus (LC) neurons send heavy projections to the VTA, and has been implicated in the pathophysiology of depression. Here, utilizing multiple circuit-dissecting techniques, we first investigated the firing activities of LC neurons specifically projecting to the VTA (LC-VTA) in the *in vitro* brain slice preparation of stress-naïve control, susceptible and resilient mice. Unexpectedly, we found that LC-VTA neurons fired significantly higher in the resilient subgroup as compared to stress-naïve control, while LC-VTA neurons of susceptible mice had a normal firing activity comparable to that of control mice. Consistently, *in vivo* recordings from anesthetized intact mice further confirmed that LC neurons of resilient mice exhibited higher firing rates and significantly increased phasic firing events as compared to control or susceptible mice. Next, we employed *in vivo* optogenetic techniques to selectively activate LC-VTA neurons with channelrhodopsin, and found that repeated (20 min/day × 10 days), but not acute (5 min during social interaction test), optogenetic activation of LC-VTA neurons in previous susceptible mice completely reversed social avoidance behavior. Interestingly, in previously susceptible mice, repeated optogenetic activation of LC-VTA neurons also induced homeostatic plasticity of VTA DA neurons projecting to nucleus accumbens (NAc), a featured self-tuning adaptive balance between excitatory I_h and inhibitory K^+ channel currents previously identified as an important active mechanism of natural resilience. Furthermore, with a circuit- and cell type-specific molecular profiling technique (*Cell*, 2014), we found that VTA-NAc DA neurons expressed significantly more α1b and β3 adrenoceptors than overall VTA DA neurons. Repeated activation of these adrenoceptors with an agonist cocktail of α1 and β3 adrenoeceptors consistently reversed social avoidance behavior in susceptible mice. These studies unravel a novel neural circuit mechanism underlying natural resilience, and provide a highly useful circuit target to promote resilience and achieve depression treatment, such as for deep brain stimulation therapy.

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Poster

254. Anatomy of Stress, Anxiety, and Fear

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Support: NIH Grant AA019793

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Title: The effects of isolation stress on UCN1 and TH immunoreactivity in the midbrain of prairie voles

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Abstract: Prairie voles (Microtus ochrogaster) are an excellent model to study mechanisms of social stress. This is because they, unlike laboratory rats and mice, are socially monogamous and form long lasting social bonds with specific animals. The corticotropin releasing factor (CRF) peptide system is important for regulation of stress responses. However, the complexity of this peptide system is only beginning to be appreciated. Although the role of CRF in stress has been extensively studied, much the functions of CRF-related peptides urocortin(UCN)1, UCN2 and UCN3 are much less understood. It has previously been shown that various acute stressors in male rats can lead to increased UCN1 expression in the centrally-projecting Edinger-Westphal nucleus (EWcp). Here we studied if male prairie voles that experience a short-term social stress would have different levels of UCN1. At the same time we also examined levels of tyrosine hydroxylase (TH) within EWcp, as a population of dopaminergic neurons are known to intermingle with UCN1 neurons in this brain region. Adult male prairie were either isolated from their cage mates for four days or kept with their same-sex cage mates during this period. We found that social isolation did not lead to significant differences in the number of UCN1- (t_9=0.33, p=0.75) or TH-positive cells (t_9=0.88, p=0.40) in the EWcp. Future studies will be completed using a longer isolation period to cause chronic social stress to understand whether the length of the social stress changes the expression of the UCN1 and TH in the EW.

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Poster

254. Anatomy of Stress, Anxiety, and Fear

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Support: 13BGIA14370026

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P20GM103641

Title: Critical role of the locus coeruleus-norepinephrine system in social stress-induced cytokine release

Authors: *J. E. FINNELL, C. M. LOMBARD, J. R. FADEL, C. S. WOOD, S. K. WOOD; Pharmacology, Physiology, and Neurosci., Univ. of South Carolina Sch. of Med., Columbia, SC

Abstract: Social stress precipitates psychiatric disorders, however only a subset of the population is susceptible. Coping strategy is known to impact one's resistance to stress; through the use of a resident-intruder paradigm in rats, we previously identified two distinct phenotypic responses to stress characterized by active or passive coping behaviors. Active rats spent more time resisting defeat in upright postures in the presence of a dominant resident rat than did passive rats. In addition, passive rats developed behavioral and neuroendocrine endpoints comparable to a depressive-like state and exaggerated neuroinflammation, while active rats did not. The locus coeruleus (LC), a major source of norepinephrine (NE) in the brain, is implicated in the pathogenesis of depression; however the mechanism by which LC-NE dysregulation may promote depressive disorders has yet to be determined. A growing body of evidence suggests that increased inflammation may play a role in the development of depression. Importantly, NE stimulates microglia activation and proinflammatory cytokine release (ie. IL-1β) in the brain and periphery, suggesting that exaggerated LC-NE activity is capable of increasing proinflammatory cytokines. The present study investigated the role of central NE in stress-induced cytokine release within passive rats susceptible to a depressive-like phenotype. Male Sprague Dawley rats were treated with vehicle or DSP-4 (400 μg/rat icv), a selective noradrenergic neurotoxin that lesions LC-NE projections, one week prior to social defeat or control exposure (30 mins/day, 5 days). 5 days after defeat/control manipulations all rats were exposed to a single social defeat episode (30 min) and brain and plasma were collected immediately following defeat. Cytokine analysis (BioRad Bioplex) showed that animals with a history of social defeat exposure had an exaggerated stress-induced inflammatory response, as evidenced by elevated IL-1β, IL-2 and TNF-α levels compared to rats previously exposed to control ($p<0.05$). Treatment with DSP-4 blocked the social stress-induced increase in inflammation in rats with both prior control and repeated defeat exposure ($p<0.05$). Together these data suggest that a history of social stress sensitizes the inflammatory response following a subsequent re-exposure to the same stressor. Furthermore, these data indicate that the LC plays a critical role in social stress-induced cytokine release. As such, the impact of the LC-NE system on pro-inflammatory cytokines may represent a putative mechanism by which stress can precipitate depressive-disorders. Research supported by 13BGIA14370026, 15SDG22430017 and P20GM103641

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Poster

254. Anatomy of Stress, Anxiety, and Fear

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Topic: F.02. Animal Cognition and Behavior

Support: Strategic Research Program for Brain Sciences from the Ministry of Education, Culture, Sports, Science & Technology (11041047)

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Title: Ventral tegmental area dopamine neurons trigger fear extinction learning

Authors: *R. LUO[1], Y. TAO[1,2], L. PRESTON[3,4], J. KOIVUMAA[3], J. JOHANSEN[3]; [1]RIKEN Brain Sci. Inst. - Wako, Wako-Shi, Saitama, Japan; [2]Joint Inst. for Advanced Biomed. Sci., Waseda Univ., Tokyo, Japan; [3]RIKEN BSI, Wako-shi, Japan; [4]Biol. Sci., Tokyo Med. and Dent. Univ., Tokyo, Japan

Abstract: Although learning to fear and predict danger is critical to survival, reversing or extinguishing fear memories is important when threats are no longer present. The neural signals which detect when fear responses should be disengaged are not known. During fear conditioning, animals learn that an auditory stimulus (conditioned stimulus CS) predicts the occurrence of an aversive shock (unconditioned stimulus US) and learn to freeze to the CS alone. If the CS is presented over many trials in the absence of shock, animals learn to reduce their freezing behavior, a process termed fear extinction. Prior work has shown that midbrain dopamine neurons in the ventral tegmental area (VTA) are activated by better than expected outcomes including rewards and the omission of expected aversive events. This suggests dopamine neurons may provide a signal to initiate extinction learning when aversive outcomes are no longer present. To investigate whether activity in VTA dopamine neurons during the omission of expected shock is necessary for extinction learning, we expressed the inhibitory halorhodopsin in TH+ VTA neurons of TH-cre rats. After animals underwent fear conditioning, the activity of this cell population was optically inhibited during the period when the expected aversive US is omitted during fear extinction. Inhibition of VTA dopamine cells specifically during the omitted shock period resulted in an inability to extinguish fear memories, as seen in elevated CS-evoked freezing during the course of extinction training as well as 24 hours after extinction. Pairings of an auditory CS with inhibition of dopamine neurons alone without shock did not produce fear learning, indicating that optical inhibition by itself did not lead to freezing behavior. To see whether activity in VTA dopamine neurons during shock omission regulates a distributed extinction learning circuit, we examined the effect of optogenetic manipulation of this cell population on MAP kinase phosphorylation (pMAPK) in the amygdala and medial prefrontal cortex (mPFC). Because pMAPK is required for, and upregulated after extinction in both mPFC and amygdala, we assayed pMAPK levels one hour after extinction learning with or without optogenetic inhibition of TH+ VTA neurons during the shock omission period. We found that extinction learning increased pMAPK levels in both amygdala and IL and that this increase was abolished when VTA dopamine cells were inhibited. Together these results suggest that

activation of VTA dopamine cells by the unexpected omission of an aversive outcome is required to initiate fear extinction learning by regulating neural plasticity across a distributed extinction learning circuit.

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Poster

254. Anatomy of Stress, Anxiety, and Fear

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Topic: E.05. Stress and the Brain

Support: NIH Grant RO1MH098348

Title: Structural and functional connectivity associated with the neuroendocrine response to acute psychosocial stress

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Abstract: Introduction. Prior work has demonstrated stress reactivity is associated with decreased activation in the vmPFC (Pruessner et al., 2008), and altered functional connectivity within the PFC-amygdala network (Kim et al., 2011). However, the changes in functional connectivity associated with stress and the relationship between structural connectivity and cortisol release have received limited attention. Therefore, the present study investigated the relationship between functional and structural indices of brain connectivity and cortisol release in response to a stressful task. Methods. Functional magnetic resonance images (fMRI), diffusion tensor images (DTI), and stress response (indexed via cortisol, heart rate (HR), and skin conductance response (SCR)) were collected from 100 participants completing the Montreal Imaging Stress Task (MIST) (Dedovic et al., 2005). The MIST is a psychosocial stress task which contains a non-stressful 'Control' condition as well as a 'Stress' condition. Effective connectivity (EC) during the MIST was assessed using Granger causality and served as an index of functional connectivity. Cortisol data was collected pre- and post-MIST. Whole brain DTI was acquired in 60 directions following the MIST and fractional anisotropy (FA) was used as an index of structural white matter connectivity. Results. HR, SCR, and stress ratings were significantly greater during the Stress condition compared to the Control condition, confirming that the experimental manipulation was successful. Functional MRI data demonstrated differential activation of dmPFC, vmPFC, and amygdala under Stress versus Control conditions during the MIST. EC values were computed using the timecourse from these regions. EC results

demonstrated greater connectivity within the dmPFC-vmPFC-amygdala network during the Control condition compared to the Stress condition. Cortisol reactivity (i.e. increase in cortisol from pre- to post-MIST) was associated with greater FA within the bilateral uncinate fasciculus (white matter tract connecting vmPFC to amygdala). Conclusions. Structural and functional indices of neural connectivity were associated with the stress response. Lower EC during the Stress compared to the Control condition suggests functional connectivity is disrupted during Stress. In addition, participants with the greatest cortisol reactivity showed the greatest FA in the uncinate fasciculus. This finding suggests the uncinate plays an important role in mediating cortisol reactivity to psychosocial stress. Current findings provide novel insights into neural mechanisms that may mediate susceptibility to psychosocial stress.

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Poster

254. Anatomy of Stress, Anxiety, and Fear

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Topic: E.05. Stress and the Brain

Support: CAPES/PDSE 99999.004844/2014-09

NIH R01 MH 052619

Title: Obese rats have enhanced panic-like sensitivity to brief hypercarbic air (CO2) exposure

Authors: *A. R. ABREU[1,3], A. I. MOLOSH[3,4], L. LI[4,5], I. F. CALIMAN[8,3], C. S. BERNABE[5,6], P. L. JOHNSON[6], R. C. A. DE MENEZES[2], A. SHEKHAR[3,4,7];
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Abstract: Brief inspiration of hypercarbic air (air containing >10% concentrations of carbon dioxide, CO2) mobilizes respiratory, sympathetic and hypothalamic-pituitary-adrenal axis responses and increases anxiety-like behavior in normal rats and humans, whereas, such levels of hypercarbia induces panic attacks in the majority of panic disorder patients. Likewise, previous experiments from our group demonstrated that obesity, induced by a high fat diet (HFD), potentiates the cardiovascular response produced by air jet stress in rats, probably due to a reduced GABA-mediated inhibition within the dorsomedial hypothalamus (DMH). Moreover, it is known that chronic reduction of GABA function in the DMH leads to the development of panic-like disorder. Therefore the aim of this study was to evaluate the influence of obesity,

induced by HFD, on the behavior responses induced by acute CO2 gas exposure. Male Wistar (100±10g) rats were fed a control diet or a HFD (45% w/w fat, both from Harlan Laboratories) for 9 weeks. Next, conscious adult rats were placed in air-flow controlled cages and exposed to either atmospheric air (ATM) or increasing environmental CO2 concentrations (from baseline concentrations up to 20%) during a 5 min period. After this period the rats were submitted to the open-field arena. During exposure, obese rats exposed to CO2 (n=6), compared to ATM (n=5) had more fecal pellets in the chamber (0.8±0.6 vs. 3.5±0.6; p=0.0090; all results by unpaired t-test). In the open field post CO(2), obese rats demonstrated increased number of freezing episodes (65.8±2.0 vs. 81.33±3.0; p=0.0028), time freezing (71.1±7.6 vs. 115.9±5.9; p=0.0011), as well as decreased total distance (0.4±0 vs. 0.2±0; p=0.0005), line crossings (312.2±16.5 vs. 204.5±12.9; p=0.0005), entries (35.6±3.7 vs. 21.8±3.1; p=0.0186) and distance in corner (0.05±0.01 vs. 0.02±0.04; p=0.0035), distance in periphery (0.2±0 vs. 0.1±0; p=0.0075) and distance in middle (0.09±0.01 vs. 0.06±0.01; p=0.0262). In contrast, no changes were observed when control rats were exposed to CO2 (n=6) compared to ATM (n=6). Additionally, CO2 increased time freezing (95.7±6.2 vs. 115.9± 5.9; p=0.0411) and the number of fecal pellets (1.4±0.7 vs. 3.5±0.6; p=0.0428) in obese (n=6) compared with control (n=6) rats. These results suggest that high fat diet-induced obesity leads to panic vulnerability following brief CO2 gas exposure in rats.

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Poster

254. Anatomy of Stress, Anxiety, and Fear

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Topic: F.02. Animal Cognition and Behavior

Support: FAPESP Grant 2014/05432-9

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CNPq

Title: Further evidence of a role for the lateral hypothalamic area juxtadorsomedial region (LHAjd) in the expression of social context-related defensive behaviors

Authors: J. D. HAHN[1], M. J. RANGEL[2], *N. S. CANTERAS[3];
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Abstract: Following a revised lateral hypothalamic area (LHA) parcellation (Swanson, L. W. 2004), several studies in recent years have reevaluated the extrinsic connections of the LHA. A major finding of these studies is indicated connectional relations between different LHA regions and different types of innate behavior: Ingestive, agonistic, and reproductive. The focus of the present research is the LHA juxtadorsomedial region (LHAjd). A recent comprehensive analysis of LHAjd connections indicated a high level of connectivity with several regions implicated in defensive behavioral responses (Hahn, J. D. & Swanson, L. W. 2012). In support of this putative role, a recent study reported increased LHAjd expression of immediate early gene product c-Fos after exposure to a social-defeat related context in a resident-intruder paradigm (Faturi, C. B., Rangel, M. J. et al., 2013). To explore this further, the present study used the same paradigm to test the hypothesis that the LHAjd is necessary for the expression of social-defeat related contextual defensive behaviors. Male Wistar rats (n = 20) received stereotaxic bilateral LHAjd NMDA injections. After a recovery period, the rats were habituated to a resident-intruder testing apparatus prior to a brief encounter with a resident male conspecific. During the encounter testing the habituated "intruder" was free to interact with the resident in the latter's home compartment. The next day the intruders were tested for behavioral responses to the encounter context in the absence of the resident. Encounter and context testing was video recorded. Intact and sham-lesion control groups were similarly tested. Behavioral scoring from the video recordings was done for stereotyped behavioral responses recorded during encounter and context testing, these included (for encounter): Passive and active defense, locomotion, grooming, and social investigation; (for context): Risk assessment, exploration, rearing, grooming, and flight. Statistical analysis revealed a significant effect on contextual behaviors: A significant decrease in risk assessment and a significant increase in exploration behaviors in LHAjd-lesioned intruders compared to LHAjd sham-lesioned and intact rats. Behavioral scores also revealed a marked encounter-associated increase in active defense and social investigation, and a marked decrease in passive defense, but these did not quite reach significance. These findings provide further evidence for LHAjd involvement in the expression of defensive behaviors, and add to the growing body of research indicating different involvement of differentiable LHA regions in the control of fundamental behaviors.

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Poster

254. Anatomy of Stress, Anxiety, and Fear

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Topic: E.05. Stress and the Brain

Support: CIHR

Title: Prolonged corticosterone exposure decreases neuronal activity and dampens glutamatergic drive to CRH neurons in the paraventricular nucleus of the hypothalamus

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Abstract: Corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus of the hypothalamus (PVN) guide the endocrine output of the hypothalamic-adrenocorticotropic axes (HPA). Activation of the HPA culminates in release of corticosterone (CORT), which feeds back to modify neural circuitry. We have previously shown that acute CORT acts a metaplastic signal at glutamate and GABA synapses in the PVN, but the consequences of chronic CORT on intrinsic excitability and synaptic drive to CRH neurons are unresolved. Here, using a mouse line in which CRH neurons can be identified based on the expression of a TdTomato fluorescent marker, we obtained on-cell recordings to examine intrinsic neuronal activity. Then, from the same cells, we obtained whole-cell recordings to examine spontaneous excitatory post-synaptic currents (sEPSCs). In naïve animals, CRH neurons have a mean firing rate of 2.83 ± 0.35 Hz (n=21), a mean sEPSCs frequency of 3.27 ± 0.46 Hz (n=21), and mean sEPSC amplitude of 22.79 ± 1.43 pA (n=21). In CORT treated animals (25μg/mL CORT in drinking water for 1 week), neuronal activity was significantly reduced (0.11 ± 0.05 Hz, n=20, $P < 0.0001$ versus controls), sEPSC frequency was unchanged (3.64 ± 0.65 Hz, n=20). We did note a significant decrease in sEPSC amplitude following chronic CORT exposure (16.06 ± 0.6 pA, n=20, $P < 0.0001$ versus controls). In addition to these changes, there was a rightward shift in the F-I plot following chronic CORT, which is consistent with decreased intrinsic excitability. These results are in line with previous work showing changes to excitatory synapses following chronic CORT exposure in the PFC and hippocampus. Because CRH neurons in the PVN are key mediators of the HPA response to stress, studying the effect chronic CORT on these cells will facilitate understanding of how chronic stress leads to HPA dysfunction and ultimately, the development of various psychiatric disorders. This study was funded by C.I.H.R.

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Poster

254. Anatomy of Stress, Anxiety, and Fear

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Topic: E.05. Stress and the Brain

Support: NIH R01 Grant DK091425

Title: Use of FDG positron emission tomography to visualize brain activation in a model of stress relief by "comfort" food

Authors: *A. E. EGAN[1,2], J. C. ELIASSEN[1], M. NORRIS[1], L. C. LEMEN[4], K. LASANCE[3], Y. M. ULRICH-LAI[1];
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Abstract: A history of limited sucrose intake reduces hypothalamic-pituitary-adrenocortical (HPA) axis, sympathetic, and behavioral stress responses to stress, similar to stress relief by "comfort" foods. Prior neurotoxic lesion studies indicate that the basolateral amygdala (BLA) and medial prefrontal cortex (mPFC) are both necessary for this sucrose stress relief. In addition, the known anatomical projections of the BLA and mPFC suggest they are unlikely to work alone. However, it is not known what other brain regions work with the BLA and mPFC to produce stress relief by palatable foods. In order to address this question, brain activation was studied with [18]F-FDG positron emission tomography (PET) scans. Adult, male Long-Evans rats with ad libitum access to chow and water were given additional brief (up to 30 min), limited (up to 4 ml per session) access to 30% sucrose drink (or water as a control) twice-daily for 14 days. On day 15, rats were fasted overnight, and half of each drink group was given a 20-minute restraint stress, with the other half remaining unstressed. Immediately following the restraint stress (or equivalent unstressed time), rats underwent a 30-minute PET scan to visualize FDG uptake in the brain. Preliminary results showed that unstressed rats have higher global brain activation regardless of drink type. Further analysis will use a combination of voxel-wise comparisons and region of interest (ROI) analyses to determine specific brain regions that are differentially activated based on stress history or drink type.

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Poster

254. Anatomy of Stress, Anxiety, and Fear

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Topic: E.05. Stress and the Brain

Title: Aged garlic extract increases neuropeptide y, superoxide dismutase 2, catalase and glutathione peroxidase mRNA levels in hypothalamus of diabetic rats

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Abstract: The hypothalamus is a brain region that regulates feeding behavior, and recent studies have shown that it has a role in glucose homeostasis. Neuropeptide Y (NPY) is an important hypothalamic orexigenic neuropeptide, related with regulation of feeding and promotion hyperphagia. Moreover, hypothalamic reactive species oxygen (ROS) production are key to the central regulation of satiety. However, during diabetes mellitus (DM), has been reported that exist hyperphagia and oxidative stress. The oxidative stress is characterized by excessive production of ROS which is involved in diabetic complications, and reduction of antioxidant defense mechanisms. It has been shown that substances with antioxidant capacity, regulate the excessive production of ROS, glucose levels and improve several complications found in the DM, but still not known molecular mechanism by which they exert this effect. Therefore, the aim of this study was to determinate whether an antioxidant as aged garlic extract (AGE) has effect on gene expression of NPY and antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX)] in normal and diabetic rats. For this purpose, male Wistar rats (280-350g) were used. AGE (200mg/kg bw) was administered daily oral or intraperitoneally (i.p.) for 4 weeks to control and diabetic rats. Diabetes was induced by treatment with streptozotocin (60mg/kg bw) i.p. 24 hours after last treatment, blood was collected for estimation of glucose, and the animals were killed by cervical decapitation. Hypothalamic region was separated from brain tissue to determinate NPY, SOD, CAT and GPx mRNA levels by performing RNA extraction, reverse transcription and real-time PCR. In our study model, diabetic rats showed increase of blood glucose (p0.05). I.p. and oral administration of AGE to diabetic rats attenuated their increment in blood sugar level. These findings suggest that the daily administration of 200 mg/kg bw AGE have anti-hyperglycemic activity. However, gene expression of NPY, SOD-2, CAT and GPX was increased significantly in hypothalamus of control and diabetic rats treated with AGE only via i.p. ($p<0.05$), probably by their ability to activate the transcription factor as Nrf2, which is activated by S-allylcisteine (SAC), an abundant component of AGE. Finally, whether the increased expression of NPY is associated with hyperphagia or not is yet to be determined.

Disclosures: **M. Barragan-Bonilla:** None. **P. Aguilera:** None. **M. Espinoza:** None.

Poster

254. Anatomy of Stress, Anxiety, and Fear

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 254.27/W27

Topic: E.05. Stress and the Brain

Title: Pharmacological inhibition of the psychiatric risk factor FKBP51 has anxiolytic properties

Authors: ***J. HARTMANN**[1], K. V. WAGNER[1], S. GAALI[1], A. KIRSCHNER[1], C. KOZANY[1], G. RÜHTER[2], N. DEDIC[1], A. S. HAEUSL[1], L. HOEIJMAKERS[1], S. WESTERHOLZ[1], C.

NAMENDORF[1], T. GERLACH[1], M. UHR[1], A. CHEN[1], J. M. DEUSSING[1], F. HOLSBOER[1], F. HAUSCH[1], M. V. SCHMIDT[1];
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Abstract: Anxiety-related psychiatric disorders represent one of the largest health burdens worldwide. Single nucleotide polymorphisms of the FK506 binding protein 51 (FKBP51) gene have been repeatedly associated with anxiety-related disorders and stress sensitivity. Given the intimate relationship of stress and anxiety, we hypothesized that amygdala FKBP51 may mediate anxiety-related behaviors. Mimicking the stress effect by specifically overexpressing FKBP51 in the basolateral amygdala (BLA) or central amygdala (CeA) resulted in increased anxiety-related behavior, respectively. In contrast, application of a highly selective FKBP51 point mutant antagonist, following FKBP51mut BLA-overexpression, reduced the anxiogenic phenotype. We subsequently used a novel FKBP51 antagonist, SAFit2, in wild-type mice via BLA micro-injections, which reduced anxiety-related behavior. Remarkably, the same effect was observed following peripheral administration of SAFit2. To our knowledge, this is the first in-vivo study using a specific FKBP51 antagonist, thereby unraveling the role of FKBP51 and its potential as a novel drug target for the improved treatment of anxiety-related disorders.

Disclosures: J. Hartmann: None. **K.V. Wagner:** None. **S. Gaali:** None. **A. Kirschner:** None. **C. Kozany:** None. **G. Rühter:** None. **N. Dedic:** None. **A.S. Haeusl:** None. **L. Hoeijmakers:** None. **S. Westerholz:** None. **C. Namendorf:** None. **T. Gerlach:** None. **M. Uhr:** None. **A. Chen:** None. **J.M. Deussing:** None. **F. Holsboer:** None. **F. Hausch:** None. **M.V. Schmidt:** None.

Poster

254. Anatomy of Stress, Anxiety, and Fear

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 254.28/W28

Topic: E.05. Stress and the Brain

Support: NSERC

Title: Prostaglandin E2 inhibits GABAergic synaptic transmission onto parvocellular neuroendocrine cells in the paraventricular nucleus of the hypothalamus

Authors: *W. INOUE;
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Abstract: Immune-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis and ensuing release of anti-inflammatory glucocorticoids are critical for the fine tuning of the inflammatory response. Although prostaglandin E2 (PGE2) has a well defined role in the immune-induced activation of parvocellular neuroendocrine cells (PNCs) in the hypothalamic

paraventricular nucleus (PVN), it remains unclear if and how PGE2 modulates synaptic inputs onto PNCs. Using whole-cell patch clamp recordings obtained from PNCs in an *ex vivo* hypothalamic slice (P21-35 male Sprague-Dawley rats), I evaluated the effect of PGE2 on GABAergic-mediated inhibitory synaptic transmission. Bath application of PGE2 (0.01−100 μM) dose dependently decreased the amplitude of evoked inhibitory postsynaptic currents (eIPSCs) with maximal effect at 10 μM (48.9 ± 7.9 % of baseline). The PGE2-mediated (10 μM) depression of eIPSCs had a rapid onset (significant inhibition by 3 min), was long-lasting (55.2 ± 10.1 % inhibition at 25 min after wash out) and accompanied by an increase in paired pulse ratio (149.5 ± 14 % of baseline). In addition, PGE2 decreased the frequency (41.1 ± 6.1 % of baseline) but not the amplitude (101.0 ± 11.0 % of baseline) of spontaneous IPSCs, suggesting that PGE2 acted at a presynaptic locus to decrease the probability of GABA release. Inclusion of BAPTA (10 mM) in the pipette solution to prevent a rise in postsynaptic Ca2+ failed to affect the PGE2-mediated inhibition of eIPSCs, thereby ruling out a role for the Ca2+-dependent release of a retrograde messenger. I demonstrate that PGE2 causes a long-lasting depression of GABA release onto PNCs, providing a plausible mechanism for the disinhibition of HPA output during inflammation.

Disclosures: W. Inoue: None.

Poster

254. Anatomy of Stress, Anxiety, and Fear

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 254.29/W29

Topic: F.02. Animal Cognition and Behavior

Support: NSERC

Title: The lateral septum and anterior hypothalamus work in tandem to regulate defensive burying

Authors: *S. J. LAMONTAGNE, M. C. OLMSTEAD, J. L. MENARD;
Psychology, Queen's Univ., Kingston, ON, Canada

Abstract: The lateral septum (LS) and anterior hypothalamus (AHA) are heavily inter-connected and independently implicated in behavioural defense regulation. The current study examined whether these two structures work in tandem to regulate rats' defensive responses toward a potential threat (as modeled in the elevated plus-maze) and a present, localizable threat (as modeled in the shock-probe burying test). A pharmacological disconnection technique was used: rats (n = 8) in the experimental condition received co-infusions of muscimol into one side of the lateral septum and contralateral anterior hypothalamus, and rats in the three control conditions received either contralateral infusions of saline (n = 9) into both structures or unilateral infusions

of muscimol into one structure (LS, n = 8; AHA n = 6), combined with saline in the contralateral side of the other structure. Five minutes after their infusions, rats were tested in the plus-maze. One week later, they received a second infusion and were tested in the burying test. Co-infusions of muscimol into one side of the LS and the contralateral AHA significantly suppressed rats' shock-probe burying without altering their open-arm avoidance in the plus-maze. No behavioural effects were observed following unilateral infusions of muscimol into either structure alone. Together, these findings suggest that the LS and AHA work in a serial fashion to regulate rats' innate defensive behaviours toward threats that are present and localizable in their immediate environment but not toward potential threats that might or might not be present.

Disclosures: S.J. Lamontagne: None. **M.C. Olmstead:** None. **J.L. Menard:** None.

Poster

254. Anatomy of Stress, Anxiety, and Fear

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 254.30/W30

Topic: E.05. Stress and the Brain

Support: NEI R00EY019547

NINDS 5P30NS069266

Title: The minor spliceosome snRNA's U4atac and U6atac are down regulated in starvation induced stress response

Authors: *N. STURROCK[1], J. SIKKA[2], M. BAUMGARTNER[2], C. LEMOINE[2], R. KANADIA[2];
[1]Physiol. and Neurobio. U3165 Kanadia, [2]Physiol. and Neurobio., Univ. of Connecticut, Storrs, CT

Abstract: The drive to eat versus satiation is mediated through the oppositional firing behavior of orexigenic neurons expressing AgRP and NPY along with anorexigenic neurons expressing POMC. This balance in neuronal activity relies on signaling from a variety of stress response pathways, which if disrupted can cause eating disorders like anorexia or hyperphagia. The consequence of hyperphagia is often obesity, a growing global epidemic. Significant effort has been placed on understanding the neurophysiological and metabolic regulation of these neurons. However, not much is known about the role of splicing in regulating the production of proteins required to execute the neuronal activity necessary for the proper functioning of these neurons. The minor spliceosome, responsible for splicing just 1% of genes, is implicated in regulating calcium homeostasis, which when disrupted can cause increased reactive oxygen species (ROS). Eating behaviors reliance on ROS levels means that changes in calcium homeostasis may affect its regulation, which suggests that the minor spliceosome might play a vital role in regulating

eating behavior. The minor spliceosome, as the name suggests is splicing machinery that parallels the ubiquitous major spliceosome. The minor spliceosome has four essential small nuclear RNAs (snRNA's) including U11, U12, U4atac, U6atac and shares U5 snRNA from the major spliceosome. Recently it was shown that U6atac levels are rate-limiting to the efficiency of minor spliceosome and is upregulated under stress to increase efficiency of minor intron splicing thereby increasing protein production. Here we explored the expression of minor spliceosome snRNA's in the ventral medial hypothalamus and the arcuate of 48-hour fasted animals. Section *in situ* hybridization and qPCR showed that the minor spliceosomes snRNAs U6atac and U4atac were downregulated in the ventral medial hypothalamus and the arcuate of 48-hour fasted animals. Importantly, this change was not observed in the cortical neurons. These data suggests the minor spliceosome is actively downregulated as a response to fasting and exploration of its targets (ongoing) will help bring to light the role of minor spliceosome in regulation of feeding behavior.

Disclosures: **N. Sturrock:** None. **J. Sikka:** None. **M. Baumgartner:** A. Employment/Salary (full or part-time):; University of Connecticut. **C. Lemoine:** A. Employment/Salary (full or part-time):; University of Connecticut. **R. Kanadia:** A. Employment/Salary (full or part-time):; University of Connecticut.

Poster

255. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 255.01/W31

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01DA027688

Title: Delays in proactive inhibition are specific to adolescence

Authors: *M. CHODAKEWITZ[1], H. C. MEYER[2], D. J. BUCCI[2];
[2]Psychological and Brain Sci., [1]Dartmouth Col., Hanover, NH

Abstract: Previous research has postulated that the differential development of subcortical reward areas and top-down control systems results in a functional imbalance that affects inhibitory control during adolescence. Specifically, that development of the prefrontal cortex lags behind development of the nucleus accubmens (NAC) and that NAC is sensitized in adolescents compared to both children and adults. This model has garnered substantial support in recent years from neuroimaging and behavioral studies in humans, as well as electrophysiological studies in lab animals. However, little research has focused on the pattern of inhibitory behavior across the adolescent period in rodents. The present research aimed to model inhibition as it most often manifests in the lives of adolescents; specifically, situations in which

the meaning of a stimulus (respond or not) is ambiguous and can change on a moment to moment basis. Thus, the present research utilized a negative occasion setting paradigm (NOS), in which the inhibitory properties of a stimulus only apply to a specific circumstance. In this paradigm, a "target" stimulus presented by itself is followed immediately by delivery of a food reward. However, when a "feature" stimulus is presented just before the target, food is not delivered. Importantly, NOS involves learning the meaning of a cue in the environment and applying it to withhold a response before it is initiated. This form of proactive inhibition is distinct from forms of inhibition present in other behavioral models, such as Stop-Signal Reaction Time task and the 5-choice serial reaction time task. Our laboratory has previously demonstrated that adolescent rats (ie, starting training on PND 35) require ~18 sessions, almost twice as many as adults, to exhibit inhibition during NOS. To determine how this behavior manifests across development, separate cohorts of rats began training on PND 40 or PND 30. Consistent with our results in 35 day old rats, we observed a delay in the ability to withhold behavior when rats began training at PND 40. Indeed, these rats required 13 sessions to exhibit inhibition, three more than required for adult rats. Importantly, this suggests that the ability to withhold behavior improves across adolescence. Conversely, rats that began training at PND 30 only required 10 sessions to exhibit inhibition. These data are consistent with evidence suggesting that although the ability to control behavior is evident earlier in development, adolescents often forgo appropriate behaviors in the face of reward. Taken together, these results indicate that NOS performance is mediated by factors that are differentially activated during adolescence.

Disclosures: **M. Chodakewitz:** None. **H.C. Meyer:** None. **D.J. Bucci:** None.

Poster

255. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 255.02/W32

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01DA027688

Title: Age differences in the extinction of Pavlovian excitatory responding

Authors: ***D. J. BUCCI**[1], H. C. MEYER[2];
[2]Psychological and Brain Sci., [1]Dartmouth Col., Hanover, NH

Abstract: Previous research suggests that environmental cues signaling a potential reward may drive incentive-seeking behavior to a greater extent in adolescents than in children or adults. Furthermore, adolescents are often unable to exert behavioral control in the face of environmentally salient cues. However, the majority of results demonstrating that reward-cue

processing differs during adolescence comes from human neuroimaging and behavioral studies. Indeed, rodent literature has primarily focused on behavioral differences during the implementation of an instrumental response in service of obtaining reinforcement. Conversely, less is known about how adolescent rodents process and respond to Pavlovian cues. Thus, the present experiments elucidated the age differences in Pavlovian anticipatory responding during an auditory stimulus indicating the impending delivery of a reinforcer. Separate cohorts of adolescent (beginning training on PND 35) and adult rats were trained in a simple discrimination consisting of 8 presentations of a reinforced stimulus (CS1+) immediately followed by a food reward and 8 presentations of a non-reinforced stimulus (CS2-). The primary variable of interest was anticipatory nose-pokes into the recessed magazine where food was delivered. We determined that adolescents and adults learned to discriminate between these stimuli at comparable rates and responded significantly more during CS1+ than CS2-. After 8 days of acquisition training, responding was extinguished during two daily sessions in which all rats were presented with 30 presentations of each stimulus and no reinforcer (CS1-; CS2-). We found that adolescent rats persisted in responding during the previously reinforced CS1 for longer than adult rats, although by the end of the second training session all rats displayed similar low levels of responding during all cues. In a subsequent test for retention 96-hours later, adolescents displayed higher levels of spontaneous recovery of responding to CS1 than adults. Our data suggest that although adolescents acquire Pavlovian approach to a novel cue at an equal rate, they experience difficulties learning a secondary inhibitory meaning of same cue during extinction. The acquisition of competing excitatory and inhibitory representations during extinction parallels the type of learning that occurs during negative occasion setting, in which rats must learn to respond differentially during presentations of one cue based on the presence or absence of a second cue. Thus, the present results may be useful in elucidating the behavioral patterns observed during adolescent negative occasion setting (see accompanying poster).

Disclosures: **D.J. Bucci:** None. **H.C. Meyer:** None.

Poster

255. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 255.03/W33

Topic: F.02. Animal Cognition and Behavior

Support: NSERC

Title: Response inhibition after extinction: optogenetic activation of the infralimbic prefrontal cortex reduces the spontaneous recovery and reinstatement of appetitive Pavlovian conditioning

Authors: *F. LACROIX, D. SPARKS, C. SANIO, A. CHAPMAN, N. CHAUDHRI; Psychology/Center for Studies in Behavioural Neurosci., Concordia Univ., Montreal, QC, Canada

Abstract: Extinction occurs when a Pavlovian conditioned stimulus (CS) that predicts a reinforcer is subsequently experienced without that reinforcer. The infralimbic prefrontal cortex (IL) is believed to play a pivotal role in inhibiting responding to a CS after extinction has taken place. Based on this hypothesis, we predicted that activating the IL during CS trials using optogenetics would decrease spontaneous recovery (SR) and reinstatement of sucrose-seeking. To investigate this research question, male Long-Evans rats (Charles River, 220-240 g) received a unilateral microinfusion (0.5 µl) of virus coding for yellow fluorescent protein (AAV-CAMKIIa-eYFP) or channelrhodopsin-2 and eYFP (AAV-CAMKIIa-hChR2-eYFP) into the IL (AP +2.9, ML ±0.6, DV -5.1), followed by an optical fiber targeting the same region. Next, rats underwent Pavlovian conditioning sessions (9 sessions, 40 min each) in which CS trials (10 s white noise, 14 trials/session, VT-120s) were paired with 10% sucrose (10S, 0.2 ml/CS). Entries into a fluid port where 10S was delivered were measured. Extinction sessions were then conducted in which the CS was presented as before, but without 10S. In Experiment 1, rats received 1 extinction session, followed by 2 additional extinction sessions 24 h and 26 days later to test SR. Photostimulation (473 nm; 20 Hz; 5 ms pulses; 10,002 ms duration) was administered during each CS trial at test. The spontaneous recovery of port entries during the CS observed in rats expressing eYFP only (n=6) was significantly reduced on a trial-by-trial basis in the ChR2 group (n=6). In Experiment 2, Pavlovian conditioning was followed by 6 extinction sessions and two reinstatement tests. At test, a 0.2 ml prime of 10S occurred before the first CS trial, and each CS trial was paired with photostimulation. A reinstatement of port entries during the CS was observed in rats expressing eYFP only (n=12), but not in rats expressing ChR2 (n=8). Subsequently, photostimulation delivered during CS trials enhanced the reacquisition of Pavlovian conditioning in rats expressing ChR2, compared to eYFP controls. *In vitro* patch clamp recordings confirmed that IL pyramidal neurons expressing ChR2 spiked with high fidelity in response to light stimulation frequencies up to 20Hz inclusively. These findings support the hypothesis that the activity of IL neurons suppresses conditioned behavior after extinction, and suggest a novel role for the IL in promoting the re-learning of excitatory, appetitive Pavlovian associations following extinction.

Disclosures: F. Lacroix: None. **D. Sparks:** None. **C. Sanio:** None. **A. Chapman:** None. **N. Chaudhri:** None.

Poster

255. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 255.04/W34

Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01DK085721

Title: Differential recruitment of the medial prefrontal cortex and hippocampal formation during renewal of conditioned responses to food cues in male and female rats

Authors: *L. C. ANDERSON, G. D. PETROVICH;
Psychology, Boston Col., Chestnut Hill, MA

Abstract: Food cues can stimulate appetite in the absence of hunger and contribute to obesity. Renewal, or reinstatement, of responding to food cues after extinction may explain the inability to resist palatable foods and change maladaptive eating habits. Recently, we found sex differences in renewal of extinguished Pavlovian conditioned responses to food cues. Here, we examined recruitment (Fos induction) of telencephalic regions important for associative learning, decision-making, and contextual processing, the medial prefrontal cortex (mPFC) and hippocampal formation, during renewal in male and female rats. We used a context-dependent renewal protocol where conditioning and extinction are conducted in different contexts and the renewal of responding is induced by return to the conditioning context (ABA renewal). Control groups remain in the same context during acquisition, extinction, and test (AAA). Rats were trained to associate a tone (conditioned stimulus, CS) with food (unconditioned stimulus) in 5 acquisition sessions. Acquisition was followed by 2 extinction sessions with CS-only presentations. Rats were then tested for renewal of responding with CS-only presentations in a single session. The measure of learning was an increase in the expression of food cup behavior (conditioned response, CR) during CSs. There were no sex differences during acquisition and responding was similar in extinction. During the test males showed renewal of CRs, while females did not. There was a main effect of sex and experimental condition interaction for CRs ($p < 0.05$), and a significant difference between male groups ($p < 0.05$), but no differences between female groups ($p > 0.05$). Following testing, brain tissue was processed with immunohistochemistry for detection of Fos and total number of neurons with Fos induction was analyzed in areas of interest. Significant differences were found within the infralimbic (ILA), prelimbic (PL), ventral subiculum (SUBv), and dorsal CA1 (CA1d). Within the ILA and PL, Fos induction in males was significantly higher in experimental compared to control groups ($p < 0.05$). The pattern of Fos induction was opposite in females: it was significantly lower in the experimental compared to control groups ($p < 0.05$). Within the CA1d, there was more Fos in the experimental compared to control groups in males, while there were no differences between the female groups ($p < 0.05$ for males). In contrast, in the SUBv, both males and females had significantly higher Fos induction in experimental compared to control groups ($p < 0.05$). These results indicate a distinct mPFC and hippocampal system is recruited during context-dependent renewal in a sex specific way.

Disclosures: L.C. Anderson: None. **G.D. Petrovich:** None.

Poster

255. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 255.05/W35

Topic: F.02. Animal Cognition and Behavior

Support: NIDDK R01 DK085721

Title: Blockade of orexin/hypocretin receptor 1 signaling attenuates Pavlovian cue-food conditioning and extinction

Authors: *S. E. KEEFER, S. COLE, H. S. MAYER, G. D. PETROVICH;
Psychology, Boston Col., Chestnut Hill, MA

Abstract: Food cues can drive feeding in the absence of hunger, and orexin/hypocretin (ORX) signaling is necessary for this type of overeating. The current study examined whether ORX also mediates cue-food learning during the initial acquisition and extinction of these associations. In Experiment 1, male, Long-Evans rats underwent two sessions of Pavlovian appetitive conditioning. Conditioning sessions included 8 presentations of a 10s tone (conditioned stimulus, CS) followed by immediate delivery of 2 palatable food pellets (unconditioned stimulus, US) distinct from standard chow. Thirty minutes prior to each session, rats received an i.p. injection of either the ORX 1 receptor antagonist SB-334867 (SB) or vehicle (V) in a crossover design: V/V, V/SB, SB/V and SB/SB (n=8/group). During conditioning session 1, SB had no effect on conditioned responses, measured as percentage of food cup behavior during the CS, or on latency to approach the food cup after the CS onset. During conditioning session 2, all groups that received SB prior to either conditioning session displayed significantly less food cup behavior and had longer latencies to approach the food cup after CS onset compared to the V/V group, signifying SB attenuated learning. In Experiment 2, another group of rats underwent 5 sessions of appetitive conditioning (drug free) followed by 2 extinction sessions, each consisting of 8 CS-only presentations. Thirty minutes prior to each extinction session, rats received an i.p. injection of either SB or V resulting in 4 groups as described above. During extinction session 1, SB had no effect on food cup behavior or latency. During extinction session 2, three distinct differences were found across groups. First, the SB/V group displayed overall more food cup behavior during the CS, and had shorter latencies to the food cup throughout the session compared to the other 3 groups. Food cup behavior of this group persisted during the 10 sec after CS offset when pellets were previously delivered during conditioning, signifying an inhibition of extinction due to exposure to SB during extinction session 1. Second, The SB/SB group displayed more CS specific food cup behavior during the first half of the session compared to the other 3 groups, but significantly decreased this behavior during the session. Third, both groups that received SB prior to session 2 displayed an increase in latency time throughout extinction session 2, while the groups treated with V prior to session 2 did not show this increase. Locomotor behavior was unaffected by SB in either experiment. Together these results suggest a role for ORX signaling during Pavlovian appetitive conditioning and extinction.

Disclosures: **S.E. Keefer:** None. **S. Cole:** None. **H.S. Mayer:** None. **G.D. Petrovich:** None.

Poster

255. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 255.06/W36

Topic: F.02. Animal Cognition and Behavior

Support: NIDDK DK085721

Title: Disconnection of the medial prefrontal cortex and lateral hypothalamus system prevents cue-potentiated feeding in sated rats

Authors: ***S. COLE**, S. E. KEEFER, H. S. MAYER, G. D. PETROVICH;
Dept. of Psychology, Boston Col., Chestnut Hill, MA

Abstract: Environmental influences, particularly cues for food, can stimulate feeding in the absence of hunger and ultimately lead to maladaptive overeating behavior. In the cue-potentiated feeding paradigm, a learned food cue can drive eating in sated rats. Previous work has shown that lesions of the medial prefrontal cortex (mPFC) prevent feeding driven by a food-associated context, and that mPFC neurons projecting to the LHA are selectively activated during feeding under a food cue. These findings suggest that communication between the mPFC and LHA is a critical component of the network mediating cue-potentiated feeding. To examine this possibility, here we functionally disconnected the mPFC and LHA prior to a discriminative cue-potentiated feeding procedure. Male, Long-Evans rats received unilateral neurotoxic lesions of the mPFC and LHA in opposite hemispheres (group CONTRA; n=7), the same hemisphere (group IPSI; n=7), or vehicle infusions (group SHAM; n=6). After recovery from surgery, rats were food-deprived and trained across eight days. On each training day all rats received one session consisting of cue-food pairings (CS+), and a second session consisting of a different cue presented alone (CS-). The two cues were a 180s tone and a 180s light, which were counterbalanced across groups. At the completion of training rats were allowed ad libitum access to chow for 3 days prior to the beginning of testing. All animals were tested for consumption during presentations of the cues, CS+ and CS- separately, in a counterbalanced order across two consecutive days. On each day, consumption was first measured before presentations of the cues (baseline), and then during cue-food presentations (CS+ and CS- tests). There were no differences in baseline consumption between groups immediately prior to either CS test (p values > 0.05). During the CS tests, rats consumed significantly more food during the CS+ presentations than during CS- presentations ($p < 0.05$), confirming cue-potentiated feeding. Importantly, this difference was reduced significantly in the CONTRA group compared to the IPSI and SHAM groups ($p < 0.05$), but this difference in consumption between CS+ and CS- tests did not differ significantly between these two control groups ($p > 0.05$). These findings demonstrated that

disconnection of the mPFC and LHA system prevented cue-potentiated feeding in sated rats. Furthermore, the effect was specific to eating driven by a food cue, because the disconnection had no effect on baseline consumption. Future work will examine the specific neurotrasmitter and neuromodulator mechanisms within this mPFC-LHA system that drive this non-homeostatic eating.

Disclosures: S. Cole: None. **S.E. Keefer:** None. **H.S. Mayer:** None. **G.D. Petrovich:** None.

Poster

255. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 255.07/W37

Topic: F.02. Animal Cognition and Behavior

Support: IRP/NIDA/NIH.

Title: Individual variability in outcome devaluation and second- order conditioning predict sign-tracking behavior

Authors: *H. M. NASSER, Y.-W. CHEN, K. A. FISCELLA, A. B. KAWA, D. J. CALU; Behavioral Neurosci. Res. Br., Natl. Inst. of Drug Abuse, Baltimore, MD

Abstract: During autoshaping, where the extension of a lever precedes the delivery of reward, rats show individual differences in conditioned responding; sign-tracking (ST) rats approach/contact the lever, while goal-tracking (GT) rats approach/contact the food cup more than ST. It is hypothesized that ST behavior results from heightened incentive motivation for reward-associated cues, together with reduced goal-directed behavior driven by the current value of the reward. Rats were tested in outcome devaluation (Exp. 1) or second order conditioning (Exp 2) prior to the ST/GT assessment to determine whether ST rats show reduced goal-directed behavior and heightened incentive motivation. To assess goal-directed behavior (Exp. 1), we trained rats to associate a light conditioned stimulus (CS) with food reward. Paired rats received homecage access to the food reward, followed by injections of LiCl (0.3M, 5 ml/kg i.p.), which causes gastric malaise. Unpaired rats received homecage exposure to food reward and LiCl injections, separated by 24 h. Conditioned responding to the light CS was measured in a single probe session. The level of conditioned responding in paired rats during outcome devaluation correlated with the rats' subsequent ST/GT preference scores, with ST rats failing to suppress responding after outcome devaluation. To assess incentive motivation (Exp. 2), we trained rats to discriminate between two light CSs, one light (A) was reinforced with food reward while the other light (B) was non-reinforced. During second order conditioning, one auditory CS predicted A, where another auditory CS predicted B. Conditioned responding to the auditory CS was measured in a single probe session. Second-order conditioning was observed in all rats. ST/GT

scores were correlated with responding to B. That is, ST, but not non-ST rats, show intact auditory CS discrimination, suggesting the incentive properties of A can support second order conditioning in ST rats. The results support the view that ST rats are more sensitive to incentive properties of rewarded cues and have deficits in using information about devalued outcomes to guide goal-directed behavior. This work was supported by IRP/NIDA/NIH.

Disclosures: **H.M. Nasser:** None. **Y. Chen:** None. **K.A. Fiscella:** None. **A.B. Kawa:** None. **D.J. Calu:** None.

Poster

255. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 255.08/W38

Topic: F.02. Animal Cognition and Behavior

Support: Whitehall Foundation

NIH Grant F32MH105125

NIH Grant F32MH106178

Title: Disconnection of ventral pallidum and nucleus accumbens shell with DREADDs enhances sign-tracking in rats

Authors: *S. E. CHANG, T. P. TODD, K. S. SMITH;
Dartmouth Col., Hanover, NH

Abstract: An initially neutral conditioned stimulus (CS) that signals delivery of a food unconditioned stimulus (US) can acquire rewarding properties due to the attribution of incentive salience, in which the incentive motivational value of the US is transferred to the CS. As a result, subsequent presentations of the CS may lead to behavior directed towards the CS itself (i.e., sign-tracking) rather than the site of US delivery. Recently, we have shown that disrupting activity in the ventral pallidum (VP) attenuates the acquisition of sign-tracking using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), a technology that allows for repeated activation of engineered receptors by systemic injection of the otherwise inert ligand clozapine N-oxide (CNO). Previous studies have shown that the nucleus accumbens (NAc) and its dopaminergic inputs are critical for sign-tracking. The VP and NAc are bidirectionally connected, and their disconnection has previously been found to disrupt the ability of a CS to direct instrumental choice behavior. Here we investigated the effects of disconnecting VP and NAc shell on the acquisition of sign-tracking using DREADDs. Two groups of rats received surgery in which unilateral infusions of the inhibitory hM4Di DREADD were virally inserted into the VP and NAc shell. Half of the rats received hM4Di delivery into the VP and NAc shell

in contralateral hemispheres (Group Contra), while the other half received ipsilateral infusions (Group Ipsi). Rats underwent 12 days of training in which each session consisted of 25 CS+ and 25 CS- trials. CS+ trials consisted of insertion of one lever for 10 s that resulted in the delivery of a food US (2 grain pellets) upon retraction, while CS- trials consisted of insertion of another lever that was followed by nothing. Rats received systemic injections of CNO prior to each session. Thus when hM4Di receptors were activated, the VP-NAc shell pathway was disrupted for rats in Group Contra, while the VP-NAc shell pathway was left intact in one hemisphere of Group Ipsi. In contrast to bilateral VP disruption, disconnection of VP and NAc shell dramatically enhanced levels of sign-tracking in Group Contra relative to Group Ipsi. In addition, disconnection of VP and NAc shell reduced food cup behavior during CS presentations. These results suggest that disrupting the VP-NAc pathway can magnify the incentive value attributed to reward-paired cues. This conclusion paradoxical in the context of viewing the NAc and VP as complementing one another for motivation, and suggests that for incentive salience there may be mutual inhibition between them (which current electrophysiological efforts are examining).

Disclosures: **S.E. Chang:** None. **T.P. Todd:** None. **K.S. Smith:** None.

Poster

255. Appetitive and Incentive Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: Whitehall Foundation

NIH Grant F32MH106178

Title: Inhibiting ventral pallidum disrupts adaptive salt-seeking behavior in rats

Authors: *K. J. STANSFIELD, S. E. CHANG, K. S. SMITH;
Dartmouth Col., Hanover, NH

Abstract: Much of reinforcement learning involves a process of trial-and-error in which ongoing behavior is iteratively updated when outcomes are received. However, prior experience is not always required for changes in reward seeking. Such non-incremental learning is illustrated by the “salt appetite” phenomenon: animals that have learned to associate a cue with aversively concentrated salt taste will, if deprived of sodium, immediately orient to the cue and seek out salt before the salt has ever been tasted in the sodium-deprived state. The ventral pallidum (VP) plays an important role in aspects of reward learning, motivation, and hedonics, and represents salt appetite in neural dynamics. In a homeostatic state, VP activity occurs to cues associated with pleasant sugar tastes, but not to cues for aversive salt tastes. Yet, immediately after the sodium deprivation, VP neurons become activated by salt-paired cues similarly to sugar-paired cues. The

present study investigated the causal influence of VP on adaptive salt seeking in rats using optogenetics. Half of the rats received infusions of halorhopdopsin (Group Halo) or a control virus (Group Control) into the VP. All rats received bilateral intra-VP fiber implants. Rats then underwent 8 days of place preference training in which they learned to associate one context with a pleasant flavored sucrose solution (4 days) and another context with a distinctly flavored salt solution (4 days). Rats were given a baseline test session in which they were given access to both contexts for the first time with the sucrose and salt removed to measure their baseline preference. Following 2 retraining days in each context, rats were sodium-depleted through systemic injections of furosemide. After 48 hours, rats were placed into the chamber with access to both contexts without sucrose or salt to measure their preference. All rats received yellow laser stimulation (3 s on/off pulses) throughout the entire test session. Thus, VP was inhibited for rats in Group Halo but not for rats in Group Control. We found that rats in Group Control showed a normal "salt appetite", spending more time in the salt-paired context than the sucrose-paired context following sodium depletion (a form of "unconditioned place preference"). In contrast, rats in Group Halo were indifferent and spent equal amounts of time in the contexts. VP inhibition did not affect general motor activity nor salt/sucrose consumption when the solutions were made available. Thus, inhibiting VP selectively impaired the ability of rats to use environmental cues to guide adaptive salt-seeking behavior, highlighting a critical role for this area in adaptive reward seeking.

Disclosures: K.J. Stansfield: None. **S.E. Chang:** None. **K.S. Smith:** None.

Poster

255. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 255.10/W40

Topic: F.02. Animal Cognition and Behavior

Support: NIH/NIDA Grant R21 DA036672

Title: Genome-wide association study for the propensity to attribute incentive salience to reward cues in outbred rats

Authors: *A. GILETA[1], S. GOPALAKRISHNAN[1], J. GAO[1], C. J. FITZPATRICK[2], S. B. FLAGEL[2], T. E. ROBINSON[3], A. A. PALMER[1];
[1]Human Genet., Univ. of Chicago, Chicago, IL; [2]Dept. of Psychiatry, [3]Dept. of Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: Incentive salience refers to the motivational value attributed to a reward-predicting stimulus, which causes it to become desirable and able to bias attention and elicit approach. In the case of addiction, reward-associated stimuli (conditioned stimuli) that acquire incentive

salience can trigger powerful craving and motivate drug-seeking/taking behaviors. Pavlovian Conditioned Approach (PCA) is a behavioral paradigm used to reliably quantitate the propensity of rats to approach and interact with a reward-paired cue. This conditioned response, known as sign-tracking, reflects the extent to which the cue is attributed with incentive salience. This trait has been shown to be both highly heritable and variable in Sprague-Dawley (SD) rats. Over the past 3 years, our collaborators phenotyped a cohort of ~4,000 outbred SD rats from multiple vendors for PCA. We will use this cohort to perform the first large-scale genome-wide association study (GWAS) for the attribution of incentive salience. A subset of these rats (n=80) are being whole-genome sequenced to assay the standing genetic variation and linkage structure in the component subpopulations. To obtain genotypes for the remaining rats, we utilize double digest genotype-by-sequencing (GBS), a reduced-representation sequencing approach we have optimized for this study. Due to the extensive structure observed in SD rat populations, association analyses will be performed using mixed models containing a random effect term with a genetic relatedness matrix estimated from the SNP genotype data. The marker data will also be used to precisely estimate the narrow-sense heritability of this trait.

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Poster

255. Appetitive and Incentive Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: NIDA Training Grant T32-DA007281

NIH Grant P30 DK020572

Michigan Nutrition Obesity Research Center (DK089503).

Title: Enhanced cue-triggered motivation in obesity-prone rats; interactions between predisposition and junk-food exposure

Authors: *R. C. DERMAN[1], C. R. FERRARIO[2];
[2]Pharmacol., [1]Univ. of Michigan, Ann Arbor, MI

Abstract: While the decision to seek out food can be controlled by endogenous hunger signals, it can also be influenced by external Pavlovian cues that predict food availability. Human studies suggest that enhanced sensitivity to food-cues may promote obesity and hamper weight loss in susceptible individuals. Here, we asked whether the incentive-motivational properties of a food-cue are enhanced in obesity-prone vs. obesity-resistant rats in the absence of overt obesity. We

used a Pavlovian-to-instrumental transfer (PIT) procedure to assess the motivational strength of food-associated cues and their ability to invigorate ongoing food-seeking behavior. First, rats were trained to lever-press for food. Next, they received 8 Pavlovian training sessions where one cue (CS+) was paired with the delivery of food pellet and another cue (CS-) was presented without food delivery. During PIT testing, no food was available, rats had continual access to the levers, and each CS was presented 4 times. PIT was demonstrated by enhanced active lever responding in the presence of the CS+ relative to the CS-. During PIT testing, obesity-prone rats showed enhanced conditioned approach to the food cup during CS+ presentation and expressed strong and persistent PIT. In contrast, PIT was nearly absent in obesity-resistant rats, though they did show conditioned food-cup approach. Thus, in the absence of obesity, the ability of the CS+ to invigorate instrumental responding was stronger in obesity-prone rats. These data show that individual susceptibility to obesity is associated with pre-existing differences in the motivational significance of Pavlovian food-cues and are consistent with our recent findings in outbred rats (Robinson et al., 2015). Next, we determined the effects of exposure to a sugary, fatty, "junk-food" diet on PIT. Training and testing were identical, except that prior to initial training rats were given intermittent access to discrete amounts of "junk-food". We found that exposure to "junk-food" abolished PIT, but not conditioned approach, in obesity-resistant rats. Conversely, "junk-food" exposure in obesity-prone rats resulted in a highly focused PIT effect, with responding in the presence of the CS+ almost exclusively dedicated to lever pressing, and very little approach to the food cup. Thus, in susceptible individuals junk-food exposure further enhanced PIT. These data show that interactions between pre-disposition and consumption of fatty, sugary, foods enhance the incentive motivational properties of food-paired cues and will be discussed in light of the role of altered mesolimbic reward circuits in obesity.

Disclosures: R.C. Derman: None. **C.R. Ferrario:** None.

Poster

255. Appetitive and Incentive Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: CONACyT 152208

DGAPA-PAPIIT IN209911

Technical assistant Gabriela Vera

Technical assistant Alejandro Rangel-Hernández

Title: Differential changes in appetitive taste memory and aversive new learning induced by prolonged consumption of sugar or high fructose corn syrup

Authors: *D. BADILLO JUAREZ, M. MIRANDA;
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Abstract: Sweet food induces taste preference that increases consumption; brain reward pathways are involved in such preference. Accordingly, it has been reported that after long term, but intermittent, sucrose intake some brain areas present neurochemical changes similar to those produced by addictive substances, suggesting the convergence of structures involved during learning and memory, and in the regulation of rewarded-related behavior. Currently, high fructose corn syrup 55 (HFCS-55) and sucrose are the sweeteners most used in America. Thus the objective of this work was to evaluate the effect of sweet withdrawal, after prolonged, permanent or intermittent intake of sucrose or HFCS-55 solution, on anxiety, taste preference, and during a new aversive learning with the same taste stimulus. Thus, Wistar male rats for the intermittent group, had access to food and sweet solution (10% sucrose or 8% HFCS-55) only for 6 hours a day, during 21 days; other rats in the permanent group, had *ad libitum* chow and sweet solution (10% sucrose or 8% HFCS-55) during 21 days; all groups had water *ad libitum*. After 21 days rats were deprived of sweet solution during 2 days. Then anxiety levels were tested in the elevated plus-maze task, and subsequently taste preference and the ability to acquire conditioned taste aversion (CTA) were also evaluated. The results demonstrated that rats after withdrawal of intermittent sucrose consumption or after withdrawal HFCS-55 showed less time on the open arm, indicating more anxiety after withdrawal of intermittent sucrose consumption; rats after withdrawal of intermittent HFCS-55 consumption showed less time on the open arm, compared with permanent HFCS-55 group. In addition, withdrawal of intermittent sucrose induced a significant taste preference compared with permanent sucrose consumption; however similar taste preference was observed after removal permanent or intermittent, HFCS-55 intake. Moreover, after intermittent sucrose consumption, rats required more CTA training sessions than rats after HFCS-55 consumption, to achieve aversive memory. These results suggest that sucrose induced stronger taste preference and higher latent inhibition of CTA compared with HFCS-55. Altogether this data indicates that withdrawal of prolonged but intermittent sucrose consumption generates anxiety, significant increase in consumption (like binge-eating) and decreases the ability to acquire new aversive memory association of the same taste. Acknowledgments: Technical assistants Gabriela Vera and Alejandro Rangel-Hernández. Research supported by DGAPA-PAPIIT IN209911, and CONACyT 152208.

Disclosures: D. Badillo Juarez: None. **M. Miranda:** None.

Poster

255. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 255.13/W43

Topic: F.02. Animal Cognition and Behavior

Title: Differences in cue-directed appetitive behavior and its relation to c-fos expression in the prefrontal cortex

Authors: *S. M. LEWIS[1], D. J. KRAAN[2], M. H. MONFILS[3], H. J. LEE[3];
[1]Univ. of Washington, Seattle, WA; [2]Univ. of Texas, Austint, TX; [3]Univ. of Texas, Austin, TX

Abstract: Repeated illumination of a panel light (the conditioned stimulus; CS) followed by release of food pellets (the unconditioned stimulus; US) reliably results in acquisition of a US-directed response in rats: conditioned food cup approach. However, some rats also acquire a CS-directed response: conditioned orienting. We have previously shown that rats that acquire conditioned orienting in our paradigm (Orienters) tend to make more risky and impulsive choices, be more distracted in an attentional task, and to display more 50 kHz ultrasonic vocalizations (USVs) in response to amphetamine, than those who do not acquire conditioned orienting (Non-Orienters). We were interested in which specific brain regions could be responsible for these differences in the behavioral phenotypes between the two groups. Research has already shown that the central amygdala and its connections to the nigrostriatal pathway are important in conditioned orienting. However, the role of prefrontal cortical regions in conditioned orienting is yet unknown. We hypothesized that prefrontal cortical functions might be different between Orienters and Nonorienters given that prefrontal cortex plays an important role in impulsive/risky decision-making and attentional function. Therefore, we investigated neural activity as measured by c-fos in prefrontal cortical regions of Orienters and Non-orienters. Adult Sprague-Dawley rats were conditioned using our standard light-food pairing paradigm. Rats were perfused immediately after the last training session and brain sections were immunostained for c-fos. Minimal c-fos expression in the pre- and infra- limbic cortical areas is found in either group, but significantly higher expression in the orbitofrontal cortex. Furthermore, our data suggests reduced c-fos expression in the orbitofrontal cortex of Orienters.

Disclosures: **S.M. Lewis:** None. **D.J. Kraan:** None. **M.H. Monfils:** None. **H.J. Lee:** None.

Poster

255. Appetitive and Incentive Learning and Memory I

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 255.14/W44

Topic: F.02. Animal Cognition and Behavior

Support: CAPES

CNPq

Title: The reinforcement omission effect on the behavioral repertoire of rats with lesions on the nucleus accumbens

Authors: *J. O. BUENO, E. F. BERNARDES;
Univ. São Paulo, Ribeirao Preto, Brazil

Abstract: The reinforcement omission procedure, in fixed-interval schedules of reinforcement, produces a reduction in post-reinforcement pause and, consequently, an increase in frequency responses in the next interval. There are different interpretations related to reinforcement omission effect (ROE), based upon motivational and/or attentional components. Preliminary studies have examined the role of activation of some amygdala nuclei to modulate these components. Recent studies suggest that the substructures of the amygdala may be involved in different processes, and connections between different amygdala nuclei and cortical/subcortical structures also seem to be involved in processes related to rewards and expectancy. Other studies suggest that the interaction between the amygdala and nucleus accumbens (NAC) is important for the modulation of motivational processes. However, there are no studies in the literature assessing whether neurotoxic lesions in different cortical and subcortical regions may interfere in ROEs. This study aimed to examine the ROEs on the behavioral repertoire of rats with lesions of the NAC, in classical conditioning procedures and non-contingent reinforcement. Thirty male Wistar rats, divided in NAC and SHAM groups, were submitted to 28 training sessions with 8 trials each one: 20 pre-lesion, two retraining sessions and six post-lesions sessions with omission of reinforcement. Each trial constituted of a 20 seconds signal (tone), followed by the release of a drop of water in the 19th second. In sessions with omission, the water was released in the half of trials. Ten categories of behaviors were analyzed. Comparison between duration rates during omission and reinforcement trials showed that NAC and SHAM groups showed the ROEs. NAC group was less sensitive to the ROEs. Regarding the behavioral categories Magazine sniffing and Near magazine sniffing, the duration rates of SHAM group during omission were higher in relation to rates of NAC group. For the categories Magazine licking, Far from magazine sniffing, Rearing, Locomotion and Grooming duration rates of SHAM group were lower than the NAC group. The results suggest that NAC can be part of circuitry involved in the modulation of ROEs and also indicate the need to consider the involvement of more complex neural network for evaluating the ROEs.

Disclosures: J.O. Bueno: None. **E.F. Bernardes:** None.

Poster

255. Appetitive and Incentive Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant DA033386

Title: Role of dopamine in learning Pavlovian cues associated with different rates of reward

Authors: K. GIRVEN, K. FONZI, I. OLIVA, *M. J. WANAT;
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Abstract: Learning the multiple cues that predict a significant outcome is critical for an animal's survival. These neurobiological processes are thought to involve the mesolimbic dopamine system. Specifically, dopamine neurotransmission encodes the expected value of an anticipated reward in response to a reward-predictive cue. Recently we identified that reward rate is another parameter encoded by cue-evoked dopamine release. Dopamine is also critical for certain forms of learning which involve a single cue - reward relationship. However, real world situations often entail learning about multiple cue - reward relationships simultaneously; though it is not clear how dopamine is involved in these processes. Here, we examined the role of dopamine during the acquisition of a Pavlovian conditioning task incorporating multiple cue-reward relationships. In this paradigm, distinct auditory cues were associated with different reward rates. We recorded phasic dopamine release in the nucleus accumbens throughout learning with fast scan cyclic voltammetry. Preliminary results indicate that dopamine release in the nucleus accumbens core encodes reward rate early in training. In contrast, the pattern of dopamine release in the nucleus accumbens shell is dynamic throughout learning. Antagonizing dopamine receptor function during different phases of training suggests dopamine is involved solely in the expression of Pavlovian conditioned responding, and not in the learning or in the performance of the task. Collectively, these results illustrate the heterogeneity in the pattern of dopamine release throughout the nucleus accumbens in a Pavlovian conditioning task that incorporates multiple cue-reward relationships. Furthermore, our results suggest a selective role of dopamine in the expression of conditioned responding in this task.

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Poster

255. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 255.16/W46

Topic: F.02. Animal Cognition and Behavior

Title: How does reward devaluation control behavior? Expectancy and S-R habits in appetitive conditioning

Authors: *M. R. PAPINI, A. C. GLUECK, S. E. CONRAD;
Dept of Psychology, Texas Christian Univ., Fort Worth, TX

Abstract: Transitions from outcome-dependent behavior to outcome-independent, habitual behavior have been described in the course of instrumental learning, spatial learning, and during the development of addictive behavior. Outcome-dependent behavior is sensitive to current

reward value, whereas habitual behavior is released by the training stimulus and is less dependent on current reward value, at least in the short term. These two modes of behavioral control are usually depicted as arising sequentially, with outcome expectancy guiding behavior early in training, whereas habit becomes typical of extensively trained responses. It is thought that this behavioral transition is mediated by a shift in control from the dorsomedial striatum to the dorsolateral striatum. To test this sequential view, rats received autoshaping training in which the presentation of a lever (L) was paired with the presentation of 12 pellets per trial (6 trials/session). Consistent with the sequential view, Experiment 1 showed that presession feeding (a reward-devaluation procedure) suppressed lever pressing early in training (after 5 sessions), but not late in training (after 20 sessions). Thus, autoshaping exhibits both outcome-dependent and outcome-independent properties as a function of amount of practice. In Experiment 2, rats received pairings of one lever presentation (L1) with 12 pellets per trial, while a second lever (L2) was paired with 2 pellets per trial (3 trials with each lever per session). In late training, when one lever was presented in a given trial (L1 or L2), there was no evidence that a 12-to-2 pellet reduction in L1 (another reward-devaluation procedure) controlled lever pressing_outcome-independent, habitual behavior. However, occasional choice trials with both L1 and L2 present simultaneously yielded evidence of reduced responding for L1 (the downshifted lever) relative to L2 (the unshifted lever)_outcome-dependent behavior. These results suggest that even after the kind of extensive training that yields evidence of habitual behavior, control of appetitive behavior by outcome expectancy can be rescued by encouraging comparisons among alternative outcome expectancies. It is hypothesized that both dorsal striatal streams coexist in late training and are differentially activated by testing conditions that either encourage (two-lever choice) or discourage (single lever) incentive comparisons.

Disclosures: **M.R. Papini:** None. **A.C. Glueck:** None. **S.E. Conrad:** None.

Poster

255. Appetitive and Incentive Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: University of Sussex Strategic Development Funds

BBSRC BB/M009017/1

Title: Neuronal ensemble characterisation following exposure to reward-associated cues

Authors: J. J. ZIMINSKI[1], *E. KOYA[2];
[1]Sch. of Psychology, [2]Univ. of Sussex, Brighton, United Kingdom

Abstract: Learned associations between natural rewards and the environmental cues that predict their availability are encoded by a minority of sparsely distributed, behaviorally activated neurons, called ‘neuronal ensembles’. The glutamate synapse plays an important role in many learning and memory processes. Yet, to date very little is known about the alterations at glutamate synapses specifically on these neurons that encode this type of association. The aims of our laboratory are to identify neuronal ensembles activated by environmental cues associated with sucrose in corticostriatal brain areas, and to characterise their physiology at glutamate synapses. To that end, we measured conditioned behavioural and neuronal ensemble activity (using GFP immunohistochemistry) using Fos-GFP mice that express GFP in strongly activated neurons. We trained these mice to associate an auditory cue with non-contingent sucrose delivery following multiple training sessions. Five to seven days following conditioning, sucrose cue exposure elicited conditioned approach towards the sucrose delivery site, and enhanced ensemble activity in the orbitofrontal cortex and nucleus accumbens. These data suggest that neuronal ensembles that encode sucrose memories reside in these areas. Investigations are underway into characterizing the synaptic properties in these activated neurons using Fos-GFP mice. Also, we plan to compare these synaptic properties with neurons activated by environmental cues associated with non-contingent cocaine.

Disclosures: **J.J. Ziminski:** None. **E. Koya:** None.

Poster

255. Appetitive and Incentive Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: NRF-2014R1A1A2058480

Title: Contribution of lateral habenula to midbrain dopaminergic neurons in the Pavlovian appetitive conditioning

Authors: ***B.-R. CHOI**, D.-H. KIM, J.-S. HAN;
Konkuk Univ., Seoul, Korea, Republic of

Abstract: Lateral habenula (LHb) is known as major dopamine (DA) inhibition center, which is contribute to reward learning. In the previous study, DA system is activated by reward delivery and inhibited by reward emission and LHb works in an opposite manner, with DA system. However, no study has done to examine how LHb acts in Pavlovian appetitive conditioning. The present study, therefore, examined the neuronal activity of LHb in Pavlovian appetitive conditioning, with paired group and unpaired group. In a paired group, light was presented for 10 sec and immediately delivered two reward pellets, and 16 trials per one session for 64 min with 4

min variable inter-trial interval and conditioning was performed for 8 days. For rats in a unpaired control group, the same number of lights and food pellets were pseudorandomly presented using an unpaired procedure in which the CS and US were noncontingent, with the provision that the identical stimulus did not appear three consecutive times. Three control groups were included to exclude alterations of neural activity by stimuli presentation and apparatus exposure (groups that received either light or food only and naïve group). Neural activity in LHb and other brain area, substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) and basolateral amygdala (BLA), central amygdala (CeA) were measured by c-fos expression. LHb neural activity was only increased in unpaired group. And SNc, VTA, BLA, and CeA neural activity were increased only in paired group. These results indicate that increased LHb activity in unpaired group contributed to inhibition of midbrain DA neurons. Additionally, using retrograde tracer fluoro gold (FG) and cholera toxin B subunit (CTb), we examined the neural activity which was projected from LHb to SNc or VTA in Pavlovian associative conditioning. We unilaterally injected FG or CTb into SNc or VTA of rats. These rats received Pavlovian appetitive conditioning for 8 days. Most LHb neurons expressing c-fos in unpaired group innervated neurons in SNc or VTA, suggesting that LHb neurons that were activated by unpaired training condition inhibit midbrain DA neurons. Supported by the Korea Research Foundation Grant funded by the Korean Government (NRF-2014R1A1A2058480) to Jung-Soo Han

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Poster

255. Appetitive and Incentive Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH/NIDA

MOST 103-2911-I-038-501

Title: Pontomesencephalic tegmental afferents to VTA are necessary to develop prepotent response inhibition

Authors: ***H.-J. YAU**[1], D. V. WANG[1], J.-H. TSOU[1], B. T. CHEN[1], K. DEISSEROTH[2,3], S. IKEMOTO[1], A. BONCI[1,4];
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Abstract: Dopamine neurons fire phasic burst activity during presentation of reward or reward predictive cues. It is believed that DA neuron burst activity depends on afferent inputs. Several

lines of evidence lead us to study the regulatory roles of the pedunculopontine tegmental nucleus (PPTg) in VTA circuits and its role in modulating behaviors. Anatomically, PPTg provides ascending cholinergic and non-cholinergic inputs to the VTA. Further, inactivation of PPTg decreases VTA DA burst activity to conditioned stimuli. These studies suggest that PPTg may relay sensory information onto VTA to modulate firing activity of midbrain DA cells. In line with this evidence, lesion of PPTg impairs stimulus-reward learning. Nevertheless, direct evidence linking PPTg-to-VTA inputs in the acquisition of stimulus-reward association is lacking. Conventional methods of lesion or pharmacological inactivation in PPTg can neither conclusively establish a causal link between specific PPTg outputs and observed behavioral or cellular deficits, nor distinguish the relative contributions of different PPTg neuronal populations. To overcome these limitations, we employed optogenetic approach to selectively inhibit PPTg glutamatergic or cholinergic inputs to the VTA in order to examine the contribution of these inputs in Pavlovian appetitive conditioning. Furthermore, we performed *in vivo* optetrode recording in freely moving mice as they performed a Pavlovian task to examine the regulation of VTA circuits by PPTg glutamatergic or cholinergic afferents. Here we report that PPTg-to-VTA excitatory inputs play a critical role in the acquisition of stimulus-reward associations by influencing development of prepotent response inhibition. We also identify an unprecedented role of PPTg-to-VTA pathways in modulating the activity of VTA non-DA neurons.

Disclosures: **H. Yau:** None. **D.V. Wang:** None. **J. Tsou:** None. **B.T. Chen:** None. **K. Deisseroth:** None. **S. Ikemoto:** None. **A. Bonci:** None.

Poster

255. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 255.20/X2

Topic: F.02. Animal Cognition and Behavior

Support: R01AG045380

R01DK098709

R01DA029035

Title: Microstructural analysis of cue evoked sucrose consumption and its dependence on dopamine

Authors: ***A. T. LIU**, B. HALBOUT, S. B. OSTLUND;
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Abstract: It is widely believed that cues associated with palatable foods are capable of triggering food cravings and over-eating. The current study was designed to probe if cues associated with a palatable sucrose solution potentiate feeding through Pavlovian (stimulus-reward) incentive motivation and to characterize cue-triggered changes in the microstructure of sucrose licking, focusing on measures associated with palatability (lick bout duration) and motivation (lick bout frequency). In the first experiment, food restricted rats were given Pavlovian conditioning to associate an auditory cue (CS+) with small aliquots of 20% sucrose solution located in a liquid well in one end of the behavioral chamber. A control cue (CS-) was not paired with sucrose delivery. After training, rats were shifted to a state of general satiety with ad lib access to home chow and given a pair of tests to characterize cue-triggered sucrose licking. Sucrose solution concentration varied across the two tests (2% vs. 20%, order counterbalanced). During each test, rats were given continuous access to sucrose, and the CS+ and CS- were noncontingently presented in pseudo-random order to assess their influence on sucrose licking. We found that the CS+ was effective in eliciting sucrose licking, an effect that was more prominent when 2% sucrose was available, which, consistent with a motivational interpretation, seems to be driven by an increase in the frequency of licking bouts during the CS+. To more directly assay the influence of Pavlovian incentive motivation on sucrose licking, we conducted an experiment identical to the first, except that sucrose solution was consumed from a distinctive bottle dispenser located on the opposite side of the chamber as the well used during Pavlovian conditioning. This ensured that previous stimulus-response habits were incompatible with sucrose licking at test, letting us attribute any cue-related increases in behavior to Pavlovian incentive motivation. We found that the effects of the CS+ on licking were most evident in the 2% sucrose test and were most clearly associated with increases in licking bout frequency rather than bout length. Additional testing examined the dependence of cue-triggered sucrose drinking on dopamine signaling, which is known to be critical for the expression of Pavlovian incentive motivation in other experimental settings. We found that pre-treatment with the D1 dopamine antagonist SCH-23390 abolished the sensitivity of sucrose licking to the CS+. These findings indicate that cues associated with palatable foods can trigger excessive food intake through a dopamine-dependent incentive motivational process.

Disclosures: **A.T. Liu:** None. **B. Halbout:** None. **S.B. Ostlund:** None.

Poster

255. Appetitive and Incentive Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant DA029035

NIH Grant DK098709

NIH Grant AG045380

Title: Modulation of cue-triggered reward seeking by cholinergic signaling in the dorsomedial striatum

Authors: *S. B. OSTLUND[1], A. T. LIU[1], B. HALBOUT[1], K. M. WASSUM[2], N. T. MAIDMENT[3];
[1]Dept. of Anesthesiol. and Perioperative Care, UCI Sch. of Med., Irvine, CA; [2]Psychology, UCLA, Los Angeles, CA; [3]Psychiatry and Biobehavioral Sci., UCLA Sch. of Med., Los Angeles, CA

Abstract: Reward-predictive cues acquire the ability to motivate and guide the selection of reward-seeking behaviors. This tendency for external cues to promote the pursuit of rewards - sometimes independently of, or even at odds with, more pressing physiological needs - is widely believed to contribute to over-eating and excessive drug and alcohol use. The influence of reward-paired cues on reward seeking can be studied in rats using the Pavlovian-to-instrumental transfer (PIT) task. Previous PIT studies have established that the dorsomedial striatum (DMS) is required for the expression of action selection based on cue-elicited reward expectations. While acetylcholine (ACh) is known to be an important modulator of local neurotransmission and cellular activity within the DMS, how this contributes to cue-evoked reward seeking is largely unknown. To investigate this issue, hungry rats were pre-trained using an outcome-specific PIT protocol known to depend on the DMS. At test, rats were allowed to freely perform two different lever-press actions, each associated with a different food reward. Cues that were separately trained with these rewards were noncontingently presented at test to assess their ability to influence reward-seeking behavior. Prior to testing, rats received bilateral intra-DMS injections of vehicle, mecamylamine (nicotinic ACh receptor antagonist), or scopolamine (muscarinic ACh receptor antagonist). We found that rats tested after vehicle treatment showed normal sensitivity to the outcome-specific influence of reward-paired cues, selectively increasing their performance of whichever action was associated with the reward they expected based on the current cue. Interestingly, nicotinic and muscarinic antagonists had distinct effects on PIT performance; whereas mecamylamine facilitated expression of PIT, scopolamine abolished this effect. These findings indicate that ACh signaling in the DMS modulates the expression of cue-triggered reward seeking, with its actions at nicotinic and muscarinic receptors playing opposing roles. Further study of the mechanisms underlying this behavior may guide efforts to treat disorders associated excessive reward seeking, like overeating and drug addiction.

Disclosures: S.B. Ostlund: None. **A.T. Liu:** None. **B. Halbout:** None. **K.M. Wassum:** None. **N.T. Maidment:** None.

Poster

255. Appetitive and Incentive Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: VU Summer Research Fellowship

Title: Temporal information is transferred in a general Pavlovian-Instrumental transfer task

Authors: R. B. DELLA VALLE, *M. S. MATELL;
Psychology, Villanova Univ., Villanova, PA

Abstract: The temporal relationship between stimuli plays an integral role in associative learning, but is not often studied using Pavlovian-Instrumental transfer designs (PIT). Previous work has shown that systematic variation of the time between a conditioned stimulus (CS) and unconditioned stimulus (US) mediates the timing of operant responses in sensory-specific PIT procedure (Delamater & Holland, 2008). The current experiments aimed to extend these results to general PIT, and then examine the involvement of the amygdala on such temporal information transfer. In two experiments, forty rats were trained on a Pavlovian delay conditioning procedure (CS-US interval 60s (Exp. 1) or 120s (Exp. 2)) using a house light and a 4 KHz tone as a CS+ and CS-, counterbalanced. Following training, temporal control was assessed using non-reinforced probe trials that lasted 3-4 times the CS-US interval. After temporal control was established, as evidenced by maximal responding around the time of the US on probe trials, subjects were trained to nosepoke on a low density reinforcement schedule. Finally, nosepoke responding was tested in extinction, and PIT was assessed by presenting the CS+ and CS- as probes. In both procedures, general PIT was seen as rats were found to increase response rates during the CS+ compared to the CS-. Importantly, maximal operant responding occurred at the CS-US interval learned in the Pavlovian phase of training. These results indicate that temporal knowledge can be transferred from a Pavlovian procedure to an instrumental procedure under general PIT conditions, thereby suggesting temporal modulation of arousal. A subsequent experiment, currently ongoing, aims to determine if the retrieval of temporal knowledge in testing is prevented by lesions of the central and basolateral amygdala, which have been previously shown to differentially mediate the general and outcome-specific forms of PIT (Corbitt and Balleine, 2005). The results of this experiment are expected to suggest whether or not temporal knowledge is mediated via the same pathway as the associative knowledge or whether it is generated via a parallel pathway.

Disclosures: R.B. Della Valle: None. **M.S. Matell:** None.

Poster

255. Appetitive and Incentive Learning and Memory I

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Topic: F.02. Animal Cognition and Behavior

Support: NIH Grant R01DA029035

Title: Effects of protracted withdrawal from repeated cocaine exposure on goal-directed learning

Authors: *B. HALBOUT[1,2], A. T. LIU[2], S. B. OSTLUND[2];
[1]UC Irvine, Irvine, CA; [2]Anesthesiol., Univ. of California Irvine, Irvine, CA

Abstract: Repeated exposure to drugs of abuse facilitates habitual learning processes that may contribute to compulsive drug taking and seeking. Recently, several studies have shown that exposure to pyschostimulants accelerate the onset of habitual control over food-seeking behaviors, possibly to the detriment of goal-directed control. The present study used behavioral tests that strongly implicate action-outcome learning and investigated more directly cocaine exposure effects on goal-directed actions. We tested whether prolonged withdrawal from repeated cocaine exposure alters goal-directed behaviors mediated by the representation of an outcome value associated with a particular action (outcome specific devaluation) and by the detection of a change in the causal relation between an action and its consequence (contingency degradation). Male Long Evans adult rats (n = 10 / group) received 5 or 15 days of daily cocaine (0, 15 or 30 mg/kg, i.p.). Following a 30-day withdrawal period they were trained to perform two different lever press actions for distinct food pellets (e.g., Left press - Grain & Right press - Chocolate). For the outcome specific devaluation test, rats were selectively satiated on one of the two pellets immediately before undergoing a test to assess their ability to flexibly adjust their choice between the two actions in accord with current goal values. During contingency degradation training one of the outcomes (Grain or Chocolate) was delivered non-contingently with the same probability regardless of whether the rat performed the appropriate lever-press response or not. An extinction test assessed how this contingency manipulation altered rats' choice between the two actions. Overall cocaine-treated rats did not differ from saline-treated rats in their ability to use up-to-date value assignments for expected outcomes when selecting between reward-seeking actions, suggesting preserved goal-directed control. Surprisingly, prolonged cocaine exposure facilitated the sensitivity of action selection to action-outcome contingency degradation. While further investigation should confirm the robustness of these effects, our findings indicate that withdrawal from chronic cocaine can actually promote processes that support certain flexible behavior.

Disclosures: **B. Halbout:** A. Employment/Salary (full or part-time):; University of California, Irvine. **A.T. Liu:** A. Employment/Salary (full or part-time):; University of California, Irvine. **S.B. Ostlund:** A. Employment/Salary (full or part-time):; University of California, Irvine.

Poster

255. Appetitive and Incentive Learning and Memory I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 255.24/X6

Topic: F.02. Animal Cognition and Behavior

Support: Natural Sciences and Engineering Research Council

Title: Effect of glycogen synthase kinase-3 inhibition on cocaine cue-conditioned activity

Authors: *J. F. ROCCA, R. J. BENINGER;
Psychology, Queen's Univ., Kingston, ON, Canada

Abstract: Exposure to contextual cues previously associated with reinforcers, such as drugs of abuse, can elicit drug craving in humans and approach and other responses in laboratory animals. Incentive learning is the process by which contextual cues gain the ability to elicit responses. Conditioned activity is a paradigm used to study incentive learning and to model drug craving in rodents. In this paradigm, rodents are given cocaine paired with a specific test environment (cocaine-paired), while control groups are given saline during testing and cocaine later in their homecage (cocaine-unpaired). When these groups are tested with saline-only in the test environment, paired animals display conditioned locomotor activity. Prior research has implicated dopamine D2-like receptors in the acquisition and expression of conditioned activity. D2-like receptor activity decreases the production of the second messenger, cyclic adenosine monophosphate, and consequently reduces protein kinase A activity. Recently, however, another cell-signaling pathway, the β-arrestin2/Akt/glycogen synthase kinase-3β (GSK-3β) pathway, has been shown to be affected by D2-like receptor activity. We investigated the potential role of GSK-3 in conditioned activity involving 3 days of cocaine (10 mg/kg)- or saline-paired exposure to the test environment followed by a test with saline for both groups. The selective GSK-3 inhibitor SB-216763 was given to Wistar rats during the conditioning phase or test day phase. Preliminary results show a trend towards a block of expression at lower doses than those needed to block acquisition of conditioned activity. Several more doses are currently being tested. Results may implicate the β-arrestin2/Akt/GSK-3β pathway more strongly in the expression than acquisition of incentive learning in some conditioning paradigms.

Disclosures: J.F. Rocca: None. **R.J. Beninger:** None.

Poster

256. Molecular Mechanisms of Memory Consolidation

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Topic: F.02. Animal Cognition and Behavior

Support: CNPq Grant 306468/2014-0

FAPERGS

PROAP-CAPES

Title: Improvement of memory reconsolidation induced by polyaminergic agents involves calcium-dependent protein kinase in rats

Authors: *M. A. RUBIN, B. A. GIRARDI, C. SIGNOR, D. A. RIBEIRO, C. F. MELLO; Federal Univ. of Santa Maria (UFSM), Santa Maria, Brazil

Abstract: The reactivation of a memory results in its destabilization, requiring a process of memory reconsolidation to maintain it. Spermidine is an endogenous aliphatic amine with polycationic structure that modulates N-methyl-D-aspartate (NMDA) receptor activity and improves memory. Recent evidence suggests that systemic administration of spermidine improves the reconsolidation of fear memory. In the current study we determined whether the calcium-dependent protein kinase (PKC) signaling pathway is involved in the improvement of fear memory reconsolidation induced by intrahippocampal (ih) administration of spermidine in rats. Male Wistar rats were trained in a fear conditioning apparatus using a 0.4 mA footshock as unconditioned stimulus. Twenty-four hours after training, animals were re-exposed to the apparatus in the absence of shock (reactivation session). Immediately after the reactivation session, spermidine (2 - 200 ρmol/site); the PKC inhibitor, 3-[1 (dimethylaminopropyl)indol-3-yl]-4-(indol-3-yl) maleimide hydrochloride (GF 109203X, 0.3 - 30 ρg/site); the antagonist of the polyamine-binding site at the NMDA receptor, arcaine (0.2 - 200 ρmol/site) or the PKC activator, phorbol 12-myristate 13-acetate (PMA, 0.02 - 2 nmol/site) were injected intra-hipocampally. Testing was carried out in the same apparatus, twenty-four hours after reactivation. Freezing scores at testing were considered a measure of memory. While the post-reactivation administration of spermidine (20 and 200 ρmol/site) improved, GF 109203X (1, 10 and 30 ρg/site) impaired memory reconsolidation. GF 109203X (0.3 ρg/site) prevented spermidine (200 ρmol/site)-induced improvement of memory reconsolidation. The post-reactivation administration of arcaine (200 pmol/site) impaired and the PMA (2 nmol/site) improved memory reconsolidation. PMA (0.2 nmol/site) prevented arcaine (200 ρmol/site)-induced impairment of memory reconsolidation. These drugs had no effect on memory if they were administered in the absence of reactivation. These results suggest that the enhancement of memory reconsolidation induced by the administration of spermidine involves PKC activation.

Disclosures: **M.A. Rubin:** None. **B.A. Girardi:** None. **C. Signor:** None. **D.A. Ribeiro:** None. **C.F. Mello:** None.

Poster

256. Molecular Mechanisms of Memory Consolidation

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Topic: F.02. Animal Cognition and Behavior

Support: ATIPE AVENIR (CNRS)

Emergence Paris

Title: Sleep-scoring in mice using gamma frequency in the olfactory bulb

Authors: ***S. BAGUR**, M. LACROIX, G. LAVILLEON, K. BENCHENANE;
ESPCI, Paris, France

Abstract: The growing interest in the physiology of various behavioral states demands reliable and systematic methods of wake and sleep stage scoring. Traditional methods identify sleep by monitoring movement or muscular activity, however if sleep and wake are truly different brain states the two should be identifiable using information on neuronal activity alone. It has been shown that gamma oscillations in the olfactory bulb (OB) of the mouse are strongly reduced during sleep (Manabe et al. 2013). Here, we show that low gamma power in the 50-70Hz band recorded from the OB shows a bimodal distribution that allows to separate sleep and waking states. Moreover rapid-eye-movement (REM) and non-REM (NREM) sleep can be distinguished using theta-band power (6-10Hz) recorded in the hippocampus (HPC). We therefore present a method of constructing a two-dimensional phase space allowing to distinguish between waking, REM and NREM sleep that relies exclusively on LFP recordings from the HPC and the OB. This method can be automatically calibrated and is robust from day to day and across multiple states of vigilance including task-driven behaviour. This technique allows for identification of brief periods of arousal and dozing, it is therefore a promising tool for the fine study of sleep microstructure.

Disclosures: **S. Bagur:** None. **M. Lacroix:** None. **G. Lavilleon:** None. **K. Benchenane:** None.

Poster

256. Molecular Mechanisms of Memory Consolidation

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Topic: F.02. Animal Cognition and Behavior

Support: National Honor Scientist Program of Korea

Title: Gene regulatory events in the hippocampus following the contextual fear conditioning

Authors: ***N.-K. YU**, H. KIM, J. CHO, J.-H. CHOI, S.-E. SIM, S. J. KANG, C. KWAK, J.-I. KIM, S. LEE, D. CHOI, N. V. KIM, B.-K. KAANG;
Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: A novel experience induces dynamic gene expression changes in the brain. The learned information through the experience is encoded as memory in the brain, and the memory becomes stable through processes called consolidation. It is well-known that memory consolidation requires the gene expression regulation. Therefore, to understand the molecular mechanism of how long-term memory is formed, it is of critical importance to map how gene expression is regulated following the learning. Although there have been extensive studies focusing on the regulation and action mechanism of individual genes during long-term memory formation, genome-wide landscape of gene expression following the learning process is still elusive. Through unbiased genomic analyses, we have identified previously undiscovered gene regulatory events and pathways in the mouse hippocampus following the contextual fear conditioning. With accompanied validation experiments, our analysis is thought to open a new window for investigating the molecular mechanism of learning and memory. N.-K. Yu and H. Kim contributed equally to this work.

Disclosures: **N. Yu:** None. **H. Kim:** None. **J. Cho:** None. **J. Choi:** None. **S. Sim:** None. **S.J. Kang:** None. **C. Kwak:** None. **J. Kim:** None. **S. Lee:** None. **D. Choi:** None. **N.V. Kim:** None. **B. Kaang:** None.

Poster

256. Molecular Mechanisms of Memory Consolidation

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Topic: F.02. Animal Cognition and Behavior

Support: FWF P 21930 – B09

Title: Chronic *in vitro* imaging in a H2B::Pa-GFP-expressing transgenic mouse model reveals experience-induced nucleus-scale remodeling of chromatin in neocortical neurons

Authors: ***S. RUMPEL**[1], D. ASCHAUER[1], F. GROESSEL[2], W. HAUBENSAK[2], M. PETER[3]; [1]Johannes Gutenberg Univ. Mainz, Mainz, Germany; [2]Res. Inst. of Mol. Pathology (IMP), Vienna, Austria; [3]Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Dynamic remodeling of gene expression patterns in response to behavioral experiences is believed to crucially contribute to the brain's ability to adapt the structure of neuronal networks in a dynamic manner and to mediate higher cognitive functions such as memory formation. A body of evidence for short- and long-lasting changes in expression patterns induced by experiences has been obtained in recent years, primarily highlighting regulatory processes at the molecular level. Furthermore, it is long known that neuronal activity can induce remodeling of neuronal chromatin also at the scale of the whole nucleus *in vivo* (1). However, most of this data stems from post-hoc analyses that cannot provide information on the

longitudinal dynamics. Here, we describe the generation and functional characterization of a novel transgenic mouse model conditionally expressing a fusion protein of H2B and a photoactivatable form GFP (PA-GFP). We take advantage of this mouse model to chronically label and image chromatin in neurons of the mouse auditory cortex *in vivo* using two-photon microscopy through a chronically implanted cranial window. We observed that photolabeled patterns on the chromatin remain stable over several hours in most neurons, while it appears dynamic in other neurons. Combining chronic imaging with classical auditory cued fear conditioning, we observe an increase in the fraction of neurons showing remodeling of chromatin, indicating an experience-dependent regulation of chromatin dynamics. We furthermore demonstrate in acute brain slices prepared from transgenic mice that pharmacological manipulation of neuronal activity impacts on the dynamics of chromatin on the time scales of few hours, similar to the observations *in vivo*. In summary, we establish a methodology to follow chromatin dynamics chronically *in vivo*. We furthermore provide a first longitudinal description of neuronal chromatin during behavioral procedures that have been previously shown to induce alterations in gene expression in the auditory cortex and the formation of sound-related memories (2). 1)Barr, M. L. & Bertram, G. E. (1949) Nature 163, 676-677 2)Peter, M. et al., (2012) Genes Brain Behav. 11:314-324

Disclosures: **S. Rumpel:** None. **D. Aschauer:** None. **F. Groessel:** None. **W. Haubensak:** None. **M. Peter:** None.

Poster

256. Molecular Mechanisms of Memory Consolidation

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Topic: F.02. Animal Cognition and Behavior

Title: The NMDA receptor antagonist (±)-3-(2-Carboxypiperazin-4-yl) propyl-1-phosphonic acid impairs consolidation, but not acquisition, of hippocampal spatial memory in the rat

Authors: *C. MARSHALL, D. C. GIDYK, R. J. MCDONALD;
Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: ***N-methyl-D-aspartate*** receptors (NMDAR) are a class of post-synaptic glutamate receptors located in the hippocampus, a brain area critical for spatial memory. A large body of evidence supports its role in the induction of synaptic plasticity and the acquisition of hippocampal memories. However, previous findings from our group have suggested that NMDARs may instead be involved in the consolidation of learned information into long term memory, as opposed to its acquisition. One caveat associated with our work was that only NMDA receptors in the dorsal hippocampus were blocked. The purpose of the current experiment was to determine whether NMDARs are involved in the acquisition or consolidation

of spatial information in the rat hippocampus when NMDA receptors are blocked across the entire septal/temporal poles of the structure. We used 16 Long Evans rats for each part of the experiment. Rats were given bilateral, dorsal and ventral hippocampal cannulations. After recovery, a 3 phase rapid acquisition version of the Morris water task was used to train and test the animals. This consisted of 4 days of spatial pre-training to a fixed hidden platform, intrahippocampal infusion of the NMDAR antagonist **(±)-3-(2-Carboxypiperazin-4-yl) propyl-1-phosphonic acid** (CPP) or saline followed by 2 hours of mass training to a new position, and a probe test. For part 1 of the experiment all procedures occurred in a single spatial context and for part 2 the mass training and probe test will occur in a different spatial context. Within the same spatial context, rats given saline before mass training learned the new platform position and showed a preference for this new position when given a probe 24 hours later. Rats given the CPP before mass training learned the new platform location but when given the probe test 24 hours later showed no preference for the quadrant it was located in. Contrary to the leading theory in the field, These results suggest that NMDARs in hippocampus are essential for consolidation of spatial information acquired in the spatial version of the water task but not acquisition.

Disclosures: C. Marshall: None. **D.C. Gidyk:** None. **R.J. McDonald:** None.

Poster

256. Molecular Mechanisms of Memory Consolidation

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UBACYT (B018; 2011– 2013 – 20020100100683 and 2014–2017 – 20020130100881BA)

Title: Memory reconsolidation of an inhibitory avoidance task in mice involves cytosolic ERK2 bidirectional modulation

Authors: *M. M. BOCCIA[1], M. C. KRAWCZYK[2], M. G. BLAKE[2], C. M. BARATTI[2], A. G. ROMANO[3], M. FELD[3];
[1]Univ. of Buenos Aires, Capital Federal, Argentina; [2]Cátedra de Farmacologìa, Facultad de Farmacia y Bioquìmica - UBA, Argentina; [3]Dto. Fisiología, Biología Mol. y Celular, Lab. de Neurobiología de la Memoria., Fac.Cs.Exactas y Naturales, UBA/ IFIByNE, CONICET, Argentina

Abstract: Reconsolidation has been defined as the process of memory stabilization after retrieval involving, among others, gene expression regulation and post-translational modifications. Many of these mechanisms are shared with memory consolidation. Here, we studied hippocampal ERK participation on memory reconsolidation of an inhibitory avoidance task in CF-1 mice. We found a retrieval-induced cytosolic ERK2 activation in the hippocampus (HIP) 15 min after memory reactivation, and an inhibition at 45 min. PD098059, a MEK1/2 (MAPK/ERK kinase) inhibitor, administered in the HIP immediately after retrieval impaired memory in a dose-dependent fashion. However, infusions of the highest dose of PD098059 performed 40 min after retrieval enhanced memory in mice trained with a weaker footshock. These results suggest for the first time that ERK2 is involved in memory reconsolidation in a biphasic fashion. Furthermore, the inhibition of ERK could either impair or enhance mice performance depending on ERK state of activation.

Disclosures: **M.M. Boccia:** None. **M.C. Krawczyk:** None. **M.G. Blake:** None. **C.M. Baratti:** None. **A.G. Romano:** None. **M. Feld:** None.

Poster

256. Molecular Mechanisms of Memory Consolidation

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Topic: F.02. Animal Cognition and Behavior

Title: Associative and affective contributions to context fear conditioning

Authors: L. M. TURNBULL, A. A. SCHMELING, E. J. DONZIS, B. R. SCHWARCZ, *N. C. TRONSON;
Dept. of Psychology, Univ. of Michigan, Ann Arbor, MI

Abstract: Two commonly used context and fear-associated conditioning paradigms show discrepancies in susceptibility to manipulations of memory reconsolidation, the process that maintains or updates memory after retrieval. There are several hypotheses as to why these protocols differ: differences in molecular mechanisms or brain regions activated after retrieval, or the different relative contribution of associative components and affective responses elicited at test. The goal of the current study was to directly compare the circuit and molecular pathways activated after retrieval in context fear conditioning (CFC) and inhibitory avoidance (IA), and directly manipulate the relative contributions of associative strength (ie, CS-US association) and affective load (ie. shock level) to each. We show that at low levels of shock (0.2mA), mice effectively learn to avoid a shock-paired context in inhibitory avoidance. However, in context fear conditioning, mice fail to show a robust freezing response. In contrast, at high levels of shock (0.8mA), we observed both avoidance in IA and freezing in CFC. Using immunohistochemistry for immediate early genes cFos, Arc, and Egr1 and the promiscuous

signaling kinase Erk/MAPK, we found similar patterns of activation after retrieval of either IA or CFC in dentate gyrus, CA3 and anterior cingulate cortex. In contrast, greater activation of amygdala, retrosplenial cortex, and CA1 occurred after retrieval of CFC compared with IA. Differences in brain regions and signaling pathways activated after retrieval of a high shock-intensity fear memory vs. low-intensity pairings suggest new potential targets for disrupting affective components of a memory. This may also pose novel treatments for disorders of emotion and memory, including Post-Traumatic Stress Disorder.

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Poster

256. Molecular Mechanisms of Memory Consolidation

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Title: Involvement of δCalcium/calmodulin-dependent protein kinase II in persistent forms of memory

Authors: *G. P. ZALCMAN[1], N. FEDERMAN[3], A. FISZBEIN[2], A. ROMANO[1];
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Abstract: Calcium/calmodulin-dependent protein kinase II (CaMKII) is an abundant synaptic signaling molecule that is essential for both memory formation and synaptic potentiation. In mammals, CaMKII exists in multiple isoforms that are the product of four closely related genes: α, β, γ, and δ. Little information is available on the role of the δCaMKII in memory processes. In a previous study, we showed that Camk2d gene promoter is acetylated in the mouse hippocampus one hour after strong novel object recognition (NOR) training and that its mRNA levels were specifically induced 3h post-training. Strong training induces long-term-memory (LTM) formation, which can be assessed up-to 7 days after training. In the present work, we carried out experiments aimed at determining the epigenetic regulation, expression and role of CamK2d on recognition memory. In the first place, we studied the Camk2d promoter and found

there was nucleosome remodeling during NOR memory consolidation and 7 days after NOR training. Secondly, we measured δCamKII mRNA expression outside the memory consolidation window, more specifically 24 hs and 7 days after training, and we found that mRNA expression levels for animals trained with a strong protocol were significantly increased when compared to a non-trained control group at both time points. Finally, to study the requirement of δCaMKII in recognition memory we knocked down δCaMKII expression with an oligodeoxynucleotide antisense to its mRNA 3 hs after strong training and tested the memory effect 24hs and 7days afterwards. We found that NOR memory was intact 24hs after training but it was impaired when assessed on day 7, indicating that δCamKII knock-down during memory consolidation specifically affects persistent forms of recognition memories. Altogether, our results support a key role for δCaMKII isoform in persistent forms of memory and suggest that Camk2d may have a sustained expression throughout the "lifetime" of this kind of memory. Furthermore, this is the first work that provides insight information about nucleosome remodeling during memory formation and maintenance.

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Poster

256. Molecular Mechanisms of Memory Consolidation

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Evelyn F. McKnight Institute

Civitan International

Title: H2B ubiquitination-dependent histone "cross-talk" is a critical regulator of synaptic plasticity and fear memory formation

Authors: *T. J. JAROME, W. M. WEBB, R. B. CREED, F. D. LUBIN;
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Abstract: Monoubiquitination of histones is a dynamic epigenetic controller of gene transcription changes in mammalian cells. Some evidence suggests that H2B monoubiquitination mediates "cross-talk" between histone methylation marks at gene promoters (Sun & Allis, 2002). However, while histone methylation has been shown to play critical roles in synaptic plasticity and memory formation, histone monoubiquitination has not been previously examined in this context. Here, we found that learning in a contextual fear conditioning (CFC) paradigm

transiently increased levels of H2B monoubiquitination at lysine 120 (H2BubiK120) in area CA1 of the hippocampus. Further experiments revealed accumulation of H2BubiK120 levels at gene promoters congruent with H3 lysine 4 trimethylation (H3K4me3) and H3 lysine 9 dimethylation (H3K9me2) histone methylation marks. Additionally, intra-CA1 siRNA-mediated knockdown of the ubiquitin E3 ligase for H2BubiK120, RNF20, prevented the learning-dependent increases in global and promoter-specific H2BubiK120 levels. RNF20 Knockdown also blocked H3K4me3 and H3K9me2 levels and altered mRNA expression of the H3K4me3 and H3K9me2 effector genes, c-fos and G9a respectively. This suggests that RNF20 mediated H2BubiK120 is involved in establishing histone methylation marks at gene promoters during CFC memory consolidation. Interestingly, we found that H2BubiK120 levels at the c-fos promoter was associated with increased binding of the 19S proteasomal subunit Rpt6, which has been shown to "bridge" H2B monoubiquitination to H3K4me3 marks in yeast (Ezhkova & Tansey, 2004). Intra-CA1 RNF20 knockdown impaired CFC memory and late, but not early, long-term potentiation (LTP), suggesting that H2BubiK120 was critical for activity-dependent synaptic plasticity and memory formation. Remarkably, the memory impairments resulting from RNF20 knockdown were completely rescued by simultaneous knockdown of the histone lysine-specific demethylase 1 (LSD1), which resulted in histone hypermethylation in the hippocampus. Collectively, these results provide the first evidence of histone monoubiquitination as a critical regulator of activity-dependent synaptic plasticity and memory formation, and suggest that H2B monoubiquitination dynamically regulates histone methylation cross-talk to mediate gene transcription control during long-term memory formation.

Disclosures: **T.J. Jarome:** None. **W.M. Webb:** None. **R.B. Creed:** None. **F.D. Lubin:** None.

Poster

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Title: Cognitive impairments caused by sleep deprivation is prevented by increasing protein synthesis in the hippocampus

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Abstract: Sleep loss produces deficits in hippocampus-dependent memory storage, but the molecular and cellular mechanisms that underlie these effects of sleep deprivation remain unclear. Several studies have suggested that signaling pathways associated with translation are altered during sleep and after periods of sleep deprivation. Here, we demonstrate that five hours of total sleep deprivation increases phosphorylated AMP-activated protein kinase (AMPK) alpha, reduces mTOR complex 1 (mTORC1) and reduces phosphorylated eukaryotic translation initiation factor 4E binding protein 2 (4EBP2), which subsequently leads to impaired protein synthesis in the hippocampus. However it is yet to be determined whether restoring protein synthesis in the hippocampus is sufficient to prevent the cognitive deficits associated with sleep deprivation. Viral expression of 4EBP2 selectively in hippocampal excitatory neurons in mice that were sleep deprived for five hours increased phosphorylated 4EBP2 levels, which was sufficient to restore hippocampal protein synthesis to non-sleep deprivation levels. Furthermore, viral expression of 4EBP2 prevents the memory deficits associated with sleep deprivation in the object place recognition task. These findings indicate that AMPK-mTORC1-4EBP2 signaling and subsequent impaired protein synthesis is the critical component underlying the memory deficits associated with sleep deprivation in hippocampus-dependent learning tasks. Furthermore, this study defines the molecular mechanism by which loss of sleep impairs cognitive processes and highlights a vital role for protein synthesis and mTOR signaling on long-term memory formation.

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Poster

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Support: CONACYT 155242

PAPIIT IN209413/24

Title: Dopamine in hippocampus is required for spatial novelty detection and consolidation of an updated memory

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Abstract: The ability to identify objects in a particular context and differentiate the experience of a previous familiar configuration from a novel configuration depends largely on the maintenance of such spatial information of the object and the successful discrimination between actual experience and the internal representation of the environment. It has been hypothesized that this ability depend mostly on the hippocampal formation particularly on CA1 region on which occurs the match-mismatch between the previous spatial information and the current novel configuration. Also, it's known that the CA1 region receives dopamine projections, this projections seem to play a role in the novel context detection. In the present study was evaluated the dopamine role during an context memory update model for the novelty detection as for the consolidation of the new context. For this study were used 4 weeks old Balb/c mice cannulated in the dorsal hippocampus. For the memory updating it was used the Object Location Memory protocol, consisting on a two day for the acquisition of two different objects, on the third day,in which memory update occurs, one of the objects was displaced to a novel position, finally on the fourth day the updated memory was evaluated by repeating the third day configuration. The role of dopamine function was evaluated by the D1-D5 antagonist SCH 23390 administration prior the novelty context presentation or immediately after. The results indicate that dopamine activity is required for both, novelty detection and for the consolidation of the updated memory.

Disclosures: D. Avila Aguirre: None. **F. Bermudez Rattoni:** None.

Poster

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Title: The essential role of BAF53b in learning-related synaptic structural plasticity and long-term fear memory formation in the lateral amygdala

Authors: *M. YOO[1], K.-Y. CHOI[2], J. SHIM[3], J.-H. CHOI[3], J. KIM[1], J. OH[1], B.-K. KAANG[3], J.-H. HAN[1];
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Abstract: Epigenetic regulation of gene expression has been implicated in long-lasting memory formation. However, little is known about the role of chromatin remodeling mechanism in learning and memory. Recent study reports that BAF53b, a post-mitotic neuron specific subunit of Brg/Brm-associated factor (BAF) chromatin remodeling complex is involved in hippocampus-

dependent long-term memory formation. However, whether BAF53b plays a role for long-term fear memory formation in the lateral amygdala (LA), a key brain site for fear memory storage, and its underlying mechanisms remain unknown. To address these issues, we used viral vectors to manipulate the expression level of BAF53b specifically in the LA neurons during memory formation and investigated its effects on learning-related synaptic structural plasticity and long-term fear memory formation using auditory fear conditioning paradigm. First, we found that BAF53b knockdown in the LA neurons impaired long-term, but not short-term, fear memory formation, proving that BAF53b function in the LA is essential for long-term fear memory formation. Second, transient BAF53b overexpression in the LA enhanced long-term memory up to one month with no effect on short-term memory. Third, at the synapse level, confocal imaging of synaptic spines, which was visualized with GFP fluorescence or dye injection, in the LA brain sections after fear learning revealed that the density of thin-type spine was specifically increased in BAF53b-overexpressing LA neurons one hour after auditory fear conditioning compared to GFP expressing control neurons, while head volume of mushroom-type spine in BAF53b knockdown neurons was specifically smaller compared to that in control neurons 24 hours after auditory fear conditioning. In parallel to this result, our RT-PCR analysis showed that BAF53b knockdown blocked activity-dependent upregulation of miR132, which is known to be involved in regulation of activity-dependent spine enlargement. Taken together, our results demonstrate that BAF53b in the LA is essential for long-term fear memory formation of auditory fear conditioning and reveal novel underlying mechanisms of BAF53b during long-lasting memory formation.

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Poster

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Title: The co-repressor SIN3A is a novel transcriptional regulator of Homer1/mGluR5 signaling during memory consolidation

Authors: H. SCHOCH[1], M. S. BRIDI[2], C. FLORIAN[5], S. G. POPLAWSKI[3], G. S. PORCARI[4], *T. ABEL[1];

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Abstract: Long-term memory consolidation is a tightly regulated process that requires de novo gene expression and protein synthesis. Histone deacetylases (HDACs) have been well studied as negative regulators of memory consolidation, and pharmacologically relieving HDAC-mediated repression elicits robust long-term memory enhancements in multiple rodent learning paradigms. In contrast, little is known about the transcriptional repressor complexes that localize HDACs to their target genes. One co-repressor, SIN3A, is a strong candidate memory regulator that forms a complex with HDAC1 and HDAC2 and represses transcription via interactions with MECP2, REST, MEF2, and NCOR. To elucidate the role of SIN3A in memory consolidation, we generated conditional SIN3A neuronal hypomorph (Sin3aNH) mice with reduced expression of SIN3A in forebrain excitatory neurons. Here we show that Sin3aNH animals have enhanced long-term memory for contextual fear and enhanced hippocampal synaptic plasticity. We identified Homer1 as a gene target of SIN3A, and showed that Sin3aNH animals show activity-dependent upregulation of Homer1 transcripts in the hippocampus, increased localization of Homer1b/c and mGluR5 to the post-synaptic density, and expanded mGluR5 activity. These studies support a role for SIN3A as a novel memory repressor molecule that regulates synaptic plasticity and memory consolidation via Homer1/mGluR5 signaling.

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Poster

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Title: Hippocampal BDNF-dependent mechanisms mediate the formation of long-lasting latent memory traces during the infantile amnesia period

Authors: *A. TRAVAGLIA, R. BISAZ, C. M. ALBERINI;
New York Univ., New York, NY

Abstract: Aversive experiences in early life profoundly affect the neural systems critically involved in cognition and emotions. However, in adulthood, there is very little conscious recollection of episodic experiences that occurred during the early period of life, a phenomenon

known as infantile amnesia. This points to the paradox of how early memories can be so powerful if they cannot be remembered. We found that rats trained in inhibitory avoidance (IA) at postnatal day (PN) 17, which corresponds to early childhood in humans, acquire and express memory immediately after training, but they lose it rapidly and lack long-term memory 1 and 7 days after training, recapitulating the phenomenon of infantile amnesia. However, a later reactivation (up to a month after training) of the trace with both context exposure and footshock presented in a temporally unpaired manner, which per se does not evoke IA response, produces a significant memory reinstatement. The memory is context-specific. These data suggest that reminders occurring later in life can reinstate an early life memory trace that remained latent for a long time. The reinstatement of memory does not occur if the dorsal hippocampus is inactivated with muscimol at the time of training, whereas the same inactivation at the time of the reminders has no effect. Quantitative western blot analyses of dorsal hippocampal extracts indicated that training at PN17 leads to the activation of the BDNF/TrkB pathway and bilateral injection of blocking antibody to BDNF (anti-BDNF) into the dorsal hippocampus at the time of training prevents memory reinstatement, suggesting that dorsal hippocampal BDNF is induced and required for the formation of a latent memory trace. We conclude that BDNF-dependent mechanisms in the hippocampus are required for the formation of latent memory traces during the period of infantile amnesia. These traces are stored long-term, despite the fact that memories are not expressed; however, memories can be reinstated at later times by reminders.

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Poster

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Title: Sex differences in generalization and molecular mechanisms of context fear conditioning

Authors: ***A. A. SCHMELING**, E. J. DONZIS, L. M. TURNBULL, N. C. TRONSON; Univ. of Michigan, Ann Arbor, MI

Abstract: Prevalence for disorders of fear and anxiety are more than twice as common in women compared with men, possibly due to sex differences in learning about trauma-related contexts and cues. Supporting this view, studies of both human fear conditioning and animal models have demonstrated differences between males and females in models of fear conditioning. Such sex differences in fear-related memories may emerge from several distinct processes including learning about context, learning about the aversive event, the association of

context and shock, or differences in the modulatory effects of aversive stimuli on memory formation. Here we aimed to determine whether differences in context learning in males versus females contribute to sex differences in context fear conditioning. To do this, we examined generalization of fear after context fear conditioning from the training context to a similar context in male and female mice. We demonstrated that females, but not males, show generalization of fear responses in a similar context, suggesting sex differences in how - and what - information is learned during context fear conditioning (CFC). Pre-exposure to the training context 24 hours before CFC alleviated generalization of fear in females, but only under some conditions. These data demonstrate stronger context learning in males compared with females, and imply that females use an alternative strategy for learning CFC. To determine activation of brain regions mediating fear conditioning in males and females, we examined activation of immediate early genes (IEGs). We observed similar activity in the hippocampus and amygdala in males and females after context fear conditioning. However, in the pre-exposure + CFC group males showed significantly lower activation in hippocampus compared with CFC only group, whereas there was no difference in females. In contrast, females in the pre-exposure + CFC group showed significantly reduced basal amygdala IEG activity compared with the CFC only group. Overall, these data suggest that males and females differentially utilize context information in context fear conditioning memories. Broadly, these findings highlight the concept that males and females may rely on different neural mechanisms even when behavioral outcomes are similar.

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Poster

256. Molecular Mechanisms of Memory Consolidation

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Topic: F.02. Animal Cognition and Behavior

Support: ATIPE AVENIR (CNRS)

Emergence Paris

Title: Characterizing sub-stages of Non REM sleep: importance for memory consolidation

Authors: ***M. M. LACROIX**[1], S. BAGUR[2], K. BENCHENANE[2];
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Abstract: Non Rapid-Eye-Movement Sleep (NREM) is characterized by different patterns of oscillations that are conserved from humans to rodents (Iber et al. 2007): thalamo-cortical spindles (8-14Hz), hippocampal sharp-wave ripples (SPW-Rs, 150-200Hz) and cortical slow

oscillations including delta waves (cortical down state <1Hz) and delta rhythm (1-4Hz). Accumulation of evidence shows the importance of those patterns in memory consolidation. Indeed, SPW-Rs have been shown to support neuronal reactivations in hippocampus, replaying previous experience after spatial learning in rodents, and are necessary for memory consolidation during sleep (Girardeau, Benchenane et al. 2009; de Lavilléon, Lacroix et al. 2015). Slow oscillations and spindles are increased after declarative learning in humans and rodents, and this increase is correlated with subsequent performances at memory recall (Genzel et al. 2014). Accordingly, artificial increase of slow oscillations in humans improves memory performance and is associated with an increase in spindles occurrence (Marshall et al. 2006). This suggests that coordination between rhythms might be crucial for memory consolidation, as hypothesized by many studies characterizing the time relationship of those patterns. Yet, if a relationship between these rhythms is regularly found in various studies, the precise relationship between them fluctuates. We hypothesized that this discrepancy might be influenced by the different NREM stages that cannot be distinguished easily in rodents. Indeed, although different sleep stages are well characterized in humans, only few attempts to differentiate between several phases in rodents were reported (Bergmann et al. 1987, Gottesmann et al. 1992). Using chronic recordings of the local field potential at multiple brain sites together with tetrodes recording of neuronal spiking activity in the prefrontal cortex in mice, we were able to clearly establish three sub-stages of NREM: 1) a light sleep, characterized by low occurrence of delta waves and few spindles, 2) a sparse slow wave sleep, characterized by the presence of spindles and some delta waves, and 3) a dense slow wave sleep, characterized by presence of spindles and very high density of delta waves. SPW-Rs activity followed the profile of spindles and not delta waves'. These sub-stages were very reproducible across animals in terms of duration and patterns distribution. In accordance with previous study, this new approach will allow to further decipher the role of each oscillatory pattern in memory processes.

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Title: Promoter specific effects of DREADD modulation on synaptic plasticity and hippocampal learning

Authors: *A. J. LOPEZ[1], E. KRAMAR[2], J. KWAPIS[3], A. O. WHITE[3], D. MATHEOS[3], A. VOGEL-CIERNIA[4], M. WOOD[3];
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Abstract: Chemogenetics can lead to a dramatic change in how neural circuits are studied and understood. Particularly, Designer Receptors Exclusively Activated by Designer Drug (DREADDs) are a novel tool with the potential to bi-directionally drive cellular, circuit, and, ultimately, behavioral changes. These receptors have no endogenous ligand and can only be activated with exposure to clozapine-n-oxide (CNO), which is otherwise an inert ligand. To examine how this technique can be used to manipulate the activity of specific cell populations, we used this chemogenetic system to test memory formation in a hippocampus-dependent task. Here, we use DREADD technology to show the explicit role of the CA1 hippocampal subfield in object location memory (OLM), but not object recognition memory (ORM) formation. Excitatory DREADD (HM3D), inhibitory DREADD (HM4D), or GFP control were expressed virally in the CA1 subfield of the hippocampus. The lab has previously shown that for both OLM and ORM tasks, a 3 minute training session is a subthreshold event for long-term memory (LTM) formation, while a 10 minute training session is sufficient for LTM formation. For activation experiments, mice were given a 3 minute training session in the OLM and ORM tasks paired with systemic CNO administration. For inactivation experiments, mice were given a 10 minute training session in the OLM and ORM tasks paired with system CNO administration. Mice were tested for LTM 24 hours following each respective training event. Compared to control animals, HM3D mice showed dramatic increase in LTM formation in the OLM task, but not ORM task. In contrast, HM4D mice showed LTM impairments in the OLM task, but showed no such impairments in LTM formation for the ORM task. However, DREADDs expressed under the hSyn promoter led to an inverse electrophysiological phenotype, compared to DREADDs expressed under the CaMKIIα promoter. Together, these experiments show a novel way of modulating LTM formation in behaving animals while further demonstrating hippocampal dependence in OLM but not ORM task LTM formation. Moreover, this provides evidence for promoter-specific effects on neural circuit function.

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Support: NRF-2013R1A1A1006766

NRF-2014R1A3A1063542

Title: Expression of a Noonan syndrome-associated mutant PTPN11 in excitatory neurons impairs learning

Authors: M. KANG[1], T. KIM[2], H.-H. RYU[1], A. J. SILVA[3], B.-K. KAANG[2], *Y.-S. LEE[1]; [1]Chung-Ang Univ., Seoul, Korea, Republic of; [2]Seoul Natl. Unviersity, Seoul, Korea, Republic of; [3]Univ. of California Los Angeles, Los Angeles, CA

Abstract: Noonan syndrome (NS) is a neurodevelopmental disorder highly associated with congenital heart disease, short stature, facial abnormalities and cognitive deficits such as learning disabilities. Mutations in *Ptpn11* which encodes SHP-2, a positive regulator for Ras-Erk signaling are responsible for ~ 50% of NS cases. Previously, we showed that the mutant mouse harboring NS-associated mutant *Ptpn11* show enhanced excitatory synaptic function, suggesting that the imbalance between excitatory and inhibitory synaptic transmission could be the cellular mechanism underlying learning and memory deficits in the NS mouse models. Here, we examined the effect of cell type-specific ectopic expression of a NS-associated mutant SHP-2 on spatial learning and memory in adult mice. We infused floxed adeno-associated viral vector containing a NS-associated mutant *PTPN11* into the hippocampal CA areas of neuronal cell type-specific Cre mouse lines. We found that overexpressing SHP-2^{D61G} in the hippocampal excitatory neurons, but not in the inhibitory neurons impairs spatial learning and memory, demonstrating that deregulation of SHP-2 signaling cascade in the excitatory neuron is responsible for the learning deficits in NS mouse models. Our results also show that SHP-2 in the excitatory neuron is critically involved in hippocampus-dependent learning and memory.

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Poster

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Support: NIH Grant R01NS073974

Title: Temporal profile of BDNF-dependent protein synthesis requirement for rat hippocampal memory consolidation: A computational analysis

Authors: *Y. ZHANG, P. SMOLEN, D. A. BAXTER, J. H. BYRNE;
Neurobio. & Anat., Univ. Texas, Houston, TX

Abstract: Long-term memory (LTM) is labile to disruption for up to two days after acquisition, after which it is more resistant to disruption and remains intact for prolonged time periods (days to lifetimes). The dynamical properties of the mechanisms that underlie consolidation are not well understood. To gain insights into molecular underpinnings of consolidation, we developed a differential-equation based model of key regulatory elements including BDNF, CREB, and C/EBP that underlie consolidation. To the extent possible, the model was constrained by data from an empirical study (Bambah-Mukku et al., J Neurosci 34:12547, 2014). These data suggest that a positive BDNF-autoregulatory feedback loop mediates hippocampal memory consolidation following inhibitory avoidance (IA) training. Interesting features of the data are a significant delay of bdnf transcription and a relatively abrupt termination of the feedback loop at or about 48 h. To examine the role(s) of feedback, the model was driven by a stimulus analogous to IA training. Sustained increases in the levels of phosphorylated CREB and C/EBP represented consolidation of LTM. The model simulated the complete time courses of transcription and translation for BDNF and the time courses of CREB phosphorylation and C/EBP expression after training, and fit the empirical measurements at 30 min and at 6, 12, 20 and 48 h after training. Simulations showed two waves of increased BDNF activity, which were accompanied by two phases of CREB phosphorylation and C/EBP expression. These simulations suggest that the first phase of CREB phosphorylation and C/EBP expression was independent of the feedback loop. The second phase was induced by the delayed increase of bdnf transcription. To simulate this delayed increase it was necessary to hypothesize that the same transcriptional repressors implicated in the termination of the loop at 48 h (Bambah-Mukku et al., 2014) are also active initially to inhibit the loop and then are inactivated, allowing closure of the positive feedback. Therefore we predict that the delayed initiation and termination of the BDNF feedback loop are controlled by the biphasic regulation of bdnf transcription by a group of transcriptional repressors. We also predict a time window during which late CREB phosphorylation and C/EBP expression is required for developing resistance of IA memory to disruption.

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Poster

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Support: Defense Threat Reduction Agency (DTRA)

Title: The Role of IL-1 signaling in behavior and neurodegeneration after soman (GD) exposure

Authors: *T. M. FERRARA-BOWENS[1], J. CHANDLER[1], J. IRWIN[1], K. LAITIPAYA[1], L. SHUMWAY[1], M. WEGNER[2], E. A. JOHNSON[1];
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Abstract: Chemical warfare nerve agent (CWNA) exposure can initiate status epilepticus (SE), which can lead to severe neuropathology and behavioral impairment. 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 inflammatory cytokines, including the pro-inflammatory cytokine interleukin (IL)-1. This creates a positive feedback loop to exacerbate neurodegeneration and to damage potentially healthy neuronal tissue. Inhibition of neuroinflammation reduces acute neurodegeneration in various CNS injury models and may also be beneficial following CWNA exposure. 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. Results showed that the WT and IL-1Ra KO mice were more hyperactive and anxious compared to the IL-1R1 KO mice, and that the absence of IL-1 signaling may attenuate anxious behavior. There was also progressive brain damage in the amygdala and hippocampus between 24 hours and 25 days of exposure. In addition, the IL-1 signaling inhibitor anakinra produced post-exposure neuroprotection in these brain regions. 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.

Disclosures: T.M. Ferrara-Bowens: None. **J. Chandler:** None. **J. Irwin:** None. **K. Laitipaya:** None. **L. Shumway:** None. **M. Wegner:** None. **E.A. Johnson:** None.

Poster

256. Molecular Mechanisms of Memory Consolidation

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Topic: F.02. Animal Cognition and Behavior

Support: CIHR 74650

CIHR 130522

Title: Learning regulates the mRNA demethylase fto and mRNA methylation

Authors: *B. J. WALTERS, V. MERCALDO, P. W. FRANKLAND, S. A. JOSSELYN;
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Abstract: Translation of mRNA into proteins is vital for memory formation, as evidenced by local, spine-specific mRNA translation occurring in response to synaptic stimulation and the inhibition of memory expression produced by the administration of translational inhibitors. Although the role of translation in memory is well accepted, the actual mechanism that underlies local control of translation is poorly understood. In this study, we explored whether mRNA methylation is involved in regulating learning-induced translational control, providing a first test of this novel mechanism in learning and memory. RNA can be heavily modified, with the most abundant modification being the methylation of adenine, but technical difficulties involved in interrogating this modification have left its function elusive for nearly 5 decades. Two recent advances brought RNA methylation back into the spotlight and have begun to elucidate the potential roles of mRNA methylation in particular. First, the identification of the first mRNA demethylase Fto and its link to human obesity and cognitive decline demonstrated diseases which can be influenced by dysregulation of RNA methylation. Second, the advent of next generation sequencing allowed the potential functions of RNA methylation to be investigated. Using these techniques, a plethora of potential functions of RNA methylation were identified, with its most promising role being the potential to control mRNA translation. Specifically, mRNA methylation dictates the incorporation of mRNA into P-bodies that store or degrade mRNA, providing a probable mechanism for translational control. The ability to locally control translation via mRNA methylation makes this modification particularly enticing for regulating synaptic activity, where local control of translation in synapses is vital for synaptic plasticity. Here, we provide the first direct exploration of the role of mRNA methylation and the mRNA demethylase Fto in cognition. Using contextual fear conditioning in mice, we demonstrate that associative learning dynamically regulates Fto expression and mRNA methylation in area CA1 of the dorsal hippocampus. This regulation appears to be specific for Fto as Alkbh5, the other identified mRNA demethylase, is unchanged. Similarly, even though every type of RNA can be methylated, it appears that only mRNA is differentially methylated after learning. These studies represent the first attempt to bridge the gap between transcriptional control and the local translational control in learning and memory, thus identifying an essential missing link and a new layer of regulation in learning and memory.

Disclosures: B.J. Walters: None. **V. Mercaldo:** None. **P.W. Frankland:** None. **S.A. Josselyn:** None.

Poster

256. Molecular Mechanisms of Memory Consolidation

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Title: NF-kappaB regulates DNA demethylation in the hippocampus via Gadd45beta during fear memory formation

Authors: *F. D. LUBIN, T. J. JAROME, A. A. BUTLER, N. L. PACHECO, J. N. NICHOLS; Dept. Neurobiol, Univ. Alabama Birmingham, Birmingham, AL

Abstract: Experience-driven learning triggers critical mechanisms for turning genes on and off in neurons across several brain regions during long-term memory formation. In addition to transcription factors, epigenetic mechanisms such as DNA methylation and ***de***methylation have emerged as critical regulators of these gene expression changes required for memory "consolidation" or formation. While several mechanisms have been identified for DNA methylation events during memory formation, very little is known about how the DNA ***de***methylation process is regulated as a function of learning. Recently, Gadd45β has been identified as a potential mechanism for regulation of active DNA ***de***methylation in the hippocampus during memory consolidation; however how *Gadd45β* activity is regulated remains equivocal. Here, we found that learning in a contextual fear conditioning (CFC) paradigm increased *Gadd45β*, but not *Gadd45α* or *Gadd45γ*, gene expression in area CA1 of the hippocampus. The Gadd45β gene contains several κB consensus sequences for the nuclear factor kappa B (NF-κB) transcription factor, a critical regulator of memory formation. Thus, we found that pharmacological inhibition of NF-κB prevented the learning-induced increases in *Gadd45β* mRNA levels and decreased DNA methylation at the learning permissive gene, *Bdnf*, suggesting that NF-κB is involved in the regulation Gadd45β expression and DNA ***de***methylation events during the memory consolidation process. Next, we determined the subunit make-up of the active NF-κB dimer complex involved in Gadd45β-mediated DNA ***de***methylation. We found that while conditional *relA/p65* mutations in pyramidal neurons impaired hippocampus-dependent memory formation in the CFC learning paradigm, we observed no alterations in Gadd45β mRNA levels in area CA1. Intriguingly, in *c-rel -/-* mice, learning failed to induce increases in Gadd45β mRNA levels in the hippocampus and prevented increases in Gadd45β protein levels at the *Bdnf* gene and subsequent *Bdnf* DNA ***de***methylation. Congruently, intra-CA1 region specific *c-rel* knockdown prevented Gadd45β expression and *Bdnf* DNA ***de***methylation following CFC learning in adult animals. Collectively, these results identify a novel role for activity-dependent NF-κB in the process of DNA ***de***methylation events in hippocampal neurons during fear memory formation and nominate Gadd45β as a target effector of NF-κB in this process. Together, these studies further increase our understanding of epigenetic DNA ***de***methylation mechanisms necessary for fear memory processing, which will have broad relevance for the treatment of phobias and anxiety.

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Poster

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Title: HDAC3: An epigenetic key to ameliorating synaptic plasticity and memory impairments in the aging brain

Authors: *J. L. KWAPIS, Y. ALAGHBAND, E. A. KRAMÁR, D. P. MATHEOS, D. RHEE, A. J. LOPEZ, M. A. WOOD;
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Abstract: Aging is accompanied by cognitive impairments, including difficulty forming long-term memories. Long-term memory formation requires gene expression, a process that may be disrupted with age (Rowe et al., 2007; Berchtold et al., 2008). Epigenetic alterations (changes in gene expression that occur through alterations in chromatin structure) may therefore contribute to age-related impairments in both gene expression and long-term memory. One major epigenetic mechanism important for memory is histone acetylation, in which acetyl groups are added or removed from histone tails by acetyltransferases (HATs) or histone deacetylases (HDACs), respectively. Increasing histone acetylation by blocking HDAC activity generally enhances both gene expression and long-term memory. In particular, histone deacetylase 3 (HDAC3) appears to be a key negative regulator of long-term memory formation, as blocking HDAC3 produces persistent object location memory following subthreshold training. Here, we tested whether HDAC3 activity also contributes to age-related impairments in synaptic plasticity and long-term memory. We hypothesized that dysregulated HDAC3 activity in the aging brain contributes to an unusually repressive chromatin structure that limits synaptic plasticity and memory formation. To test this, we focally deleted HDAC3 in the dorsal hippocampi of aging (18-month-old) mice before training them in the hippocampus-dependent object location memory (OLM) task. As predicted, aging wild type mice showed severe impairments in long-term memory for OLM. Deleting HDAC3 in the hippocampus rescued this deficit; 18-month-old mice lacking hippocampal HDAC3 showed robust long-term memory. This suggests that HDAC3 negatively regulates hippocampus-dependent memory formation in the aging brain. To test whether HDAC3 also limits synaptic plasticity, we next examined long-term potentiation (LTP) in hippocampal slices from 18-month-old mice. A single train of 5 theta bursts failed to produce stable LTP in

these slices, suggesting that LTP, like memory, is impaired with age. Blocking HDAC3 activity with a dominant negative mutant (HDAC3Y298H), however, ameliorated LTP deficits, resulting in a robust and stable potentiation. Together, our results indicate that HDAC3 is a key negative regulator of both long-term memory and synaptic plasticity in aging mice. We are currently working to understand how gene expression dynamics downstream of HDAC3 change with age to result in these impairments in synaptic plasticity and long-term memory.

Disclosures: **J.L. Kwapis:** None. **Y. Alaghband:** None. **E.A. Kramár:** None. **D.P. Matheos:** None. **D. Rhee:** None. **A.J. Lopez:** None. **M.A. Wood:** None.

Poster

256. Molecular Mechanisms of Memory Consolidation

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Support: DC00769

Title: Extension of the sensitive period for imprinting in chickens by manipulating experience-dependent translation initiation

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Abstract: The brain can be transiently sensitive to learning during short periods of time, known as sensitive periods (SP). Extending SPs may thus permit behavioral plasticity beyond early stages in life. Experience-dependent protein synthesis underlying long-term memory formation is a potential target for SPs extension. We tested this idea using imprinting in chickens as a learning task. We trained chickens to recognize an object paired to a sound on a computer screen, to test visual and auditory imprinting. Using Western Blotting and pharmacology we investigated whether eIF2α and mTORC1, which can independently regulate translation initiation, affect imprinting. Non-phophorylated eIF2α, which enhances translation initiation, was increased in the area involved in auditory imprinting but not in the site for visual imprinting. Accordingly, manipulation of this pathway either blocked or triggered consolidation of auditory, but not visual, memory. Notably, facilitating translation initiation through eIF2α also extended the critical period for auditory imprinting. The mTORC1 pathway was activated in both visual and auditory areas. However, blocking mTORC1 with Rapamycin only disrupted the formation of visual memories. In addition, we found that thyroid hormones, which can extend the critical period for visual imprinting, induced mTORC1 activation. Together, these data strongly suggest that the formation of auditory and visual long-term memory is mediated by two independent

pathways that regulate translation initiation. Furthermore, manipulating these pathways can extend the SP for imprinting in each sensory modality.

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Poster

256. Molecular Mechanisms of Memory Consolidation

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Topic: F.02. Animal Cognition and Behavior

Title: The effects of post-acquisition blockade of hippocampal NMDA receptor subunit GluN2A and/or GluN2B on spatial memory forgetting

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Abstract: Many studies have demonstrated that the hippocampal N-methyl-D-aspartate type glutamate receptors (NMDARs) are involved in the acquisition of spatial memory. However, we have repeatedly shown that the antagonism of the NMDARs suppresses “forgetting,” which is defined as the deterioration of a previously acquired memory. Here, we investigate the roles of the GluN2A and GluN2B NMDAR subunits in forgetting using the Morris water maze place task. Thirty-one rats were divided into four groups: artificial cerebrospinal fluid infused (aCSF; n = 8), NVP-AAM077 (the GluN2A selective antagonist) infused (NVP; n = 8), Ro 25-6981 (the selective GluN2B antagonist) infused (Ro; n = 7), or joint NVP/Ro infused (NVP+Ro; n = 8). Following four days of training (four trials per day), each rat received a chronic infusion into the bilateral dorsal hippocampus for 5 days using Alzet osmotic pumps (0.5 μl per hour). In the first probe test, which was delivered 7 days after the end of the acquisition training, both NVP-infused groups (i.e., NVP and NVP+Ro) spent significantly more time in target quadrant than the other two groups and compared to the chance level (25%). In the second probe test, which was delivered 24 hours later, only the NVP group spent significantly more time in the target quadrant compared to chance. In summary, the post-acquisition blockade of the GluN2A subunits enhances spatial memory retention, which suggests that hippocampal GluN2A-containing NMDARs play an important role in forgetting of a previously acquired spatial reference memory. In addition, the blockade of the GluN2B subunits suppresses the facilitative effect of the GluN2A antagonist, as indicated by the results of the NVP+Ro group during the second probe test.

Disclosures: **K. Shinohara:** None. **T. Hata:** None.

Poster

256. Molecular Mechanisms of Memory Consolidation

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Title: Correlation analysis between remote memory and CREB phosphorylation in cerebral cortex using *in vitro* imaging

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Abstract: Cyclic adenosine monophosphate response element binding protein (CREB) is a transcription factor that is considered important for memory consolidation and recovery process of depression. Once serine 133 of CREB is phosphorylated, kinase inducible domain (KID) of CREB binds to KIX domain of CREB binding protein, and the downstream gene expression is induced. However, the spatiotemporal pattern of the phosphorylation of CREB *in vivo* has not been fully analyzed because of technical difficulty. We employed the split luciferase technique to monitor the phosphorylation of CREB in live animals. In this technique, firefly luciferase was cleaved into N-terminal and C-terminal segments. The KID and KIX domains were fused with N-terminal and C-terminal segments of luciferase, respectively. By the interaction between these two fusion proteins mediated by serine 133 phosphorylation of KID, split segments complement each other to be a functional luciferase that can emit light. After we confirmed that these probe proteins emitted light serine 133 phosphorylation-dependently using HEK293T cells, we generated a transgenic mouse line that expressed probe proteins. Contextual fear memory is first formed hippocampus-dependently (recent memory) and then becomes hippocampus-independent over time (remote memory). It is considered remote memory is encoded in cerebral cortex, but molecular basis of remote memory consolidation is unclear. We measured the light emission from cerebral cortex every week after contextual fear conditioning using our transgenic mice. Four weeks after conditioning, remote memory consolidation was tested, and we found that CREB phosphorylation in prefrontal cortex 1 week after conditioning was correlated with remote memory. This result indicates the importance of CREB-dependent gene expression in prefrontal cortex for remote memory consolidation.

Disclosures: **T. Ishimoto:** None. **H. Mori:** None.

Poster

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Title: The role of neuron-specific nucleosome remodeling complex subunit baf53b in cocaine-associated memories

Authors: *A. O. WHITE, D. P. MATHEOS, E. A. KRAMÁR, A. J. LÓPEZ, J. DOAN, M. DAVATOLHAGH, M. WOOD;
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Abstract: Cocaine exposure in a distinct environment can lead to the formation of persistent drug-associated memories. Re-exposure to that environment in the absence of cocaine can lead to robust drug-associated behaviors. Recent evidence has implicated epigenetic mechanisms in drug-associated behavior and memory formation. Hitherto, there has been little research into a possible role for one major epigenetic mechanism, nucleosome remodeling, in drug-associated behaviors and memories. We examined the role of the neuron-specific nucleosome remodeling Brg1-Associated Factor (nBAF) complex in cocaine-associated behaviors and memories. Genetic manipulations targeting the BAF53b subunit of nBAF establish a differential role for nucleosome remodeling in the acquisition/consolidation cocaine-associated memories but not cocaine sensitization behavior. The deficits in cocaine-associated memories were concomitant with aberrant gene expression in the nucleus accumbens during cocaine memory consolidation. Additionally, mutant mice displayed disrupted synaptic plasticity in the nucleus accumbens. This study provides the first evidence that a neuron-specific nucleosome remodeling has a role in drug-associated memory formation.

Disclosures: A.O. White: None. **D.P. Matheos:** None. **E.A. Kramár:** None. **A.J. López:** None. **J. Doan:** None. **M. Davatolhagh:** None. **M. Wood:** None.

Poster

256. Molecular Mechanisms of Memory Consolidation

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UBACyT 2014-2017

Title: Hippocampal synaptic NF-kappa B during inhibitory avoidance long-term memory consolidation in mice

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Abstract: NF-kappa B is a transcription factor whose nuclear activity has been proven to be necessary for consolidation of long term memory in several species ranging from crabs to mice. This transcription factor has a wide distribution in the nervous system, with a well-reported presence in dendrites and synaptic terminals. This localization of the transcription factor, plus evidence pointing to different functions, is what gave rise to two general hypotheses for synaptic NF-kappa B: (a) The transcription factor plays a role in the synapse to nucleus communication, and it is retrogradely transported from polarized localizations to regulate gene expression; (b) The transcription factor modulates the synaptic function locally. Evidence indicates that both mechanisms can operate simultaneously. Our work focuses on the mice hippocampus in an associative learning paradigm such as the inhibitory avoidance learning task. We pay special attention to the local role of the transcription factor at the synapse. We describe its activation and localization dynamics during memory consolidation in this task and also we report NF-kappa B strongly bound to synaptic membranes. Furthermore we explore the role of synaptic NF-kappa B in this associative learning paradigm and how its activation is independent of the aversive or appetitive nature of the stimulus.

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Poster

257. Temporal Processing in Entorhinal and Hippocampal Circuits

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Title: To extract and interpret dynamics of functional and effective connectivity

Authors: *H. WANG, P. P. QUILICHINI, C. BERNARD;
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Abstract: Many analysis methods have been proposed to extract functional connectivity (or statistical dependency) graphs from neuroimaging data; however, they cannot identify the directed coupling or effective connectivity among network nodes. We introduce MULAN - a method that uses fuzzy inference and statistical tools, to optimally integrate information from multiple analysis methods, and to infer effective connectivity. We evaluated MULAN using thousands of simulated datasets under different scenarios, such as varying the number of nodes, link densities, connectivity motifs, the presence of hidden nodes and different connection strengths. MULAN outperformed all individual analysis methods and can reliably infer effective connectivity graphs. We have shown how to cross-validate the inference, using sub-datasets with hidden nodes. Moreover, we provided a concrete example of how to apply MULAN to empirical data. After evaluation of MULAN on the analyzed network is in a steady state regime, here we determine how to compute dynamic effective connectivity graphs, when the connectivity fluctuates over time. We used functional connectivity dynamics (both the correlation and consine similarity across the time windows) to capture the major switching time of the underlying structure. We used 27 methods to build the functional connectivity dynamics matrices. Among them, methods - GGC, BTED, PTED, BCorrU- can distinguish switching time when the underlying ground-truth structure undergoes major changes but with stable periods larger than 12 seconds (the minimal time is 4 seconds). Then we proved MULAN algorithm is able to extract the underlying structures within each stable period. We applied the dynamics of connectivity analysis on local field potential recordings in the hippocampal CA1 region and in the entorhinal cortex. The dynamics of functional connectivity matrix could distinguish the slow wave from theta oscillation. We extracted and explained the dynamics of effective connectivity from MULAN algorithm.

Disclosures: **H. Wang:** None. **P.P. Quilichini:** None. **C. Bernard:** None.

Poster

257. Temporal Processing in Entorhinal and Hippocampal Circuits

Location: Hall A

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Topic: F.02. Animal Cognition and Behavior

Title: Reprogramming of the hippocampal circadian clock in epilepsy

Authors: *C. BERNARD[1], A. GHESTEM[1], K. LUKASIUK[2], K. DEBSKI[2], S. SCHOCH[3], A. BECKER[3], P. SASSONE-CORSI[4], N. CEGLIA[5], P. BALDI[5];
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Abstract: Numerous behavioral and physiological activities are regulated in a circadian manner, including core temperature, feeding and sleep. In the brain, the master time keeping machine is the suprachiasmatic nucleus (SCN), and the core time keeping mechanism is made of several transcriptional/translational feedback loops. As a result, 10-20% of the genes oscillate, resulting in a daily remapping of the SCN neuronal network at the protein level. Such remapping allows switching between different physiological/behavioral states to optimize function. Many neurological/psychiatric disorders show altered circadian patterns (in particular sleep), and disruption of circadian rhythms can lead to pathological states (e.g. obesity). Whether genes and proteins oscillate during the night/day cycle in the brain outside the SCN, thus changing the functioning mode of networks, and whether such circadian activity is reprogrammed in pathologies is not clearly established. Here, we demonstrate: (i) that a circadian gene/protein remapping occurs in the mouse hippocampus, thus changing its functional mode during the night/day cycle, and (ii) that there is a reprogramming of this circadian regulation in experimental temporal lobe epilepsy (TLE). More than 1200 genes are oscillating in control conditions, and 1600 in epileptic animals. Only 400 genes oscillated in both conditions; with a change of phase. Proteomics supported the gene array data. We conclude that the molecular landscape undergoes a continuous remapping during the night/day cycle in the hippocampus; and that these dynamic rules are considerably modified in epilepsy.

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Poster

257. Temporal Processing in Entorhinal and Hippocampal Circuits

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Pediatric Scientist Development Program, March of Dimes

Title: Interictal epileptiform discharges induce pathological hippocampal-cortical coupling in temporal lobe epilepsy

Authors: *J. GELINAS[1], D. KHODAGHOLY[4], T. THESEN[2], O. DEVINSKY[2], G. BUZSAKI[4,3];
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Abstract: Interictal epileptiform discharges (IEDs) are consistent indicators of epileptic brain regions, but their interactions with other brain regions and known physiologic network patterns are mostly undefined. Physiological hippocampal ripples are weakly correlated with medial prefrontal cortex (mPFC) spindles, and this coupling is implicated in memory consolidation during sleep. We show that spontaneously occurring hippocampal IEDs in freely moving rats effectively induce DOWN states and timed spindle oscillations in the mPFC during NREM sleep. IEDs occurring during REM sleep and wakefulness also generate patterns resembling DOWN states and spindle oscillations in the mPFC. Closed-loop cortical electrical stimulation triggered by IEDs can modulate this cortical response. Patients with focal epilepsy exhibit similar correlation of frontotemporal IEDs with spindles over anatomically restricted cortical regions. These findings reveal that IEDs can hijack physiologic coupling mechanisms between structures critical for memory processes, suggesting a possible contribution to cognitive impairment in epilepsy and new options for therapeutic intervention.

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Poster

257. Temporal Processing in Entorhinal and Hippocampal Circuits

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R01MH102840

China Scholarship Council

Title: A contained closed-loop electrical stimulation system

Authors: ***Z. ZHAO**[1,2], D. KHODAGHOLY[1], J. GELINAS[1], Y. WAN[2], G. BUZSAKI[1];
[1]Neurosci., NYU Langone Med. Ctr., New York, NY; [2]Neurosci., Peking Univ., Beijing, China

Abstract: Closed-loop electrical stimulation is increasingly used as a therapy in neuropsychiatric disorders, epilepsy and other neurological diseases. Here, we introduce a novel contained closed-loop system with potential applications to a broad range of neurophysiological questions. By developing a novel analog front-end design, the number of components used in the system is greatly reduced. Our design provides a scalable solution for high speed, high channel count, simultaneous acquisition and, importantly, stimulation of neural network activity. We have used our system to record high quality neural signals from silicon probes implanted in the hippocampus of freely moving rats and stimulate the hippocampal commissure through a bipolar electrode to successfully evoke a hippocampal population response. Furthermore, our system was used to apply transcranial electrical stimulation (TES) to the rat skull and bias the firing rates of hippocampal neurons. Lastly, we have used the onboard digital signal processing unit to detect interictal epileptiform discharges (IEDs) from rats with epilepsy and control feedback stimulation. The combination of acquisition, detection, and stimulation in a contained form potentially allows for stable, long-term, wireless monitoring of physiological and pathological neural networks.

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Poster

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Title: Neurogrid: large-scale hippocampal-cortical interactions

Authors: ***D. KHODAGHOLY**, J. N. GELINAS, G. BUZSÁKI;
NYU Langone Med. Ctr., New York, NY

Abstract: Large-scale recording from neural networks and their interactions is critical for understanding how information is processed and transmitted in the brain. Coupling between specific limbic and cortical brain regions is implicated in aspects of memory acquisition, consolidation, and retrieval, but how these interactions are coordinated over topographically diverse functional networks during online task performance and in offline states is unclear. Here we demonstrate large-scale hippocampal and cortical interactions by simultaneous acquisition of local field potential (LFP) and spiking activity from a large part of the dorsal cortical surface and multiple locations in the hippocampus in rats. We investigate the spatiotemporal interactions of the hippocampus with diverse functional cortical regions, as well as the physiological interactions of these cortical regions with each other. Moreover, we define the neuronal volumes involved in generation of LFP oscillations with varied frequencies and determine their capacity for traveling across the cortical surface. The ability to acquire and analyze LFP simultaneously from multiple functionally distinct brain regions will enhance comprehension of neural network processes and has implications for brain disorders characterized by disordered network function such as epilepsy.

Disclosures: D. Khodagholy: None. **J.N. Gelinas:** None. **G. Buzsáki:** None.

Poster

257. Temporal Processing in Entorhinal and Hippocampal Circuits

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: F.02. Animal Cognition and Behavior

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R01MH102840

Title: Replay is supported by a sub-population of CA1 pyramidal cells

Authors: *A. D. GROSMARK[1], G. BUZSAKI[2];
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Abstract: Hippocampal sequence replay, the preservation from a 'Maze' epoch to a subsequent 'Post' epoch of the spike-timing relationships between groups of cells, has been hailed as a key advance of systems neuroscience in the understanding of memory and systems consolidation. However, the interpretation of the replay phenomenon has been complicated by recent findings suggesting that A) network activity patterns change due to non-learning specific (homeostatic) mechanisms B) hippocampal network patterns subsequently associated with the coding of novel

stimuli are observable before the first exposure to these stimuli (pre-play) and C) mean firing and coactivation rates vary by orders of magnitude between pyramidal neurons but are largely preserved within units across time and behavioral states. The replay phenomena, to date, has most convincingly been demonstrated by the use of 'higher-order' (ensemble-based) methods but the relationship between these measures and physiological changes occurring at the level of individual cells or synapses has not been addressed. Here we use a higher-order (Bayesian) replay analysis to re-examine previous reports of replay and pre-play. We developed a novel analytic method for estimating the relative contribution of individual cells to the higher-order pre/replay signal. Using this method we find that replay (after correction by the 'pre-play' signal) of a novel experience is supported by a minority of hippocampal CA1 cells. We find that these 'replay cells' tend to be deep (dorsal) in the CA1 layer and bursty. Furthermore, we find that while 'replay cells' display increased ripple modulation (firing rate gain) from the pre to the post epoch, these cells already display elevated levels of ripple modulation in the pre-epoch. These findings support the view of learning-induced plasticity as underlying subtle and circumscribed changes within the context of a predominantly rigid hippocampal network organization.

Disclosures: A.D. Grosmark: None. **G. Buzsaki:** None.

Poster

257. Temporal Processing in Entorhinal and Hippocampal Circuits

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Title: Modulation of neurons and assemblies by sleep

Authors: *B. O. WATSON[1,2], J. P. GREENE[3], G. BUZSAKI[1];
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Abstract: The mammalian brain is a complex dynamical system with two major functional states: waking and sleep. During waking experience the brain accomplish a variety of tasks, many of which involve plastic changes moving the system state away from homeostatic baseline. Sleep may serve the purpose of re-balancing the brain to both ensure it does not become dis-

equilibrated and prepare it for future work and learning. We have pursued a technique of monitoring multiple neurons as well as local electrical potentials simultaneously in frontal neocortex in order to study the major changes accomplished by sleep. First we re-capitulate prior findings that overall cortical neuronal spike rates decrease over the course of sleep and extend this observation by demonstrating that this drop in spike rate is exponential in nature. Second, we look at 1-4ms timescale interactions between pairs of neurons which may be indicative of synapses and find that these functional synaptic-timescale interactions also weaken over sleep. We then observe how a major functional unit of the neocortex, the assembly, is altered over sleep. Assemblies naturally defined by the slow waves of slow wave sleep have been shown to replay waking activity and we show that they become less coherent as sleep progresses. Finally we examine assemblies defined during the wake state and find that they are differentially activated over the many sleep oscillations including slow waves, sleep spindles and Rapid Eye Movement sleep. Furthermore, different assemblies are differentially modulated across these brain states. This heterogeneous modulation of waking assemblies, when set on a background of largely homeostatic decreases in various forms of activity over sleep opens the door for future experiments aimed at understanding how sleep differentially effects learning-related versus learning-unrelated neuronal, synaptic and assembly activity.

Disclosures: **B.O. Watson:** None. **J.P. Greene:** None. **G. Buzsaki:** None.

Poster

257. Temporal Processing in Entorhinal and Hippocampal Circuits

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Title: Physiological properties of the hippocampus-amygdala networks during sleep and wakefulness

Authors: *G. GIRARDEAU, I. INEMA, G. BUZSÁKI;
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Abstract: Amygdala network dynamics during the acquisition and retrieval of classical and contextual fear conditioning have been extensively studied in rodents. However, little is known about the basal physiological properties of the amygdala and its interactions with the hippocampus during wakefulness, slow-wave-sleep (SWS) and REM-sleep. Here we designed a new task combining extensive spatial sampling of a linear track with a location-specific fearful element (airpuff), to investigate neuronal dynamics in the hippocampo-amygdala networks. Large neuronal ensembles were recorded in the amygdala and dorsal hippocampus in rats, during experimental sessions including extensive sleep periods before and after training on the task. We first characterized oscillatory activity in the basolateral amygdala (BLA) : we showed strong 20Hz (beta) and 50Hz(gamma) frequency bands that decrease in power during active running. Theta is strongest in REM-sleep, while there is a broad increase in low frequencies (1-12Hz) during slow-wave sleep. Subpopulations of cells in BLA and neighbouring nuclei are differentially modulated by these specific frequencies. Interestingly, the firing rate of BLA pyramidal cells (but not interneurons) specifically increases during REM-sleep compared to SWS and wakefulness. Finally, while the proportion of BLA cells modulated by hippocampal theta is low (12%), we were able to identify two populations of BLA cells that respond to hippocampal SWS ripples by increasing their firing rate during ripples (15%) or decreasing it (19%), suggesting amygdalo-hippocampal communication during SWS. Further analysis will examine the link between different subpopulations and task-related activity (response to reward/airpuff).

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Poster

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Title: Local circuit dynamics and spatial information coding in the hippocampal dentate gyrus

Authors: *Y. SENZAI[1], L. ROUX[1], E. STARK[1,2], G. BUZSÁKI[1];
[1]New York Univ., New York, NY; [2]Tel Aviv Univ., Tel Aviv, Israel

Abstract: The dentate gyrus (DG) is a 'gate' to the trisynaptic DG-CA3-CA1 pathway of the hippocampus. Local field potentials (LFP) in DG show different dynamic patterns during different brain states. However, it is not clear how the local circuit of DG supports those dynamics as well as computation in DG. To address these questions, we performed large-scale recordings of LFP and unit firing from DG of freely moving mice, combined with optogenetic and physiologic identification of different cell types. For optogenetic identification, we used promoter-specific Cre lines to express channelrhodopsin or archaerhodopsin in specific cell types. For physiologic identification, we used parameters such as the trough-to-peak latency of the averaged waveform, the burst index based on the autocorrelogram, and the amplitude versus depth profile of the dentate spikes types 2 (DS2) observed during slow wave sleep (SWS). The anatomical location of each recorded unit was estimated based on amplitude depth distribution. Based on these measures, we classified recorded units into six subgroups: somatostatin (SST) positive cells, narrow-waveform cells, wide-waveform cells, putative molecular layer perforant pathway associated (MOPP) cells, putative granule cells, and hilar bursty cells. We then analyzed how those classified DG units were recruited to local circuit dynamics such as DS1 and DS2 during SWS, as well as theta and gamma oscillations during the awake state. We found that, compared to all other cell types except wide-waveform cells, SST cells (1) were less recruited to DS1 and DS2, and that (2) tended to fire in later phase in both theta and gamma LFP oscillations. Moreover, we analyzed the spatial correlates of identified DG neurons while mice were running on a linear track for water rewards. We observed that (1) only a few putative granule cells had place fields while almost all hilar bursty cells had place fields, and that (2) several interneurons also had stable spatial firing modulation. We will also investigate how each cell type in DG contributes to behavior by examining their behavioral correlates in different environments.

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Poster

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FACES Postdoctoral Fellowship

Title: Functional labeling of restricted populations of head direction neurons in the postsubiculum

Authors: *D. F. ENGLISH, A. PEYRACHE, S. KILJAN, G. BUZSAKI;
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Abstract: The rodent navigation system includes three types of neurons whose activity codes for spatial information: place cells, grid cells and head direction (HD) cells. HD cells are components of a neural compass whose population activity encodes the directional heading of the animal in the yaw axis irrespective of other behavioral variables. This signal is generated by vestibular information and stabilized by visual landmarks. The postsubiculum (PoS; previously referred to as the dorsal presubiculum) is the first cortical area to receive the HD signal from the vestibular system via the anterodorsal nucleus of the thalamus, and where the vestibular and visual systems converge to produce a precise and stable representation of the animal's current heading. While the behaviorally related firing patterns of HD neurons in the PoS and other areas have been thoroughly investigated, there is a paucity of knowledge about the fine structural organization of the circuits which support the HD signal. To facilitate interrogation of this circuitry we aimed to express fluorescent proteins and opsins in restricted populations of HD cells with overlapping tuning fields to enable the visualization and control of a cohesive section of the neural compass. FosCreEr transgenic mice (Guenthner et al. 2013) express tamoxifen-dependent Cre recombinase under control of the Fos promoter, allowing for Cre dependent transgenes to be selectively expressed in cells active during temporally restricted tamoxifen exposure. FosCreEr mice were injected intracranially with AAV encoding either DIO-mCherry or DIO-ChR2-EYFP and subsequently injected with 4-hydroxytamoxifen during unidirectional head fixation in a familiar environment, yielding transgene expression in a subpopulation of PoS neurons. The accuracy of the restriction of transgene expression to HD cells with overlapping tuning fields will be tested using diode probes (Stark et al. 2012), which enable optical tagging of ChR2 expressing single units in freely moving mice. Guenthner CJ, Miyamichi K, Yang HH, Heller HC, Luo L. Permanent genetic access to transiently active neurons via TRAP: targeted recombination in active populations. Neuron. 2013 Jun 5;78(5):773-84. Stark E, Koos T, Buzsáki G. Diode probes for spatiotemporal optical control of multiple neurons in freely moving animals J Neurophysiol. 2012 Jul;108(1):349-63.

Disclosures: **D.F. English:** None. **A. Peyrache:** None. **S. Kiljan:** None. **G. Buzsaki:** None.

Poster

257. Temporal Processing in Entorhinal and Hippocampal Circuits

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Title: Transformation of a head-direction signal into a spatial code

Authors: *A. PEYRACHE[1], N. SCHIEFERSTEIN[2], G. BUZSAKI[2];
[1]Neurosci. Inst., New York Univ. Langone Med. Ctr., New York, NY; [2]Neurosci. Inst., New York Univ. Langone Med. Ctr., New York, NY

Abstract: The brain's cognitive map is represented by neurons firing in specific regions of the environment explored by the animals. The head-direction (HD) signal is critical for the establishment of this representation of space and for navigation capabilities. However, how this signal is transformed into higher spatial representation remains unknown. The HD signal is relayed to the parahippocampal areas by the antero-dorsal nucleus (ADn) of the thalamus, primarily to the post-subiculum (PoS). Head-directions cells, tuned for a specific orientation of the animal's head in the horizontal plane, are present in the two brain areas. Here, we show that the amount of HD and spatial information conveyed by HD cells in both structures is correlated, suggesting that HD cells convey an actual spatial signal. However, for each ADn cell, the spatial information conveyed by a generic cell spiking only in function of animal’s heading and the same HD tuning curve as the original neuron is quantitatively identical. This reveals that the spatial information conveyed by thalamic HD cells results from the animal's head direction behavior, which is non-uniform and therefore informative due to environmental constraints such as boundaries. This was not the case for a subpopulation of PoS cells which conveyed a conjunction of HD and spatial information. Thus, this thalamo-cortical circuit may transform a pure HD signal into a spatial code under geometric constraints from the environment.

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Poster

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Title: A re-examination of the relationship between rodent dCA1 and behavior

Authors: *J. D. LONG II, G. BUZSAKI;
Neurosci. Inst., New York Univ., New York, NY

Abstract: Research into the function of the hippocampus over the past several decades has resulted in a paradoxical description of the hippocampus as necessary for both declarative memory formation and spatial navigation. A conceptual tension uniting these interpretations is the implicit assumption of a homunculus that retrieves memories and reads maps. The central hypothesis of this work is that the hippocampus serves both these apparent functions by forming associations between complex stimuli, composed of many elements, and sequences of behavior, composed of many movements. This hypothesis predicts that neural correlates of specific behaviors exist within rodent dCA1 hippocampus above and beyond spatial location and head direction. Several conditions must be met prior to testing this hypothesis in the rodent dCA1 hippocampus. Firstly, the subjects must not be placed in highly constrained environments which induce strong correlations between their trajectories and behaviors e.g. linear/circular tracks, T-mazes, radial arm mazes. In this study, we utilize a cheeseboard maze, allowing the subjects (n = 4) to form stereotyped, idiosyncratic behavioral plans while foraging for water reward. Next, the time-series of the subjects' behavioral output must be accurately quantified. We use a custom-made markerless motion capture system to generate accurate 3D kinematic tracking data of our subjects at 50 frames per second (to be open source released). Lastly, the behavioral states of the subjects must be quantified to calculate correlations between these and the time-series of neural data (all subjects were implanted with 6-8 shank silicon probes bilaterally in dCA1). We define behaviors as stereotyped dynamics in the kinematic data and leverage recently developed techniques in machine learning to identify behaviors in an unsupervised manner (van der Maaten and Hinton 2008). This allows for both generalization across subjects as well as the discovery of novel behaviors (Berman et al. 2014). With this framework in place, we apply standard analysis methods in systems neuroscience to search for neural correlates of behavior in the rodent dCA1 hippocampus. At the single unit level, reverse correlation is used to determine whether the firing of individual neurons map onto specific behaviors. At the population level, ensemble decoding techniques are used to determine whether information about the moment to moment variations in the subjects' behaviors are available within the population activity. This ongoing research offers a unified interpretation of the function of the hippocampus.

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Poster

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Title: Segmentation of space by theta sequences recorded across the longitudinal axis of the hippocampus

Authors: *S. A. MCKENZIE[1], J. PATEL[1], A. CHADWICK[2], G. BUZSÁKI[1]; [1]NYUMC, New York, NY; [2]Inst. for Adaptive and Neural Computation, Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: For hippocampal principal cells, movement through space is associated with spiking at earlier phases of the theta rhythm,a phenomenon known as theta phase precession. Relatedly, cells with adjacent place fields are sequentially active within each theta period. Recent evidence suggests that these theta sequences reflect more than a fixed amount of space around the subject and could code for meaningful segments of the environment. Variations in place field size and the systematic shift in theta phase across the longitudinal axis complicate these models of temporal coding. We recorded ensembles across the septotemporal axis of CA1 to test how cells coordinate in time to represent space. Consistent with prior reports that slowly evolving neural patterns show about a ten times compression into theta sequences, the cross-correlograms between cell pairs revealed a correlation between the timing of the peak lag observed on theta time scales and that observed on behavioral time scales. This correlation was observed for cell pairs recorded from tetrodes separated by up to 5mm in which the theta phase offset of the LFP exceeds 90º. Next, we computed the translation in space at every time bin that best predicted spiking given a cell's trial-averaged place field. The mean translational offset was calculated for binned phases of local theta or for a global, dorsal reference. Remarkably, cells across the longitudinal axis showed temporally coherent retrospective and prospective coding with reference to the dorsal theta rhythm. Next, we performed Bayesian decoding of the rat's position using ensembles of spikes binned according to dorsal theta or ensembles in which the instantaneous spike counts were time-shifted to globally align theta phases. We found better decoding accuracy for the observed, non-shifted ensembles. Finally, Bayesian decoding of cell sequences bounded by peaks in dorsal theta revealed long sweeps of decoded positions that

began at a route's start location and finished at the route's end. Combined these results suggest interlaminar coordination across the septotemporal axis. The complex phase by position relationships of the more temporal cells may explain the deviations of the current results from predictions of simple models that generate theta sequences from linear phase precession over Gaussian place fields and a traveling theta wave. We propose that ensemble activity during theta is embedded within the temporal context of a past and future bounded by representations of salient events.

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Poster

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Topic: G.04. Physiological Methods

Title: Monolithicalyl integrated micro-LED probe for high-precision optogenetics

Authors: ***F. WU**[1], E. STARK[2,3], K. KIM[1], G. BUZSAKI[2], E. YOON[1];
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Abstract: Recently, optogenetics has opened up a new way to analyze neural circuits by introducing photo-sensitive proteins (opsins) into specific cell types. In principle, this type of cell-specific targeting allows precise manipulation of neural activity, testing spike timing during specific neural computations and behaviors at a temporal resolution of a few milliseconds, in the intact brain. Despite the rapid advancement of optogenetics in recent years, supporting technology to reliably deliver light to and record electrical signals from deep brain structures in freely-moving animals has been lagging. Early work involving *in vivo* optogenetics relied on manual assembly of commercially available recording components such as metal electrodes or passive high-density probes with optical fibers, which may suffer from misalignment errors and cause tissue damage. Moreover, the spatial resolution of fiber-based optogenetic devices is limited by the bulk of the implanted fibers. This work describes an innovative solution to enhance the spatial resolution and scalability of optogenetic stimulation and recording probes. We monolithically integrated 12 InGaN μLEDs (10 μm x 15 μm) and 32 recording electrodes (11 μm x 13 μm) on a 4-shank silicon probe. Combined with the measured electroluminescence characteristics of the fabricated μLEDs, thermal modeling using COMSOL Multiphysics provided a reference to drive the μLEDs to produce sufficient light intensity without overheating the tissue (< 1°C). To understand the interference between stimulation and recording channels, an equivalent circuit model of the μLED probes was built using COMSOL and SPICE,.and validated in comparison with the *in vivo* data; the analysis suggested specific fabrication steps to

further minimize interference. We implanted the μLED probes into the hippocampal CA1 pyramidal layer of 6 mice for chronic studies. We observed optically induced activity in all CaMKII::ChR2 animals (n=4) but not in wild type animals (n=2). Specifically, spikes were robustly induced by as low as 60 nW light power, and fast population oscillations were induced at the microwatt range. To demonstrate the spatiotemporal precision of parallel stimulation and recording, we achieved independent control of distinct cells ~50 μm apart and of different somato-dendritic compartments of single neurons. The scalability and spatiotemporal resolution of this novel monolithic optogenetic tool are unprecedented, providing versatility and precision for cellular level circuit analysis in deep structures of intact freely-moving animals.

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Poster

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Title: Mechanisms of theta sequence formation during exploration of a novel environment

Authors: C. DRIEU[1], R. TODOROVA[1], *M. B. ZUGARO[2];
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Abstract: The hippocampal formation plays a critical role in spatial and episodic memories. In freely behaving rats, hippocampal 'place' cells selectively discharge in specific locations of the environment ('firing fields'). When a rat crosses successive overlapping firing fields, the spike trains of the corresponding place cells overlap in time. Strikingly, at a fast time scale of ~100 ms corresponding to one cycle of the theta rhythm (7-10 Hz), these cells fire in a stereotyped order, forming sequences that reflect the ongoing trajectory. These 'theta' sequences could be related to episodic-like memory. Although the underlying mechanisms remain unknown, two theoretical frameworks have been proposed. The first posits that sequences result from asymmetric connectivity between cell assemblies. Each active cell assembly drives subsequent activation of the next assembly to generate a sequence. In the alternative model, external inputs determine when each assembly starts firing, and sequences result from independent advancement of each assembly relative to theta as the animal moves ahead (phase precession). Asymmetric

connectivity then results from, rather than causes, theta sequences. We have designed an experimental protocol that perturbs the precise spiking dynamics of hippocampal assemblies in a novel environment. Animals were transported on a miniature treadmill mounted on a model train. We have previously shown that when the treadmill is turned on (ACTIVE), place cells phase precess and form theta sequences, but when the treadmill is turned off (PASSIVE), few cells phase precess. We asked whether theta sequences would be disrupted in the passive condition. The rats underwent three successive sessions: PASSIVE-1, ACTIVE, PASSIVE-2. Spatial specificity and theta modulation of place cell activity decreased during both passive sessions compared to the active session. Fields formed during PASSIVE-1 often shifted during ACTIVE, but remained stable between ACTIVE and PASSIVE-2. In addition, the proportion of significant theta sequences across theta cycles increased two-fold during ACTIVE compared to PASSIVE-1. This would argue against pre-established connectivity between cell assemblies underlying theta sequences. Interestingly, we found the same difference between ACTIVE and PASSIVE-2, indicating that sequences formed during the active session were not preserved during the subsequent passive session. This in turn would be inconsistent with the theta sequences yielding stable connections between assemblies. Our preliminary results appear partly at odds with both families of theoretical models of theta sequences.

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Poster

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Title: Cross-frequency coupling and network dynamics in entorhinal-hippocampal circuits

Authors: *A. FERNÁNDEZ RUIZ[1,2,3], A. OLIVA[1], G. BUZSÁKI[3], A. BERÉNYI[1,3];
[1]Sch. of Medicine, Univ. of Szeged, Szeged, Hungary; [2]Sch. of Physics, Complutense Univ., Madrid, Spain; [3]Neurosci. Inst., New York Univ., New York, NY

Abstract: In the entorhino-hippocampal circuits theta rhythm organizes the activity of interconnected cellular populations during behavior and REM sleep. Sequential activation of cell assemblies mediates information flow and processing in the different nodes of the circuit and is reflected as gamma oscillations in the local field potentials (LFPs). We employed large-scale silicon probes (up to 512 channels) to record LFPs and single-unit activity simultaneously in all hippocampal subfields and layers of the entorhinal cortex in rats during various navigational tasks and sleep. Using advanced analytical techniques we characterized the spatial distribution and spectro-temporal properties of the identified sources of hippocampal gamma oscillations to elucidate if they arise as a result of local circuit computations or reflect rhythmic inputs to the target region. We found that distinct gamma patterns mediated by the different entorhinal and intrahippocampal inputs are precisely organized within the theta cycle and are coupled preferentially to characteristic frequency bands. Moreover, changes in phase preference of gamma bursts and their cross-frequency coupling with theta oscillations display topographic changes associated to behavioral states. Hippocampal and entorhinal units display variable phase-locking with the different gamma inputs according to memory demands and environmental novelty. We found that slow gamma patterns are transmitted more reliably along the whole network than faster ones, however high-frequency oscillations originated locally as a result of excitation-inhibition loops are more efficient to synchronize spiking. Different inputs can cooperate or compete to entrain local networks allowing a fast switching between different computational modes. Beyond the traditional scheme of information flow in the entorhino-hippocampal circuit we reported the coexistence and dynamic interactions of multiple channels of bidirectional communication carrying multiplexed frequency codes. Hippocampal and entorhinal spiking appear to be coordinated in a wide range of gamma frequencies and theta phases, enabling a large computational flexibility that can support different behaviors. Our results argue in favor of theta-gamma coupling as a functional mechanism to dynamically adjust the information processing to support the broad repertoire of animal behavior.

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Poster

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Title: Targeted transcranial electrical stimulation protocols: spatially restricted intracerebral effects via improved stimulation and recording techniques

Authors: *M. VOROSLAKOS[1], A. OLIVA[1], K. BRINYICZKI[2], T. ZOMBORI[2], B. IVÁNYI[2], G. BUZSÁKI[3], A. BERÉNYI[1,3];
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Abstract: Neural activity can be induced or modulated by an exogenous electric field, which can be generated non-invasively by transcranial electrical stimulation (TES). The physiological effects of TES are determined by the spatial distribution and temporal pattern of the induced intracerebral currents. In most cases in human patients there is no direct data accessible about the local neuronal entrainment by TES, but still the a priori knowledge on the expectable local intracerebral effects of given stimulation parameters may allow to design targeted treatment plans. In principle, TES can be also spatially selective (similarly to deep brain stimulation) by modulating the distribution of the electric fields. In our experiments, we set out to measure the TES generated electric fields in human brains and to test the viability of a spatially focused TES protocol. Our assumption is that the effect of repetitively delivered high frequency (>1 kHz) Gaussian pulses on multiple bilateral electrode pairs may be temporally integrated by the neuronal membranes, leading to a stronger neuronal entrainment around the overlapping region of the diagonal fields than at the periphery. We recorded TES-generated field potentials in human cadavers and anesthetized rats. Stimulation was applied by placing Ag/AgCl EEG electrodes over the external surface of the skull. We used independently isolated stimulation pairs to deliver sinusoidally modulated TES with various parameters and repetitive high-frequency Gaussian stimulus trains in various arrangements. Custom made multiple-site electrodes (>200 contact points) and 32-channel silicon probes were used to thoroughly sample the field potentials in the brain. We also measured the shunting effect of the skin during transcutaneous stimulation. In addition to our earlier results, we found that the skin dramatically reduced the generated intracranial electric fields, and alters its geometry. We recorded the unit activity during the high-frequency pulsed TES, and estimated its effects on neuronal activity. We found that the high-frequency stimulation generates a relatively small diameter axial voltage gradient in the geometrical axis of the stimulator electrodes (<50% gradient strength off-axis vs in-axis). The multiple crossing stimulation pairs protocol resulted in a spatially focal effect after

temporal integration (>30% larger electric field magnitude at the crosspoint than at the periphery). We also suggest a protocol to selectively and unilaterally stimulate the frontal cortex via TES.

Disclosures: **M. Voroslakos:** None. **A. Oliva:** None. **K. Brinyiczki:** None. **T. Zombori:** None. **B. Iványi:** None. **G. Buzsáki:** None. **A. Berényi:** None.

Poster

257. Temporal Processing in Entorhinal and Hippocampal Circuits

Location: Hall A

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Title: Place cells properties in CA1 depend on different inputs

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Abstract: The specific spatial related firing of the various entorhinal and hippocampal cells and the computational processing schemes of these structures entail the circuit as the navigational system of the brain. However, the physiological mechanisms connecting single cell physiology with the emerging functional properties of these networks still remain unclear, thus preventing to establish a solid framework to deduct the particular role of each cell type during the navigation process. To address these questions we used large-scale high-density silicon probes to record population activity (LFP) and single unit activity simultaneously along the subiculofimbrial (transversal) axis of the hippocampus and entorhinal cortex (EC) in behaving rats. First, we found that spatial coding properties differed between the proximal and distal poles of the hippocampal axis. Proximal cells show single place fields and carry more spatial information

while distal ones have sparser place fields and are less spatially informative. Secondly, investigating the theta modulation of these neurons we found that cells located at different poles fire preferentially at different phases. Proximal cells tend to fire later in the theta cycle, closer to the preferred phase of CA3 input, and distal cells earlier, closer to the phase of the EC input. The gamma frequency modulation of spiking showed stronger slow gamma (30-50 Hz) phase-locking in the proximal site, which has been associated to the CA3 input, while distal cells showed stronger phase-locking with mid-gamma (80-100 Hz), associated with EC input. The distribution of probability of functional monosynaptic connections of CA3 or EC cells to CA1 cells along the hippocampal transversal axis support the previous findings by showing a gradually shifting balance in the contribution of EC and CA3 inputs to the CA1 local populations. Together these results suggest that the different cortical and intrahippocampal inputs have distinct contribution to control the activity of cells along the hippocampal transversal axis, and may explain the variable place coding efficacy of CA1 neurons along the transversal axis also reported in other works. Taking into account the specificity of the projections from different portions of the EC cortex and CA3 to the CA1, we propose a new scheme of CA1 functional specialization based on the role of the different inputs during navigational behaviors.

Disclosures: **A. Oliva González:** None. **A. Fernández-Ruiz:** None. **G. Buzsáki:** None. **A. Berényi:** None.

Poster

257. Temporal Processing in Entorhinal and Hippocampal Circuits

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DFG SFB936/B3

Title: I(h) attenuation impairs learning and underlying entorhinal-hippocampal network oscillations

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Abstract: The entorhinal cortex (EC)-hippocampus (HC) circuitry plays a pivotal role in memory consolidation and retrieval. We wanted to determine how network activities within and between the EC-HC circuit regulate memory formation, and how these are influenced by intrinsic resonance properties of specific neuronal populations. The hyperpolarization-activated

cyclic nucleotide-gated cation (HCN)/h channels mediating I(h) are an important determinant of the biophysical properties of neurons, regulating the RMP, resonance frequency and dendritic integration. We hypothesize that changes in the intrinsic properties of specific neuronal populations affect local network activity and hence, communication within and between the EC-hippocampal circuit. We developed transgenic mice with attenuated HCN/h channel activity in adulthood using the Tet-Off system to conditionally express a dominant-negative HCN/h channel subunit (HCN-DN) in specific neuronal populations. Network oscillations within the EC-HC circuitry were investigated by using mice with CaMKII-alpha promoter-mediated, forebrain projection neuron-restricted I(h) deficiency. To assess the coordination of activity between the two regions we also studied mice with EC-specific attenuated HCN/h channel activity in principal neurons, which was achieved by expressing the HCN-DN under control of the neuropsin promoter. Phenotypes were characterized in a battery of behavioral tests that we later additionally combined with electrophysiology. Local field potentials were recorded in freely moving mice implanted with 16-site linear silicon probes along the hippocampal CA1-dentate gyrus axis. Both transgenic mouse lines show impaired learning in a hippocampus-dependent single-trial spatial learning task. EC-specific I(h) attenuation caused additional impaired learning in the fear conditioning test. Both groups differ from controls in local hippocampal and EC-HC network patterns during sleep. CaMKII-alpha-mediated I(h) attenuation led to larger amplitude sharp waves but ripples of lower spectral frequency, as well as power changes in stratum lacunosum theta and gamma, a region that receives EC-input. In contrast, EC-specific ablation of I(h) decreased modulation of gamma by theta and reduced gamma coherence between stratum lacunosum moleculare and radiatum during REM-like sleep. Thus, changing the intrinsic properties of specific neuronal populations within the EC-HC network differentially affects the underlying network oscillations.

Disclosures: **A. Merseburg:** None. **K. Meier:** None. **S. Marguet:** None. **F. Morellini:** None. **D. Isbrandt:** None.

Poster

257. Temporal Processing in Entorhinal and Hippocampal Circuits

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Topic: F.02. Animal Cognition and Behavior

Support: IS63/5-1 (SPP 1665)

Title: Development of hippocampal network activity patterns in mice during the first two weeks of life

Authors: *R. HINSCH[1], S. MARGUET[1], A. SIROTA[2], W. FAZELI[3], D. ISBRANDT[1]; [1]DZNE, Cologne, Germany; [2]BCCN, Munich, Germany; [3]Univ. children's hospital, Cologne, Germany

Abstract: An open challenge in neuroscience is to map the structural and functional network changes that accompany early brain development. Early network oscillations, which qualitatively and quantitatively differ from adult patterns, are thought to contribute to the functional and structural maturation of different brain regions. Most studies on neonatal hippocampal activity are based on in-vitro, or anesthetized in-vivo preparations. Characterizing hippocampal activity during the transition from immaturity to adulthood in unanesthetized mice is a major step towards understanding brain maturation. During adult slow-wave sleep, hippocampal activity in mouse CA1 is dominated by sharp-wave (SPW)-ripple complexes, whereas paradoxical sleep primarily consists of theta/gamma oscillations. To characterize the development of oscillatory network activities in unanesthetized neonatal mice, we adapted acute head-fixed recording techniques with multichannel silicon microelectrodes in awake, locally anesthetized mice aged between postnatal day two (P2) and P15. The qualitative and quantitative characterization of spontaneous local field potentials (LFPs) along the CA1-dentate gyrus axis revealed oscillations in CA1 apical dendritic layers in the beta frequency range (10 to 30 Hz) that lasted 2-30 s. Their mean duration increased with age, reaching a maximum between P9 and P14. Hippocampal SPWs were detected as early as at P2. At P9, SPWs occurred simultaneously with fast oscillations (~100-150 Hz) resembling SPW-ripple complexes in adult mice. Also at P9, current source density analysis of the LFP revealed an additional current sink occurring between the sink-source pair already present in the first week of life; this may indicate early functional input from entorhinal cortex (EC) to CA1, and maturation of the CA3 input via Schaffer collaterals (SC) only around P9. This finding contrasts the current hypothesis of hippocampal SPWs generation at P1-P7, which is that early hippocampal sharp waves are correlates of CA3 population activity. To further investigate developmental changes in CA1 inputs, we electrically stimulated either the perforant path (PP) (EC input) or CA3 (SC input). Preliminary data indicate that both inputs are functional at P9. Additional recordings at earlier time points from both hippocampus and EC are needed to further characterize the maturation of SC and PP functional inputs onto CA1. Together with the data described above, these will provide a basis for the characterization of functional connectivity development in the hippocampus of awake neonatal mice.

Disclosures: R. Hinsch: None. **S. Marguet:** None. **A. Sirota:** None. **W. Fazeli:** None. **D. Isbrandt:** None.

Poster

257. Temporal Processing in Entorhinal and Hippocampal Circuits

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Title: Burst generation and propagation during hippocampal sharp waves in a lognormal recurrent network model

Authors: *T. FUKAI[1,2], Y. OMURA[3], M. M. CARVALHO[4], K. INOKUCHI[3,2];
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Abstract: The strength of cortical synapses was suggested to distribute lognormally, with a long tail of strong synapses. Here, we model a recurrent neural network of the hippocampal CA3 with the weights of recurrent excitatory connections distributed lognormally. Using multi-timescale adaptive threshold neurons, we construct a low-frequency spontaneous firing state of bursty neurons, which well replicates the observed statistical properties of population synchrony in hippocampal pyramidal cells. Furthermore, we show that various properties of neuronal activity, such as the average firing rates of neurons, the rate and magnitude of spike bursts, the magnitude of population synchrony, and the correlations between pre- and postsynaptic spikes, also obey lognormal-like skewed distributions, as recently shown in the hippocampal CA1 and CA3 areas. Our model demonstrates that bursts spread over the lognormal network much more effectively than single spikes, implying an advantage of spike bursts in information transfer. This efficiency in burst propagation is not found in neural network models with Gaussian weighted recurrent synapses. Our model proposes a potential network mechanism to generate sharp waves in CA3 and associated ripples in CA1 because bursts occur in CA3 pyramidal neurons most frequently during sharp waves.

Disclosures: **T. Fukai:** None. **Y. Omura:** None. **M.M. Carvalho:** None. **K. Inokuchi:** None.

Poster

257. Temporal Processing in Entorhinal and Hippocampal Circuits

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Ellison Medical Foundation AG-NS-0724-10

Title: Theta phase precession in spatial non-grid cells in the medial entorhinal cortex

Authors: *G. W. DIEHL[1], O. J. HON[1], C. C. CANNOVA[1], M. P. BRANDON[1], S. LEUTGEB[1,2], J. K. LEUTGEB[1];
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Abstract: The precisely timed activation of hippocampal and medial entorhinal cortex (MEC) cell populations is thought to support memory for sequences of events. As an animal travels through the spatial firing fields of cells in these brain regions, each cell fires at progressively earlier phases of subsequent theta cycles. At the network level, this phase precession results in the sequential firing of co-active neurons within a single theta cycle, and thus within a time frame that allows for spike-time-dependent plasticity processes. Theta phase precession has been observed in all hippocampal subregions and in grid cells located in layer II of the MEC, upstream of the hippocampus. Phase precession could either emerge independently in each of these cell populations, or it could propagate through the circuitry by inheritance from one phase-precessing cell population to another. In contrast to models that exclusively rely on local mechanisms, hippocampal phase precession has been shown to be disrupted by MEC lesions (Schlesiger et al, SfN Abstract #578.29, 2013). This finding is consistent with the notion that grid cells are necessary for hippocampal phase precession. To examine this possibility, we asked whether the selective disruption of MEC grid cells by inactivation of the medial septal area (Koenig et al, Science, 2011; Brandon et al, Science, 2011) would result in diminished hippocampal phase precession. Surprisingly, analysis of hippocampal firing patterns during septal inactivation revealed preserved phase precession. These results raise the possibility that inputs to the hippocampus from non-grid cells within the MEC may be sufficient to support hippocampal phase precession. We find that approximately 20 % of spatial non-grid cells recorded from the superficial layers of MEC in 5 rats (n = 15 of 75) exhibit significant phase precession ($P < 0.05$, circular-linear correlation) during random foraging in an open field environment. These cells precessed on average through 1/3 of the theta cycle (32 ± 6 % of a theta cycle). Furthermore, following septal inactivation we did not find substantial disruption of phase precession in entorhinal non-grid cells, while MEC grid cells lost both spatial periodicity and theta phase precession. Even though the MEC is necessary for hippocampal phase precession, it

appears that grid firing patterns are not essential for this network computation. Instead, our data demonstrate that spatial non-grid cells in the MEC exhibit phase precession, and we identify a new candidate MEC population for supporting theta phase precession in the hippocampus.

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Poster

257. Temporal Processing in Entorhinal and Hippocampal Circuits

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Support: The Whitehall Foundation

NIMH R21 MH100354

NINDS R01 NS084324

Title: The role of recurrent networks in the medial entorhinal cortex layer II and parasubiculum for grid cell generation

Authors: *I. ZUTSHI[1], V. LILASCHAROEN[1], M. P. BRANDON[1], J. K. LEUTGEB[1], B. K. LIM[1], S. LEUTGEB[1,2];
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Abstract: Spatial memory and navigation are thought to depend on location-selective firing of various spatially modulated cell types, including grid cells. Grid cells have periodic, hexagonally-arranged firing fields and are most abundant in layer II of the medial entorhinal cortex (MEC). To understand the functional role of grid cells, it is essential to determine how grid firing patterns are generated. Grid cell spatial tuning is disrupted by inactivating the medial septal area (MSA), or by systemic injections of scopolamine, a muscarinic antagonist. Cholinergic projections to the MEC layer II originate in the MSA and selectively terminate on patches of calbindin-positive (Cb+) pyramidal cells. Previous studies have indicated that grid cells in layer II could be either Cb+ or Cb-. Irrespective of the cell identity of Cb+ cells, we hypothesize that the effect of MSA inactivation on grid cells could be mediated by Cb+ cells and their recurrent connectivity within the MEC. A transgenic mouse line expressing cre recombinant protein under the wolfram syndrome 1 (wfs1) promoter has been described to express cre in cells that exclusively overlap with the layer II Cb+ pyramidal patches. We demonstrate that wfs1+ cells are also present in the parasubiculum, but not in other subicular areas or layers of the MEC. Retrograde viral tracing has established that the wfs1+ cells receive

inputs from the ipsilateral and contralateral MEC, pre- and para-subiculum, the anterior thalamic nucleus, and cholinergic cells within the MSA. Anterograde tracing with viral tracers that selectively label synaptic terminals reveals that wfs1+ cells exclusively project to cells in layer II of the MEC and to cells at the border between the stratum radiatum and the stratum lacunosum moleculare in the dorsal CA1. In addition, wfs1+ cells send dense axon bundles through the fimbria-fornix to the contralateral Cb+ patches and the contralateral parasubiculum. By optogenetically manipulating wfs1+ cells while performing *in vivo* multi single-unit recordings in freely moving mice, we are determining the functional role of these cells and their contribution to spatial firing patterns in the MEC. Preliminary data indicates that the wfs1+ cells are functionally coupled to most cells within layer II MEC. The results from selectively manipulating subpopulations of entorhinal cells can provide experimental data on the role of intra-entorhinal recurrent networks for generating grid cell firing. These manipulations will advance our understanding of how the brain generates and uses location-selective firing patterns to facilitate spatial memory.

Disclosures: I. Zutshi: None. **V. Lilascharoen:** None. **M.P. Brandon:** None. **J.K. Leutgeb:** None. **B.K. Lim:** None. **S. Leutgeb:** None.

Poster

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Support: NIH R01NS086947

Ellison Medical Foundation (AG-NS-0724-10)

Title: Hippocampal and medial entorhinal cortex lesions result in only a transient impairment in delayed spatial alternation: Physiological evaluation of the functional recovery

Authors: *M. SABARIEGO[1], B. L. BOUBLIL[1], G. DE GUIA[1], J. K. LEUTGEB[1], R. E. CLARK[3,2], S. LEUTGEB[1,4];
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Abstract: Medial entorhinal cortex (MEC) cells project directly to the hippocampus and are thought to be the key source of spatial and temporal information to hippocampal place cells. Accordingly, MEC lesions result in impaired spatial precision and stability of place cells and in substantially reduced phase precession in the hippocampus. However, it remains unclear to what extent the spatial and temporal firing patterns in MEC are necessary for the performance of

memory tasks. We hypothesize that a primary function of MEC is to generate the temporal organization of hippocampal firing such that the population code can bridge gaps between discontinuous events during memory performance. To test this hypothesis, rats with either a hippocampal lesion, an MEC lesion, or a sham lesion were trained to perform a continuous spatial alternation task in which the animals alternated between left and right sides of a figure-8-maze on a trial-by-trial basis to receive food reward. After animals reached criterion (90% correct for 3 days), blocks of trials were introduced with 2-second and 10-second delays. All groups performed similarly during the trials without delay, but when a delay was inserted, the hippocampus and MEC lesion groups made significantly more errors with the 2-second as well as the 10-second delay ($p<0.001$). After the initial behavioral testing, electrode arrays were implanted into the hippocampus to record hippocampal single units and local field potential. When behavioral testing resumed after electrode implantation, MEC-lesions rats reached control level performance in the delayed spatial alternation task. This suggests that neuronal computations in a circuit with only lateral entorhinal cortex projections to the hippocampal circuit were sufficient to support the spatial memory. By recording from the hippocampus while MEC-lesion rats perform the spatial alternation task, we are able to examine which spatial and temporal firing patterns in the hippocampus are preserved to support behavioral performance after permanent MEC damage. By identifying which neuronal firing patterns are spared and how the remaining circuits compensate for reduced phase precession after MEC lesions, our results can lead to therapeutic approaches that either strengthen remaining physiological functions or that restore function in diseases with damage to the medial temporal lobe.

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Poster

257. Temporal Processing in Entorhinal and Hippocampal Circuits

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Indian Institute of Science Education and Research Pune

Title: Theta oscillations shape the precise temporal response of a network of stellate cells

Authors: A. NERU, *C. G. ASSISI;
Indian Inst. of Sci. Educ. and Res., Pune, India

Abstract: Sequential activity of groups of neurons is known to coordinate complex movements, encode our perception of the world and form new memories. A number of experiments have demonstrated that sequences of spikes in neuronal populations in the hippocampal formation encode spatial and temporal information. Competitive interactions between stellate cells, the predominant cells in the entorhinal cortex, via inhibitory interneurons, form a prominent motif in the medial entorhinal cortex. Using a model network of stellate cells we show that these interactions mediated via inhibition from fast spiking interneurons are sufficient to generate sequential activity in groups of neurons. However, sequences thus generated are not reliable in the presence of noisy variations in background synaptic activity. We show that heterogeneity in the topology of the network introduced due to variability in the strengths of inhibition combined with prominent theta oscillations that drive the network can serve to stabilize these spatiotemporal sequences. The biophysical properties of stellate cells dictate the preferred range of theta frequencies that ensure reliable spike sequences and constrain the duration of these sequences.

Disclosures: **A. Neru:** None. **C.G. Assisi:** None.

Poster

258. Reward: Motivational Mechanisms I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 258.01/Y12

Topic: F.03. Motivation and Emotion

Title: Direct evidences for the involvement of orexin-1 receptor in the mesolimbic reward-related behaviors in conditioned place preference paradigm

Authors: *A. HAGHPARAST[1], Z. FATAHI[1], Z. TASLIMI[2], M. MORADI[1];
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Abstract: Orexin neurons located in the lateral hypothalamus (LH) are primarily involved in reward processing. This effect might be mediated by the LH projections to the ventral tegmental area (VTA), nucleus accumbens (NAc) dorsal hippocampus (CA1 region) and which form circuit that could be particularly important in reward processing. Our laboratory tries to clear the new aspect of orexin, for that reason we have done three project s. Male albino Wistar rats were used in these projects as a three groups. In first group cannula were implanted into the LH and VTA, second group into the LH and NAc and third into the LH and CA1. The conditioned place preference (CPP) paradigm was done; conditioning score and locomotor activity were recorded by Ethovision software. In all groups LH orexinergic projection stimulated with effective dose of carbachol (250 nM/0.5 µl saline) as a cholinergic agonist lonely or 5 min after administration of

different doses of SB334867 (1, 3, 10 and 30nM/0.5µl DMSO) as a orexin-1 receptor (OX1r) antagonist into the VTA in the first, in the NAc as a second and in the CA1 in third group during 3-day conditioning phase separately. Data showed that LH stimulation could induce CPP. On the other hand, inhibition of orexin-1 receptors in the VTA, NAc and CA1 before LH stimulation could decrease the acquisition (development) of LH stimulation-induced CPP in the rats. In conclusion, our research highlights the effect of LH orexinergic projection to the important target of the reward circuit and shows that this kind of receptor (OX1r) in these areas has an important role in inducing reward-related behaviors in CPP paradigm.

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Poster

258. Reward: Motivational Mechanisms I

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Topic: F.03. Motivation and Emotion

Support: MEXT, Japan

Title: Development of the objective method for evaluating nest building behaviors in mice using a 3-D depth camera

Authors: *A. TOYODA, T. GOTO, T. OKAYAMA;
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Abstract: Animal behaviors have been widely studied by various fields of researchers. Especially, experimental animals such as rodents have been used for analyzing both innate and learned behaviors. Behavior analyses are mainly based on the observation by human eyes or 2-D video camera, therefore the objective analysis is very difficult for 3-D behaviors of animals. Recently, we developed a novel objective method for mouse behaviors using an inexpensive 3-D depth camera. In this study, we focused on the nest-building behavior, one of the representative innate behaviors in mice. Using 3-D information from the depth camera, the objective features for assessing nest-building behavior were obtained including “volume,” “radius,” and “mean height”. The “volume” represents the change in volume of the nesting material, a pressed cotton square that a mouse shreds and untangles in order to build its nest. During the nest-building process, the total volume of the nesting material was increased. The “radius” refers to the radius of the circle enclosing the fragments of cotton, and it shows the extent of nesting material dispersion. The average “radius” was approximately 60 mm when a nest was completely finished. The “mean height” represents the change in the mean height of objects. If the nest walls were high, the “mean height” was also high. These features provided us with useful information for assessment of nest-building behavior, similar to conventional methods for the assessment of

nest building. Fortunately, we found that JF1 mice built nests with higher walls than B6 mice, and B6 mice built nests faster than JF1 mice using our novel method. Thus, it can evaluate the differences in nest-building behavior that cannot be detected or quantified by conventional methods.

Disclosures: A. Toyoda: None. **T. Goto:** None. **T. Okayama:** None.

Poster

258. Reward: Motivational Mechanisms I

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Program#/Poster#: 258.03/Y14

Topic: F.03. Motivation and Emotion

Title: Exposure to different macronutrients during post-weaning and its effects on the consumption of standard and obesogenic diets in adulthood

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Abstract: Overweight and obesity are now conceived as an epidemical issue. Currently, it is more important the palatability that an adequate nutrimental balance in food, which may produce, besides mal-nourishment, inadequate alimentary patterns and eating disorders. The food palatability is generally associated with high content of carbohydrates (carbs) and fats and it is well known that this macronutrient content of food affects eating behavior. There is evidence that infancy may be a critical period for the exposure to food with high content of carbohydrates and fat, which in turn may have an important impact in the selectivity of food and eating patterns on later life. However there are very few works that have explored the relation between these factors. On this basis, the aim of this work was to study the exposure to different macronutrients during infancy and its repercussion on the consumption of an obesogenic food in adulthood. For this purpose, male Wistar rats were used. Six groups (n=10) were exposed to a different diet in infancy during 18 days, starting at 23 postnatal day (PND); as adults, five out of the six groups received the same diet during 28 days starting at 75 PND. Food intake and body weight were registered during the study. Groups (name and diet): a)Control/Obesogenic (Ob): standard food (SF) in childhood, obesogenic diet (OD) as adult. b)Carbs/Ob: SF and carbs as child, OD as adult. c)Fat/Ob: SF and fats as child, OD as adult. d)Carbs+Fat/Ob: SF, carbs, and fats as child, OD as adult. e)MIX/Ob: SF and a mixture of carbs and fats as child, OD as adult. f)Control/Control: SF as child and as adult. Results showed that there were not significant differences between groups in caloric intake during infancy, but the Carbs group showed a tendency of consuming more calories than the other groups, this could be due to appetite is not

only mediated by the palatability of the food but beside for its post-oral actions. Changes on body weight were not observed in infancy, which agrees with the regulation of caloric intake at this period. In adulthood, results showed that MIX group significantly consumed more calories and gain more body weight than other groups; on the other hand, Carbs+Fat group consumed significant fewer calories than Control/Control and MIX, and have a tendency to gain less body weight than the other groups. These findings indicate that a free-choice between carbohydrates and fat vs. the availability of a mixture of these two macronutrients in infancy could play a critical role on the later election for food. These results gain relevance if it is considered that most of highly palatable food available to human being is an enriched mixture of these two macronutrients

Disclosures: J.A. Mata-Luévanos: None. **J. Juarez:** None.

Poster

258. Reward: Motivational Mechanisms I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 258.04/Y15

Topic: F.03. Motivation and Emotion

Title: Emotional modulation on action control in a stop-signal task

Authors: *H.-J. LEE, W.-J. KUO;
Inst. of Neurosci., Natl. Yang-Ming Univ., Taipei, Taiwan

Abstract: Emotion plays an important role in action control. In this study, we investigated interaction between emotion and action control by using the stop-signal paradigm, where emotions were elicited by monetary gains (positive feedback condition), losses (negative feedback condition) and neutral feedbacks. Behavior data indicated that the time for processing stop signals was shorter when participants receiving a feedback of monetary gains than when receiving a neutral feedback, suggesting that it was easier to inhibit an ongoing action when participants felt rewarded. In the fMRI results, there were several interesting findings. First, activation in the dorsal striatum showed descending pattern across conditions. While the dorsal striatum had strongest activity in the positive feedback condition, it showed the lowest activation level in the negative feedback condition. The pattern is consistent with the previous findings. Second, comparing to the neutral feedback condition, both positive and negative feedback conditions showed higher activity in the bilateral ventro-lateral PFC, dorso-lateral PFC, pre-SMA and superior parietal regions. The results suggested that the networks related to attention and high-level action control were sensitive to the emotion perturbation.

Disclosures: H. Lee: None. **W. Kuo:** None.

Poster

258. Reward: Motivational Mechanisms I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 258.05/Y16

Topic: F.03. Motivation and Emotion

Support: NIH Grant DA035443

Title: Basolateral amygdala mu opioid receptor activation and connections to the orbitofrontal cortex mediate outcome-specific Pavlovian-instrumental transfer

Authors: *N. T. LICHTENBERG, V. Y. GREENFIELD, A. S. WANG, K. M. WASSUM; Psychology, UCLA, Los Angeles, CA

Abstract: Environmental reward-predictive stimuli provide a major influence over reward-seeking behavior. The basolateral amygdala (BLA) is involved in this, and is particularly critical for situations in which such cues provide reward-specific information that allows them to selectively invigorate and bias selective actions. But how the BLA functions within a larger circuit to carry out this complex function is largely unknown. The BLA shares dense and reciprocal excitatory connections with several cortical areas, including the orbitofrontal cortex (OFC), which is itself implicated in the ability of environmental cues to convey information about anticipated rewards. Here using the outcome-specific Pavlovian-instrumental transfer (PIT) task we evaluated the role of OFC-BLA circuitry in the selective invigorating and action selection biasing effects of reward-predictive cues over reward seeking. Pharmacological disconnection of these structures by contralateral transient inactivation impaired the ability of reward-predictive cues to, in a choice test, selectively invigorate the performance of actions that earned the same specific reward associated with the stimulus. Unilateral inactivation of either structure and ipsilateral OFC-BLA inactivation were without effect. Interestingly, OFC-BLA disconnection appeared to spare the action-selection biasing effect of the cues; rats were able to choose actions on the basis of the specific reward predicted by the cue, but the performance of this action was not invigorated above baseline response levels. To explore the specific mechanisms of this within the BLA we examined the role of BLA opioid receptors in PIT. While selective blockade of the delta opioid receptor was without effect, blockade of BLA mu opioid receptor activity abolished both the selective excitatory and response-biasing effect of reward-predictive cues over reward-seeking actions. These data suggest that connections between the OFC and BLA are vital for representing specific rewards, in this case provided by Pavlovian conditioned stimuli, and using this information to guide reward seeking, with mu opioid receptor activation in the BLA potentially working to modulate this excitatory circuit.

Disclosures: N.T. Lichtenberg: None. **V.Y. Greenfield:** None. **A.S. Wang:** None. **K.M. Wassum:** None.

Poster

258. Reward: Motivational Mechanisms I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 258.06/Y17

Topic: F.03. Motivation and Emotion

Support: T32 DA007244

NIH DA034021

Title: Comparison of rapid dopamine signaling dynamics in the nucleus accumbens core and shell during a magnitude-based decision making task

Authors: *D. A. SACKETT[1], M. P. SADDORIS[2], X. WANG[1], R. M. CARELLI[1]; [1]Psychology Dept., UNC Chapel Hill, Chapel Hill, NC; [2]Univ. of Colorado, Boulder, Boulder, CO

Abstract: To maximize resources, organisms must choose actions that result in the most valuable outcome available and maintain that information to guide future behaviors. Integral to this decision making process is the mesolimbic dopamine system, including the nucleus accumbens (NAc) and its dopaminergic input from the VTA. In the rat, the NAc is divided into two discrete subregions, the core and the shell, believed to process information about reward learning, reward value, and decision making related to goal-directed actions. However, the precise role of those subregions in processing information about magnitude-based decisions remains unclear. Here, dopamine (DA) release was first measured in the NAc shell using fast-scan cyclic voltammetry (FSCV) during a magnitude-based decision making task. Male Sprague-Dawley rats (n= 7) were trained to lever press following distinct visual cues that predicted the magnitude of future rewards. On Forced Choice Low Magnitude trials, a cue light predicted the opportunity to press a lever for a small reward (one 45mg sucrose pellet). On Forced Choice High Magnitude trials, another distinct cue light predicted the opportunity to press a different lever for a large reward (two 45 mg sucrose pellets). Lastly, on Free Choice trials, both cue lights and levers were presented and rats were able to choose between both magnitude options. All rats accurately discriminated between cue types on Forced Choice trials and developed preferences for the high magnitude option on Free Choice trials. Electrochemical recordings from electrodes in the NAc shell show increases in rapid DA release following presentation of Forced Choice cues, with peak DA concentrations being significantly greater during the high forced cue. However, results on the Free Choice trials indicate that peak DA during the choice cue was the same regardless of whether the animal subsequently chose the large or small magnitude option. In an ongoing second study, rapid dopamine release is being monitored in the NAc core in another set of rats during the same task. Preliminary results (n= 2) suggest a similar trend in dopamine release dynamics in the NAc core to Forced and Free Choice cues. The current findings implicate the NAc shell in encoding comparative reward value during reward

magnitude-based decision making, and ongoing studies will confirm if the NAc core plays a similar role in this process. Supported by NIH DA034021 to RMC.

Disclosures: **D.A. Sackett:** None. **M.P. Saddoris:** None. **X. Wang:** None. **R.M. Carelli:** None.

Poster

258. Reward: Motivational Mechanisms I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 258.07/Y18

Topic: F.03. Motivation and Emotion

Support: NIH Grant DA034021

Title: Examination of a history of cocaine self-administration on rapid dopamine signaling during a delay discounting task

Authors: *T. M. MOSCHAK, D. R. TERRY, R. M. CARELLI;
Psychology, Univ. of North Carolina, Chapel Hill, NC

Abstract: Cocaine use has been associated with heightened impulsivity in both humans and rodents. However, the precise neuronal mechanisms through which these two behaviors are related remains unclear. Several brain regions integral to impulsive control are altered by cocaine use, including the nucleus accumbens (NAc) and its dopaminergic input. Indeed, prior work from this lab has shown that rapid dopamine (DA) signaling in the NAc core tracks reward value during delay discounting, an impulsive decision making task. To examine the relationship between cocaine history, impulsive behavior and rapid DA release dynamics, we trained animals (n= 24) in a delay discounting task where they learned that distinct cues signaled the opportunity to choose between a small reward available immediately after a response versus a large reward that was available after either no delay (0 sec), a short delay (10 sec), or long delay (20 sec). Next, rats were trained to self-administer either intravenous cocaine (n=12) or water (n=12) during 2 hr daily sessions for two weeks. Animals were tested on the delay discounting task immediately after completion of the two weeks of self-administration and following an additional 3 week abstinence period (rats put in home cage, no drug). In a subset of rats (cocaine: n = 4; water: n = 3), rapid DA release was measured in the NAc core using fast-scan cyclic voltammetry during delay discounting. All rats exhibited typical delay discounting behavior, shifting preference from the large reward to the small reward as the delay to the large reward increased. Contrary to other findings, however, a history of cocaine self-administration did not significantly alter delay discounting behavior compared to water controls when tested either immediately after completion of the two weeks of self-administration or after an additional 3 week abstinence period. Nonetheless, consistent with prior work in our lab (Saddoris et al., Biol Psyc, 2014), cues predictive of available choices evoked DA release that scaled with the rat's

preferred choices and dynamically shifted as delay to reinforcement for the large reward increased. Interestingly, although a history of cocaine did not alter delay discounting behavior, cocaine-experienced rats released significantly less DA to the cues signaling available choices in the task than did water rats. These preliminary data suggest that while a history of cocaine self-administration did not make rats more impulsive, it did dampen DA processing of cues that signaled the availability of small immediate versus large delayed rewards. Supported by: DA034021

Disclosures: **T.M. Moschak:** None. **D.R. Terry:** None. **R.M. Carelli:** None.

Poster

258. Reward: Motivational Mechanisms I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 258.08/Y19

Topic: F.03. Motivation and Emotion

Support: NIH Grant DA037733 to EAW

NIH Grant DA014339 to RMC

Title: Nucleus accumbens subregions (core vs shell) differentially encode reward-associated cues following reinforcer devaluation

Authors: *E. A. WEST, E. L. THOMAS, R. M. CARELLI;
Psychology Dept., Univ. of North Carolina, Chapel Hill, NC

Abstract: Nucleus accumbens (NAc) neurons encode features of stimulus learning and action selection associated with rewards. Additionally, the NAc is necessary for using information about expected outcome values to guide behavior as measured by reinforcer devaluation tasks. Further, evidence suggests that the distinct subregions of the NAc (core and shell) may play unique roles in guiding motivated behavior. Here, we recorded neural activity in the NAc core and shell during training (after cue-outcome associations were established) and performance of a reinforcer devaluation task. Specifically, male Long-Evans rats (n=25) were trained to press a lever following an illuminated cue light that predicted a specific reinforcer (e.g., raspberry flavored pellet). Rats received an alternative reinforcer in their home cages following training (e.g., peanut butter food pellets). Once rats achieved 90% accuracy during training, they were probed in a devaluation test under extinction conditions. Specifically, each rat was allowed ad libitum access to one of the two foods (selective satiation). On a separate day, the other food was devalued (counterbalanced). Rats lever pressed significantly less when the same reinforcer received during training was devalued (66.9 +/- 8.6) compared to devaluation of the alternative reinforcer (nondevalued, 86.8 +/- 9.3; $p<.05$) showing successful outcome specific devaluation. We recorded NAc neural activity on the last day of training, as well as the two test days

(devalued vs nondevalued). We found that in the NAc core (but not shell) there was a significant correlation between the percentage of neurons that encoded the cue on the last day of training and subsequent behavioral performance following outcome devaluation (i.e., the ability of rats to stop responding when the outcome had been devalued). Further, in the NAc shell (but not core), we observed a significant decrease in the percentage of NAc neurons that showed phasic responsiveness (i.e., cells that either increased or decreased firing) to the reward-associated cue when the same reinforcer received during training was devalued (5 out of 79, 6%) compared to the satiation of the alternative reinforcer (nondevalued, 21 out of 86, 24%). These data suggest that NAc core and shell neurons differentially encode information about reward-associated cues following outcome devaluation. Specifically, NAc core neural encoding during training predicts behavioral performance on subsequent test days. In contrast, the NAc shell dynamically encodes information about the cue with respect to the current value of the outcome.

Disclosures: E.A. West: None. **E.L. Thomas:** None. **R.M. Carelli:** None.

Poster

258. Reward: Motivational Mechanisms I

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 258.09/Y20

Topic: F.03. Motivation and Emotion

Support: DFG HU 306/27-3

DFG SO 1032/5-1

EU FP7 (MC-ITN-"In-SENS"-ESR7 607616)

CAPES 8816/11-5

DAAD scholarship

Title: Dopamine decreases during extinction and increases during signaled food reward in core, but not shell region of NAc

Authors: *J. P. HUSTON[1], C. BIESDORF[2], A.-L. WANG[1], B. TOPIC[1], D. PETRI[1], H. MILANI[2], M. A. DE SOUZA SILVA[1];
[1]Univ. of Dusseldorf, Dusseldorf, Germany; [2]Dept. of Pharmacol. and Therapeut., State Univ. of Maringá, Maringá, Brazil

Abstract: Microdialysis studies in rat have generally shown that appetitive stimuli release dopamine (DA) in the nucleus accumbens (NAc) shell and core. Here we examined the release of DA in the NAc during delivery of reward (food) and during extinction of food reward in the freely moving animal by use of *in vivo* microdialysis and HPLC. Fifty-two male Wistar rats were

trained to receive food reward associated with appearance of cue-lights in a Skinner-box during *in vivo* microdialysis. Different behavioral protocols were used to assess the effects of extinction on DA and its metabolites. Results Exp.1: (a) During a 20-min period of cued reward delivery, DA increased significantly in the NAc core, but not shell subregion; (b) For the next 60 min period half of the rats underwent immediate extinction (with the CS light presented during non-reward) and the other half did not undergo extinction to the cue lights (CS was not presented during non-reward). DA remained significantly increased in both groups, providing no evidence for a decrease in DA during extinction in either NAc core or shell regions. (c). In half of the animals of the group that was not subjected to extinction, the cue lights were turned on for 30 minutes, thus, initiating extinction to cue CS at a 1h delay from the period of reward. In this group DA in the NAc core, but not shell, significantly decreased. Behavioral analysis showed that while grooming is an indicator of extinction-induced behavior, glances toward the cue-lights (sign tracking) are an index of resistance to extinction. Results Exp. 2: (a) As in Exp. 1, during a 30-min period of cued reward delivery, DA levels again increased significantly in the NAc core but not in the NAc shell. (b) When extinction (the absence of reward with the cue lights presented) was administered 24 hrs after the last reward session, DA again significantly decreased in the NAc core, but not in the NAc shell. Conclusions: a) These results confirm the importance of DA release in the NAc for reward-related states, with DA increasing in the core, but not shell subregion. b) They provide first evidence that during the withholding of expected reward, DA decreases in the NAc core, but not shell region. c) This decrease in DA appears only after a delay between delivery of reward and extinction likely due to it being masked by persisting post-reward DA release. We hypothesize the decrease in extinction-induced release of DA in the NAc core to be a marker for the despair/depression that is known to accompany the loss of expected rewards/reinforcers.

Disclosures: J.P. Huston: None. **C. Biesdorf:** None. **A. Wang:** None. **B. Topic:** None. **D. Petri:** None. **H. Milani:** None. **M.A. de Souza Silva:** None.

Poster

258. Reward: Motivational Mechanisms I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 258.10/Y21

Topic: F.03. Motivation and Emotion

Support: Life Sciences Fellowship

Title: Assessing macronutrient diet preference in rats selectively bred to run long vs short distances

Authors: *J. R. LEE, J. MUCKERMAN, B. WAHLE, A. WRIGHT, F. BOOTH, M. WILL; Univ. of Missouri, Columbia, MO

Abstract: The current study used two novel Wistar rat phenotypes, developed by selectively breeding for either high- or low-levels of voluntary running (HVR and LVR). As of the 10th generation, HVRs ran approximately 10-fold greater distances compared to the LVRs, providing a unique model to examine influence of inactivity on diet preference and feeding behavior. Previous animal studies suggest an interaction between voluntary exercise and diet preference. Recent experiments from our lab found that in an acute diet preference test, HVRs demonstrate a slight preference for a high-fat diet, while the LVR rats strongly preferred a high-carbohydrate diet. However, six weeks of voluntary running or sedentary home cage environment had no effect on this trend. The current study extends these findings by examining home cage diet preference in both male and female HVR, LVR, and outbred rats provided ad lib access to high-fat (60%), high-sucrose (33%), and a high-corn starch (50%) diet for four weeks. Animals were housed with either access to a running wheel, starting one week prior to the diet testing, or sedentary conditions throughout the study. At the conclusion of four weeks, animals were analyzed for various biomarkers associated with chronic consumption of the diets. Microbiome samples were collected for analysis before the introduction of diets and after the four-week diet preference. Present data reveal a heterogeneous pattern of diet preference across outbred population. The outbreds demonstrate a preference for the high-fat diets, but also consume both types of high-carbohydrate diets. Like the outbred rats, LVRs also prefer the high-fat diet but consume the other two diets as well. However, there is less variation of consumption patterns within the LVR phenotype. Unlike the outbred or LVR rats, HVRs have only consumed the high-fat diet, avoiding both types of high-carbohydrate diets. Analysis of the influence of voluntary exercise versus sedentary conditions are ongoing.

Disclosures: J.R. Lee: None. **J. Muckerman:** None. **B. Wahle:** None. **A. Wright:** None. **F. Booth:** None. **M. Will:** None.

Poster

258. Reward: Motivational Mechanisms I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 258.11/Y22

Topic: F.03. Motivation and Emotion

Support: European Regional Development Fund "Food Cognition Model Systems"

Title: Neural responses to subjective and objective properties of food during a reward motivation task

Authors: *J. WEGMAN[1], E. KETEL[1], I. VAN LOON[1], M. E. VAN BOCHOVE[1], D. SCHUTTER[1], P. SMEETS[2,3], J. H. F. BULT[4], R. COOLS[5], E. AARTS[1];
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Med. Ctr. Utrecht, Utrecht, Netherlands; [4]NIZO Food Res., Ede, Netherlands; [5]Donders Inst. for Brain, Cognition and Behaviour, Radboudumc, Nijmegen, Netherlands

Abstract: Food choice is driven to a large extent by anticipated effects of food [1]. Previous research found that the vmPFC and striatum tracks caloric density of foods, using a wide range of low- and high-caloric food pictures [2]. Here, we investigate neural representations of food reward anticipation based on subjective food valuations and caloric density, relying purely on taste and using foods from a single category. The current study revealed distinct roles for the globus pallidus and the ventral striatum in these respective processes. Outside the MR scanner, participants (N=35; normal to overweight; 18 men) learned picture cue-taste mappings through a forced-choice memory training. Cues were coupled to the following food products: three commercially available ketchups that differed in caloric content, a control ketchup (mashed tomatoes), and chocolate mousse. Participants were only informed that they would be tasting different ketchups and a chocolate mousse. Next, we obtained participants' valuation of the food items through a willingness to pay measure [3]. In the fMRI session, a motivation task was used in which participants worked to obtain food rewards, while hungry. In each trial, one of the trained cues indicated which food could be earned with fast and accurate responses. In the case of a hit, they would earn one gram of toasted bread with the ketchup distributed on it. At the behavioral level, we observed a significant positive correlation between willingness to pay and calories of the food products. On the neural level, we compared responses to the taste cues of the four different ketchup tastes and related this to the subjective valuation (willingness to pay) and caloric content of the foods, within the same model. A region in the right globus pallidus was linearly activated with monetary bids for the foods. BOLD signal in the ventral striatum/subgenual cingulate, on the other hand, was positively correlated to the caloric content of the ketchups that the cues represented. These findings are reminiscent of proposed distinct roles of these brain regions, where the ventral pallidum plays a role in ‘liking’ for rewards, and the ventral striatum in ‘wanting’ food rewards [4]. Our results suggest different neural representations of subjective and objective properties of food products, based purely on taste and in the absence of top-down marketing influences. [1] Brunstrom (2007). Appetite. [2] Tang et al. (2014). Psychol Sci. [3] Becker et al. (1964). Behav Sci. [4] Berridge (2009). Physiology & Behavior.

Disclosures: J. Wegman: None. **E. Ketel:** None. **I. van Loon:** None. **M.E. van Bochove:** None. **D. Schutter:** None. **P. Smeets:** None. **J.H.F. Bult:** None. **R. Cools:** None. **E. Aarts:** None.

Poster

258. Reward: Motivational Mechanisms I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 258.12/Y23

Topic: F.03. Motivation and Emotion

Support: NIMH Grant R01MH097718

Title: MK212 disrupts maternal response through suppressing maternal motivation in rats

Authors: *R. WU, M. LI;
Dept. of psychology, Univ. of Nebraska-Lincoln, Lincoln, NE

Abstract: Abstract Our previous study has shown that activation of 5-HT_{2A} or 5-HT_{2C} receptors disrupts maternal behavior in rats. However, whether this disruptive effect was conducted through suppressing maternal motivation remains unclear. It has been shown that pup-separation treatment can significantly increase the maternal motivation. Here, we examined how activation of 5-HT_{2A} or 5-HT_{2C} receptors may influence the expression of maternal behavior under pup-separation condition. As a comparison, activation of both receptors was also examined in a food-holding test (a nonmaternal motivated task). On postpartum days (PPD) 4 and 6, Sprague-Dawley mother rats were injected with vehicle (0.9% saline), 5-HT_{2A} agonist TCB-2 (5.0 mg/kg), or 5-HT_{2C} agonist MK212 (2.0 mg/kg), and their maternal behaviors were tested under either pup-separation (pups were removed from their mothers for 4 h) or non-pup-separation condition, at 30 min before and 30 min, 2 h and 4 h after injection. On PPD 8 and 10, these rats were injected with vehicle, TCB-2, or MK212 and their food pellet retrieval behavior was tested under either food-deprivation (6 h) or non-food-deprivation condition. Injection of TCB-2 and MK212 time-dependently disrupted maternal behavior (e.g. pup retrieval). However, MK212-induced disruptions, but not those induced by TCB-2 were significantly attenuated by pup separation. On the other hand, both TCB-2 and MK212 significantly increased latency of food pellet approach and decreased the number of food pellets retrieved, independent of the food deprivation condition. These findings suggest that activation of 5-HT_{2C} impairs maternal behavior partially inhibiting maternal motivation. **Keywords** 5-Hydroxytryptamine 2A receptor, 5-Hydroxytryptamine 2C receptor, Maternal behavior, TCB-2, MK212, pup separation. This study was funded by the NIMH grant (R01MH097718) to Professor Ming Li.

Disclosures: **R. Wu:** A. Employment/Salary (full or part-time):; Full time. 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.; Research Grant. **M. Li:** None.

Poster

258. Reward: Motivational Mechanisms I

Location: Hall A

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Topic: F.03. Motivation and Emotion

Support: DS was supported by BNL LDRD 10-023 to Congwu Du, NIH R21DA029245 to DS and RF SUNY.

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Title: Positive reinforcement learning is impaired in rats bred for helplessness and not reversible by monoamine oxidase (MAO)-B inhibitor deprenyl

Authors: *D. SCHULZ[1], D. PETRI[2], J. P. HUSTON[2], F. A. HENN[3];
[1]Dept. of Psychology, Yeditepe Univ., Istanbul, Turkey; [2]Ctr. for Behavioral Neurosci., Univ. of Duesseldorf, Duesseldorf, Germany; [3]Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Principles of negative reinforcement learning may play a critical role in the etiology and treatment of depression. For example, the removal of an escape platform in the water maze induces not only extinction of a negatively reinforced response, but also depression-like behavior. Conversely, rats selectively bred for helplessness, a congenital animal model of depression, are severely impaired in the acquisition of a negatively reinforced lever press response. In the present study, we examined the integrity of positive reinforcement processes in cLH rats using a random ratio (RR) schedule and a devaluation-extinction procedure. Furthermore, we tested whether the monoamine oxidase (MAO)-B inhibitor deprenyl which reversed the deficits of cLH rats in negative reinforcement learning, would also reverse any deficits in positive reinforcement learning. We found that cLH rats (n = 9) were impaired in the acquisition of even simple operant contingencies, such as a Fixed Interval (FI) 20 schedule. cLH rats exhibited no apparent deficits in reward valuation or 'hedonia'. For example, they reacted to the devaluation of food in a manner consistent with a dose-response relationship. Reinforcer motivation as assessed by the number of lever presses across sessions with decreasing reward probabilities was highest in congenital non-helpless (cNLH, n = 10) rats as long as the reward probabilities remained relatively high. cNLH compared to wild type (WT, n = 10) rats were also more resistant to extinction across sessions. Compared to saline (n = 5), deprenyl (n = 5) reduced the duration of immobility of cLH rats in the forced swimming test, indicative of antidepressant effects, but it did not restore any deficits in the acquisition of a FI 20 schedule. We conclude that positive reinforcement learning was impaired in rats bred for helplessness due to anergia or a learning deficit, but not anhedonia. Deprenyl which exerted antidepressant effects in cLH rats did not reverse the deficits in positive reinforcement learning.

Disclosures: D. Schulz: None. **D. Petri:** None. **J.P. Huston:** None. **F.A. Henn:** None.

Poster

258. Reward: Motivational Mechanisms I

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Program#/Poster#: 258.14/Y25

Topic: F.03. Motivation and Emotion

Support: DA003906

DA012513

Title: Opposing roles for dopamine D1 and D2 receptor expressing accumbens medium spiny neurons in cocaine induced neuroplasticity and reinstated cocaine seeking

Authors: *J. A. HEINSBROEK[1], Y. M. KUPCHIK[2], W. C. GRIFFIN III[1], P. W. KALIVAS[1]; [1]Med. Univ. of South Carolina, Charleston, SC; [2]Dept. of Med. Neurobio., Inst. for Med. Res. Israel-Canada, The Hebrew Univ., Jerusalem, Israel

Abstract: Medium spiny neurons (MSN) in the nucleus accumbens link internal motivational states and environmental information to motor action within the addiction circuit. Classically, dopamine D1-receptor expressing MSNs in this region are thought to drive motivated behavior through their projections to the ventral mesencephalon. In contrast D2-expressing MSN are canonically thought to inhibit and refine motivational drive through a circuit comprising the ventral pallidum (VP) and subthalamic nucleus. Recent data from our lab demonstrates, however, that both D1- and D2-expressing neurons project to the VP and that their projection from nucleus accumbens to ventral pallidum, but not to ventral mesencephalon is critical for reinstatement of cocaine seeking behavior (Stefanik *et al.*, 2013). In addition, we recently demonstrated that after withdrawal from cocaine self-administration GABA-mediated LTD is lost in accumbens to ventral pallidum synapses due to an elevated enkephalin tone onto presynaptic μ-opiod receptors (Kupchik *et al.*, 2014). These observations demonstrate that the circuitry driving cocaine related behavior is more nuanced than previously thought. To assess whether cocaine self-administration diminishes LTD in D1- or D2-MSN projections to the VP, we expressed channelrhodopsin 2 in the nucleus accumbens of D1-Cre or D2-Cre driver mouse lines. Following ten days of cocaine self- administration and subsequent extinction acute brain slices were taken and a LTD protocol was run while recording from VP neurons. In cocaine-extinguished mice, μ-opioid driven LTD was abolished in the D2-to-VP projection while the D1-to-VP projection was unaffected, suggesting that cocaine selectively alters D2-driven plasticity in the VP. To test the hypothesis that altered D2-MSN function plays a role in the motivation to seek cocaine, we selectively expressed designer receptors exclusively activated by designer drugs (DREADDs, activated by clozapine-N-oxide) in D1-Cre or D2-Cre mice to modulate the activity of D1- or D2-MSN at the level of the nucleus accumbens. Inhibiting D2-MSN using the inhibitory Gi-coupled DREADD hM4D strongly potentiated cue induced reinstatement of cocaine seeking while inhibiting D1-MSN did not have a profound effect. Conversely, preliminary data suggests that activation of D1-MSN using the excitatory Gs-coupled rM3D DREADD drives reinstated behavior. Future studies will be aimed at the dissection of D1-to-VP and D2-to-VP contributions to cue induced cocaine seeking at the level of the ventral pallidum.

Disclosures: J.A. Heinsbroek: None. **Y.M. Kupchik:** None. **W.C. Griffin III:** None. **P.W. Kalivas:** None.

Poster

258. Reward: Motivational Mechanisms I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 258.15/Y26

Topic: F.03. Motivation and Emotion

Support: NIH DK098709

NIH DA037689

Shirley and Stefan Hatos Neuroscience Research Foundation

Title: Junk food consumption induces abnormal food-seeking responses to environmental stimuli in rats

Authors: A. R. KOSHELEFF[1,2], K. QUIZON[2], J. ZHOU[2], J. HSUEH[2], A. LE[2], S. B. OSTLUND[3], N. T. MAIDMENT[2], *N. P. MURPHY[2];
[1]Psychology, [2]Psychiatry and Biobehavioral Sci., UCLA, Los Angeles, CA; [3]Anesthesiol. and Perioperative Care, Univ. of California, Irvine, CA

Abstract: The pervasiveness of highly palatable, energy-dense foods in modern diets is considered a leading cause of obesity, which has become a significant public health concern. While the legitimacy of "food addiction" is currently under debate, overeating shares many characteristics with drug addiction, such as compulsive pursuit despite potentially dire health consequences. Cues associated with palatable foods (e.g., auditory cues such as commercial jingles, etc.) can trigger food-seeking, even when sated, which could lead to decreased inhibitory control over food intake. Here, we explored whether a junk food diet dysregulates rats' ability to respond appropriately to food-paired cues in a Pavlovian-to-instrumental transfer test. Rats were first trained to lever press for a food reward. Subsequently they learned to associate free delivery of the reward with an auditory cue. Rats were then exposed to either normal chow (Control rats) or chow and a junk food diet for either 2 hrs (Binge rats) or 24 hrs (All-Day rats) per day, for up to 6 weeks. At test, rats were sated for 1 hour on chow, and presented with food-paired (CS+) and neutral (CSo) cues, and lever presses and food-cup entries recorded. Control rats increased lever pressing and food-cup entries at the onset of the CS+, but not the CSo, as expected. Binge rats increased lever pressing to both the CS+ and the CSo, though food-cup entries increased only during the CS+. All-Day rats showed markedly reduced lever pressing and food-cup entries to both cues. These results suggest that chronic junk food consumption induces atypical responding to environmental stimuli predictive of food rewards, and that different dietary access produces unique behavioral abnormalities in response to environmental stimuli. Specifically, Binge rats appear to generalize the excitatory properties of reward-paired cues to other, neutral cues, inappropriately triggering food-seeking, while All-Day rats appear insensitive to the motivational properties of the CS+. These data emphasize that junk food diets induce aberrant food-seeking in response to environmental cues, and this action may contribute to maladaptive feeding behavior leading to obesity.

Disclosures: **A.R. Kosheleff:** None. **K. Quizon:** None. **J. Zhou:** None. **J. Hsueh:** None. **A. Le:** None. **S.B. Ostlund:** None. **N.T. Maidment:** None. **N.P. Murphy:** None.

Poster

258. Reward: Motivational Mechanisms I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 258.16/Y27

Topic: F.03. Motivation and Emotion

Support: M.J. Murdock Charitable Trust

Title: Differential effects of ghrelin on alcohol intake in C57BL/6J mice and Sprague Dawley rats

Authors: E. T. BROCKWAY[1], J. A. SELVA[1], L. J. ZALLAR[1], E. E. GARLING[1], H. M. BAUMGARTNER[1], M. B. SHESKIER[1], I. MORALES[1], R. PASTOR[1,2], *P. J. CURRIE[1]; [1]Dept. Psychology, Reed Col., Portland, OR; [2]Area de Psicobiología, Univ. Jaume I, Castellón, Spain

Abstract: Recent research in rats indicates that ghrelin receptor (GHS-R1A) antagonism decreases voluntary alcohol (ethyl alcohol; EtOH) consumption and EtOH operant self-administration. The same pharmacological agent used in rats, the GHS-R1A antagonist JMV2959, reduced EtOH intake in mice, and a similar suppression of EtOH drinking was found in ghrelin knockout (KO) mice. Ghrelin KO mice also showed reduced EtOH-induced conditioned place preference and locomotor stimulation. Blockade of central ghrelin signaling using mice lacking GHS-R1A was, additionally, found to decrease EtOH intake. Overall, these data represent a growing body of literature indicating that ghrelin mediates key aspects of EtOH reward via the GHS-R1A. Some previous results in mice, however, showed that systemic injections of ghrelin (10 or 30 mg/kg; ip) did not alter EtOH intake when measured using alcohol-preferring C57BL/6J (B6) mice and a binge-like limited access drinking test. This study, nevertheless, did show an increase in food intake induced by ghrelin (indicating that those doses of ghrelin were physiologically relevant) and a trend towards increased EtOH drinking. In the present study we used male and female B6 mice to investigate whether a slightly higher dose of systemic ghrelin (35 mg/kg), and a shorter drinking test (2 vs 4 h) would help identifying an effect of ghrelin on EtOH intake. Consistent with the previous study, our results indicated that systemic ghrelin did not alter EtOH intake in B6 mice. We then investigated the potential stimulatory effect of ghrelin on EtOH intake in male Sprague Dawley rats. The peptide was administered systemically or directly into the ventral tegmental area (VTA), the nucleus accumbens, or the hypothalamic arcuate nucleus at the onset of the nocturnal period. In this study we found that both systemic and VTA treatment increased EtOH intake at 2 and 6 h postinjection. Other brain areas appeared less responsive to ghrelin's action. Overall our work

demonstrates possible differential effects of ghrelin on alcohol reward in mice and rats with rats exhibiting robust responding to the stimulatory action of ghrelin on alcohol intake.

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Poster

258. Reward: Motivational Mechanisms I

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Program#/Poster#: 258.17/Y28

Topic: F.03. Motivation and Emotion

Support: NHMRC 1047899

Title: A role for the accumbens shell - lateral hypothalamus pathway in the inhibition of alcohol seeking

Authors: ***G. GIBSON**, G. P. MCNALLY;
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Abstract: The nucleus accumbens (Acb) plays a critical role in the expression of both reinstatement (i.e., relapse) and extinction (i.e., abstinence) of drug seeking. These roles have been observed across a variety of drug reinforcers. The accumbens shell sub-region (AcbSh) is implicated in these roles and past tracing experiments have implicated AcbSh interactions with the lateral hypothalamus (LH). Here we studied the role of the AcbSh-LH pathway in extinction and reinstatement of alcohol seeking. We applied AAV encoding eYFP or ChR2(H134R) to AcbSh and implanted bilateral fibre optic cannulae above LH. Rats were trained to self-administer 4% (v/v) alcoholic beer and then extinguished. Rats were tested for reacquisition of alcohol seeking and then later for locomotor activity and food intake. Photoactivation of the AcbSh-LH pathway had no effect on expression of extinction but significantly attenuated reacquisition, and not acquisition, of alcohol seeking. This reduction in relapse was observed on measures of alcohol seeking and alcohol con¬sumption. There was no effect of ChR2 stimulation of the AcbSh-LH pathway on locomotor activity or on food intake in hungry or sated rats. Thus, these results show that stimulation of the AcbSh-LH pathway protects against relapse. Subsequent experiments characterised the effects of this stimulation on well established, but never extinguished, alcohol seeking to determine if the protective effects of ChR2 stimulation required extinction training.

Disclosures: **G. Gibson:** None. **G.P. McNally:** None.

Poster

258. Reward: Motivational Mechanisms I

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Program#/Poster#: 258.18/Y29

Topic: F.03. Motivation and Emotion

Support: Strategic Research Program for Brain Sciences of the Ministry of Education, Culture, Sports, Science, and Technology of Japan

Title: Affective temperament and smoking history predict a response to anticipated reward in the ventral striatum

Authors: *Y. OGURA[1,2], Y. WAKATSUKI[1], T. MIYAMOTO[1], Y. TSUCHIDA[1,3], Y. NAKAI[1,2], A. TOYOMAKI[1], N. HASHIMOTO[1,4], T. INOUE[1,5], I. KUSUMI[1];
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Abstract: Major Depressive Disorder (MDD) can be associated to reduced response to reward in the striatum. Functional magnetic resonance imaging (fMRI) studies showed suppressed reward-related striatal response in MDD patients (Pizzagalli et al. 2009, Stoy et al. 2012; but see Knutson et al. 2008). Clinically, however, applying fMRI for all MDD patients is unrealistic. fMRI is unsuitable for the diagnosis due to large inter-individual variance in BOLD signals. On the other hand, questionnaires are easy to conduct and useful for the diagnosis in clinical practice. For example, the patient health questionnaire (PHQ)-9 is a reliable and valid measure of depression severity (Kroenke et al. 2001). Our previous study revealed that affective temperaments, which were measured by Temperament Evaluation of Memphis, Pisa, Paris and San Diego-autoquestionnaire version (TEMPS-A; Akiskal et al. 2005), directly and indirectly predict PHQ-9 score in the general population (Nakai et al. 2014). Affective temperaments could be a good predictor of the biological phenotype of depression. However, few studies have investigated the relationship between questionnaire scores associated with MDD and brain response measured by fMRI. We hypothesized that questionnaire scores, especially affective temperaments, predict the striatal response to reward. We administered fMRI and questionnaires to general adults (n = 63; M = 48, F = 15). In the fMRI study, we adopted a modified version of "monetary incentive delay task" (Knutson et al. 2000). The subjects were instructed to watch a number which corresponds to monetary reward he/she would receive if they succeeded in subsequent button press. We focused just on anticipatory phase and subtracted beta value of "¥500" minus "¥0" contrast in the ventral striatum. In the questionnaire study, we asked the subjects to complete 9 sets of questionnaires. We also questioned demographic data to subjects. We constructed linear model to estimate the effects of explanatory variables on the beta value, and conducted a stepwise model selection by AIC. The model with minimum AIC contained 6

explanatory variables; smoking history, TEMPS_anxiety, TEMPS_ cyclothymic, TEMPS_hyperthymic, Life Experiences Survey (LES; Sarason et al.1978)_negative events, and the total score of Child Abuse and Trauma Scale (CATS; Sanders and Becker-Lausen 1995). However, coefficients of TEMPS_hyperthymic, LES_negative events and CATS were not reliable. Therefore, (1) no smoking history, (2) higher TEMPS_anxiety, and (3) lower TEMPS_cyclothymic predict higher beta values in "¥500" minus "¥0" contrast. TEMPS-A might be related to the striatal function in general adult population.

Disclosures: Y. Ogura: None. **Y. Wakatsuki:** None. **T. Miyamoto:** None. **Y. Tsuchida:** None. **Y. Nakai:** None. **A. Toyomaki:** D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); Dainippon Sumitomo Pharma. **N. Hashimoto:** 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.; Astellas Pharma. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); Otsuka Pharmaceutical, Dainippon Sumitomo Pharma, Astellas Pharma. **T. Inoue:** 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.; Otsuka Pharmaceutical. F. Consulting Fees (e.g., advisory boards); GlaxoSmithKline, Pfizer, Astellas, EliLilly, Mitsubishi Tanabe Pharma, Mochida Pharmaceutical, Otsuka Pharmaceutical, Meiji Seika Pharma, Asahi Kasei Pharma, Shionogi, Janssen Pharmaceutical. Other; GlaxoSmithKline, Eli Lilly, Mochida Pharmaceutical, Mitsubishi Tanabe Pharma. **I. Kusumi:** 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.; Takeda Pharmaceutical, Astellas, Dainippon Sumitomo Pharma. F. Consulting Fees (e.g., advisory boards); Dainippon Sumitomo Pharma, Tanabe Mitsubishi Pharma. Other; Eli Lilly.

Poster

258. Reward: Motivational Mechanisms I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 258.19/Y30

Topic: F.03. Motivation and Emotion

Support: DA031900

DA032837

Title: A novel hypocretin receptor 1 antagonist, RTIOX-276, regulates cocaine self-administration and dopamine signaling in the nucleus accumbens core

Authors: *K. A. LEVY[1], J. K. SHAW[1], D. A. PERREY[3], Y. ZHANG[3], R. A. ESPAÑA[2]; [2]Neurobio. and Anat., [1]Drexel Univ. Col. of Med., Philadelphia, PA; [3]Res. Triangle Inst., Research Triangle Park, NC

Abstract: Accumulating evidence indicates that the hypocretins / orexins (HCRT) influence cocaine reinforcement via actions on the mesolimbic dopamine (DA) system. We previously demonstrated that blockade of the hypocretin receptor 1 with SB-334867 attenuates cocaine self-administration and reduces cocaine-induced enhancement of dopamine signaling in the nucleus accumbens core. The current study sought to access the utility of a novel hypocretin receptor 1 antagonist, RTIOX-276, which has a higher affinity and greater specificity for the hypocretin receptor 1 than SB-334867. We examined the effects of RTIOX-276 on self-administration of cocaine under a progressive ratio schedule of reinforcement. We also accessed whether RTIOX-276 decreases the effects of cocaine on dopamine signaling in the nucleus accumbens core using *in vivo* fast scan cyclic voltammetry. Results suggest that RTIOX-276 attenuates the motivation to self-administer cocaine and decreases cocaine-induced enhancement of dopamine signaling. Together with previous work using SB-334867, the current findings provide further evidence for hypocretin receptor 1 involvement in the regulation of reward and reinforcement processes, particularly as it relates to cocaine. Therefore, the hypocretin receptor 1 may be a viable target for development of pharmacotherapies for the treatment of cocaine addiction.

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Poster

258. Reward: Motivational Mechanisms I

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Program#/Poster#: 258.20/Y31

Topic: F.03. Motivation and Emotion

Support: FAPESP 2013/24986-2

NIH-IRP

Title: A new tool to exam molecular alterations in synapses of neuronal ensembles

Authors: *F. C. CRUZ[1], R. M. LEAO[2], P. C. BIANCHI[2], P. CARNEIRO-DE-OLIVEIRA[2], V. P. SELVAN[3], G. M. A. LIMA[1], F. C. COSTA[1], C. S. PLANETA[2], B. T. HOPE[3]; [1]Univ. of Sao Paulo, USP, Sao Carlos, Brazil; [2]PANT, Sch. of Pharmaceut. Sci., Lab. of Pharmacol., Universidade Estadual Paulista - UNESP, Araraquara, Brazil; [3]Behavioral Neurosci. Br., NIH, NIDA/IRP/NIH/DHHS, Baltimore, MD

Abstract: Learned associations between drug effects and stimuli in the drug environment (context) play an important role in drug addiction and are thought to be encoded by sparsely distributed patterns of neurons called neuronal ensembles that are selected by the drug-related stimuli. We are now developing methods for assessing unique alterations within pre- and post-synaptic components of synapses onto these activated neuronal ensembles. To selectively label post-synaptic components on only activated post-synaptic neuronal ensembles, we injected AAV1 virus with the transgene CMV::DIO-PSD95_Myc into nucleus accumbens of c-fos-tetop::iCre transgenic rats and injected the rats with 30 mg/kg cocaine four weeks later. The neural activity-dependent c-fos promoter in the rat transgene induces Cre recombinase protein expression that activates the DIO-PSD95-Myc viral gene in only strongly activated accumbens neurons. The fusion protein PSD95-myc is translocated to post-synaptic dendritic spines. Immunohistochemical labeling of Myc peptide indicated high levels of expression of PSD95_Myc in post-synaptic dendrites of activated neurons one week after the cocaine injection. To selectively label vmPFC presynaptic terminals in accumbens, we injected AAV1 virus with the transgene EF1α::Synaptophysin_Flag into vmPFC into wild-type rats. The fusion protein synaptophysin_Flag is translocated to presynaptic terminals. Immunohistochemical labeling of Flag peptide indicated high levels of expression of synaptophysin in vmPFC terminals in accumbens four weeks after the virus injection. Expression of both fusion proteins was confirmed using Western blotting and flow cytometry. For flow cytometry, synaptoneurosomes containing pre- and post-synaptic components were obtained from nucleus accumbens of virus-injected rats and immunolabeled for PSD95-Myc and Synaptophysin-Flag. Flow cytometry indicated expression of both fusion proteins in the nucleus accumbens as well as double-labeling of synaptoneurosomes with PSD95-Myc and Synaptophysin-Flag. Identification of synapses on activated neurons will allow us to assess unique synaptic alterations on activated neuronal ensembles that mediate context-induced reinstatement of ethanol-seeking.

Disclosures: **F.C. Cruz:** None. **R.M. Leao:** None. **P.C. Bianchi:** None. **P. Carneiro-de-Oliveira:** None. **V.P. Selvan:** None. **G.M.A. Lima:** None. **F.C. Costa:** None. **C.S. Planeta:** None. **B.T. Hope:** None.

Poster

259. Dynamic Circuitry of Stress and Anxiety

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 259.01/Y32

Topic: F.03. Motivation and Emotion

Support: NIH grant MH097320

Title: The temporal dynamics of fear learning: A single-trial fMRI study

Authors: *S. YIN[1], M. DING[1], A. KEIL[2], Y. LIU[3];
[1]Biomed. Engin., [2]Dept. of Psychology and Ctr. for the Study of Emotion & Attention, Univ. of Florida, Gainesville, FL; [3]Ctr. for Mind and Brain, Univ. of California, Davis, Davis, CA

Abstract: Research in rodent models has firmly established the amygdaloid complex as playing a central role in mediating defensive responses to conditioned threat cues. In human imaging studies, however, activation of the amygdala by conditioned stimuli is not always observed. Temporal habituation has been proposed as a possible explanation. In this study we sought to gain further insights into the problem by examining the detailed time course of neural responses during fear learning. Functional MRI was recorded from 18 subjects performing a classical differential fear conditioning task. The experiment consisted of three trial blocks: habituation, acquisition, and extinction. During the habituation block two Gabor patches (45° and 135°) were presented in random order; no response was required from the subject. During the acquisition block, one Gabor patch, denoted as CS+, was occasionally paired with an aversive human scream (US; 25% reinforcement rate), whereas the other Gabor patch (CS-) was never paired with the US. The extinction block followed the acquisition block. CS+ and CS- were presented without US pairing. Comparing BOLD activity evoked by the unpaired CS+ against the CS- during acquisition, ACC and insula, but not the amygdala, were found to be activated by CS+. To examine the temporal dynamics of the amygdala, ACC, and insula, the beta series method was employed to estimate the BOLD response to each cue presentation. We found that amygdala activity was significantly reduced over the second half of the acquisition block. Importantly, similar decrease was also found in dmPFC and insula. No such decrease was found in any structures in either habituation or extinction blocks. These results support the hypothesis that amygdala habituated over time during fear acquisition and demonstrated for the first time that such temporal habituation also occurred in dmPFC and insula.

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Poster

259. Dynamic Circuitry of Stress and Anxiety

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Program#/Poster#: 259.02/Y33

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Campbell Family Mental Health Research Institute Foundation Fund

Title: Light-induced anxiety-like behavior in the unpredictable chronic mild stress model in mice: a time-dependent trajectory based approach

Authors: K. MISQUITTA[1,2], M. BANASR[3], *E. SIBILLE[4,1];
[1]Campbell Family Mental Hlth. Res. Inst. of CAMH, Toronto, ON, Canada; [2]Pharmacol. and

Toxicology, Univ. of Toronto, Toronto, ON, Canada; [3]Psychiatry, Yale Univ., New Haven, CT; [4]CAMH - Univ. of Toronto, Toronto, ON, Canada

Abstract: Unpredictable chronic mild stress (UCMS) induces anxiety-like and depressive-like behaviors in rodents, and is a well-documented model used to investigate biological mechanisms related to depression. Classical behavioral tests designed to measure these deficits can be invasive and are rarely used repetitively. Our research analyzed the effects of UCMS on regular animal behavior and the data obtained provided information on the time-dependent trajectory of symptom development. Behavior was assessed weekly for 12 hours during the dark cycle, using Noldus Phenotyper apparatus in UCMS-exposed and control BALB/c (8-12 week-old, n=12/group) male mice. An anxiogenic white spotlight was applied for the duration of 1 hour over the food zone 4 hours into the dark cycle. Time spent within the designated zones (food, drinking and shelter) over the 12 hours was measured using EthoVision tracking software. After 5 weeks of UCMS, animals were tested in the elevated plus maze, open field, novelty suppressed feeding, coat state assessment, forced swim, sucrose consumption and cookie tests. Using the phenotypers, under baseline conditions UCMS-exposed mice exhibited a progressive and significant increase in time spent in the shelter zone [$F(1,22) = 17.223$; $p<0.001$] in disfavor of the two other zones. A 1 hour white spotlight challenge under the food zone induced reduced time spent in that zone in control mice compared to the hour before and after the white light application. Across weeks UCMS-exposed mice showed a progressive and significantly greater decrease in time spent in the food zone [$F(1,22) = 10.333$; $p<0.01$] compared to non-stressed mice. This newly designed light-induced anxiety test, demonstrates that UCMS induces a progressive effect, prominent during the 4th and 5th weeks of stress exposure. Upon completion of the chronic stress paradigm we confirmed heightened anxiety in the UCMS induced mice from the elevated plus maze [$F(1,19) = 11.603$; $p<0.05$] and novelty suppressed feeding [$F(1,19) = 5.733$; $p<0.05$]. Further studies will examine the effects of strain, sex and potential antidepressant therapeutic treatment to validate this new paradigm. Classical behavioral testing involves invasive, one-time readout for assessing the effects of UCMS. Regular behavioral monitoring allows for a simple approach to measure the progressive effects of UCMS-induced anxiety-like behavior over the course of stress exposure.

Disclosures: K. Misquitta: None. **M. Banasr:** 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.; Institut de Recherches Internationnales SERVIER (France), BioHaven (Connecticut, US). **E. Sibille:** None.

Poster

259. Dynamic Circuitry of Stress and Anxiety

Location: Hall A

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Program#/Poster#: 259.03/Y34

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Science Without Borders

CNPq

Science Foundation Ireland (SFI)

APC

Title: Hippocampal neuronal activation and neurogenesis in a pharmacological model of anxiety

Authors: *A. R. COSTA[1,2], B. R. LEVONE[3], C. LINO-DE-OLIVEIRA[4], T. G. DINAN[2], O. F. O'LEARY[2], T. C. M. DE LIMA[4], J. F. CRYAN[2];
[1]Univ. Federal De Santa Catarina, Florianópolis, Brazil; [2]Anat. and Neurosci., Alimentary Pharmabiotic Centre, Univ. Col. Cork, Cork, Ireland; [3]Anat. and Neurosci., Univ. Col. Cork, Cork, Ireland; [4]Farmacologia, Univ. Federal de Santa Catarina, Florianópolis, Brazil

Abstract: Understanding the neural basis of anxiety is important to develop novel therapeutic strategies. It is well known that the cholinergic system plays an important role in mediating emotional states related to anxiety, acting on limbic structures such as the hippocampus, amygdala and prefrontal cortex.It has recently been shown that the administration of a single systemic subconvulsant dose of the cholinergic agonist pilocarpine induce a long-term state of anxiety in rats lasting from 24 h to 3 months post-administration. This positions this paradigm to be a novel animal model of anxiety. The neural basis of these effects on anxiety are unclear. Increased neuronal activation and alterations in neurogenesis have both been implicated in anxiety. Thus, we investigated if such changes could subserve the behavioural alterations in the model. To this end, pilocarpine (150 mg/kg, i.p.) and vehicle-treated male Wistar rats (90 days of age) were exposed to the open arms of the elevated plus maze test as a stressor to induce neuronal activation patterns. Subsequently, c-Fos and NeuN/BrdU stainings were performed and used as an index for neuronal activation and neurogenesis respectively. Our data shows that the number of stress-induced c-Fos positive cells in the dorsal dentate gyrus was higher in pilocarpine treated compared with control animals. These results suggest that the increased stress responsivity of pilocarpine-treated rats could be due to an overactive dorsal hippocampus. In addition, the single treatment of pilocarpine increased the survival of newly-born neurons. This increase is observed in SGZ and hilus, but not in the granular zone, suggesting the occurrence of an ectopic neurogenesis which could alter hippocampal synaptic connections. These findings can help us understand how altered cholinergic signaling can lead to maladaptive responses to stress and induce anxiety and fear responses.

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Poster

259. Dynamic Circuitry of Stress and Anxiety

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Program#/Poster#: 259.04/Y35

Topic: F.03. Motivation and Emotion

Support: MH084906

MH048404

Title: PFC-VTA neuronal substrates for anxiety-related alteration of motivated behavior

Authors: *J. PARK[1], A. DEL ARCO[1], B. YU[2], B. MOGHADDAM[1];
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Abstract: In a variety of circumstances, motivated behavior leading to reward is associated with anxiety of unpredictable aversive outcomes; for instance, losing money in gambling or encountering a predator during foraging. Such risk of an aversive outcome must be appropriately represented and used for flexible adjustment of behavior given that either excessive risk aversion or risk taking may lead to maladaptive behavior. We investigated the neuronal representation of the anxiety-related changes in motivated behavior in two brain regions critically involved in encoding of action-outcome relationship: the prefrontal cortex (PFC) and the ventral tegmental area (VTA). The activity of PFC and VTA neurons was simultaneously recorded while rats performed a task consisting of three blocks in which an instrumental action (a nose poke) was rewarded with a sugar pellet continuously, but was intermittently punished with a mild electrical shock at varying risks (0, 6 or 10 %). A significant increase in action latency and portion of immobile time was observed in blocks with higher risk of punishment. In both regions, substantial proportions of single neurons, responding to critical task events, differentiated their firing rates as a function of the risk. The proportion of risk-encoding PFC neurons was greater than VTA neurons. To quantify the trial-to-trial discriminability of the punishment risk at the population level, we tested how accurately the risk could be decoded by considering the activity of neural population jointly in each region, using a Poisson naïve Bayes classifier. Decoding of PFC population yielded a higher accuracy than VTA population. These suggested that different neural population trajectories might underlie the risk-based behavioral alteration. Importantly, sizable trial-to-trial variability in action latency was observed, which may be associated with the trial-to-trial variability of the neural population trajectory. To test these, we extracted single-trial neural population trajectories of the simultaneously recorded PFC and VTA populations, using dimensionality reduction. When compared, the PFC population trajectories were associated with a more pronounced discriminability based on the risk, than that of the VTA population. Taken together, these findings indicate that both PFC and VTA neurons are involved in the anxiety-based control of motivated behavior, but that the PFC may better represent risk-based control of behavior.

Disclosures: **J. Park:** None. **A. Del Arco:** None. **B. Yu:** None. **B. Moghaddam:** None.

Poster

259. Dynamic Circuitry of Stress and Anxiety

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 259.05/Y36

Topic: F.03. Motivation and Emotion

Support: NIH R01 MH050479

NICHD T32 5 T32 HD 7289-29)

Title: The habenulo-raphe circuit is not sensitive to the dimension of behavioral control and mediates the anxiety-like response produced by tailshock

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Abstract: Exposure to uncontrollable stress or acute trauma is a critical etiological factor in the onset of anxiety-like behavioral states in humans and rats. Serotonergic (5-HT) neurons in the dorsal raphe nucleus (DRN) are activated during uncontrollable (inescapable) tail shock (IS), but not during controllable (escapable) tail shock (ES). This activation leads to sensitization of the DRN, resulting in acute anxiety-like behavior in rats. Moreover, activation of the DRN is both necessary and sufficient for the behavioral and neurochemical consequences of IS. The lateral habenula (LHb) provides the primary glutamatergic input to the DRN and dysregulation of the LHb and DRN is implicated in stress-related psychiatric disorders. In the present study, the role of the LHb in regulating the behavioral response to acute stress was examined. First, it was necessary to determine whether stress-induced activation of the LHb is modulated by the controllability of the stressor. ES and IS resulted in an equivalent increase in Fos protein in the LHb, as compared to home cage controls (HC). Next, Fos expression restricted to the LHb-DRN pathway was measured. The retrograde tracer Fluorogold (FG) was injected into the DRN and Fos protein was quantified in FG-positive cells in the LHb following ES, IS or HC. ES and IS yielded a similar increase in activation of the LHb-DRN pathway, with no effect of controllability of the stressor. Finally, an optogenetic strategy was implemented to determine whether silencing the LHb during IS would protect against the behavioral consequences of IS. Halorhodopsin silencing of the LHb during IS produced resistance to the anxiety-like behavioral outcome of IS, as measured in a juvenile social investigation test. These data suggest that the LHb modulates DRN activity during stress, and that silencing this pathway during uncontrollable stress may protect against stress-related psychiatric disorders.

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Poster

259. Dynamic Circuitry of Stress and Anxiety

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: JSPA Grant-in-Aid for Scientific Research 23590721

Title: Effects of neonatal dopamine depletion on anxiety-related behaviors and c-Fos expression in the dorsal raphe nucleus in adult rats

Authors: ***M. OGATA**, K. NODA, H. AKITA, H. ISHIBASHI;
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Abstract: Dopamine neurons originating in the substantia nigra and ventral tegmental area of the midbrain is involved in several brain functions, including motor control, attention and emotion. Rats with dopamine depletion during adulthood and neonatal period exhibited akinetic motor activity and spontaneous motor hyperactivity, respectively, indicating that behavioral effects of dopamine depletion depend on the period of lesion development. Ameliorative effects of amphetamine or methamphetamine treatment on the motor hyperactivity induced by neonatal dopamine depletion have been reported. Although several reports have shown that dopamine depletion during the adulthood results in a significant increase in anxiety-related behavior, effects of neonatal dopamine depletion on anxiety-related behavior in the adulthood are poorly understood. In the present study, we investigated responses to anxiogenic stimuli in the adult rats with neonatal dopamine depletion using the behavioral tests (i.e., open field test, light/dark box test and elevated plus maze test) and immunohistochemical analysis of c-Fos expression in the dorsal raphe nucleus. The adult rats that received intra-ventricular injection of 6-hydroxydopamine 4 days after birth showed significant increases in distance traveled and time spent in the center area in the open field test. Intraperitoneal treatment of methamphetamine (4 mg/kg) 40 min prior to the open field test ameliorated the increased distance traveled, but not the increased time spent in the center area of open field. Increased time spent in the open arm of elevated plus maze was also observed in the adult rats with neonatal dopamine depletion. There is no significant effect of neonatal dopamine depletion on the anxiety-related behavior in the light/dark box test. In the histological analysis, a significant increase in the number of c-Fos immunoreactive-positive neurons in the dorsal raphe nucleus was observed at 2 h after the elevated plus maze test in the adult rats with neonatal dopamine depletion. These data indicate that neonatal dopamine depletion results in a spontaneous motor hyperactivity and decrease in anxiety-related behavior during adulthood, and suggest that the mechanism of abnormal anxiety-

related behavior differs from that of spontaneous motor hyperactivity and that the neuronal hyperactivity in the dorsal raphe nucleus is implicated in the abnormal anxiety-related behavior induced by neonatal dopamine depletion.

Disclosures: **M. Ogata:** None. **K. Noda:** None. **H. Akita:** None. **H. Ishibashi:** None.

Poster

259. Dynamic Circuitry of Stress and Anxiety

Location: Hall A

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Program#/Poster#: 259.07/Y38

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Deep brain stimulation in Internal Capsule and ventral Striatum: Effects on spontaneous grooming and anxiety in Sapap3 mutant mice

Authors: C. PINHAL[1,2], F. SANTANA[1,2], L. FELLINGER[1,2], I. EHMER[1,2], R. HAMELINK[1,2], G. FENG[3], M. FEENSTRA[1,2], *I. WILLUHN[1,2], D. DENYS[1,2];
[1]Netherlands Inst. For Neurosci., Amsterdam, Netherlands; [2]Dept. of Psychiatry, Academic Med. Ctr., Amsterdam, Netherlands; [3]Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Background: Obsessive-compulsive disorder (OCD) is a psychiatric condition characterized by unwanted thoughts (obsessions), anxiety, and repetitive behaviors (compulsions). Recently, deep-brain stimulation (DBS) has been identified as a promising therapy for patients who are otherwise therapy-refractory. Clinical studies with DBS in the anterior limb of the Internal Capsule (ALIC) and ventral Striatum (vS) have shown to be effective. However, it often takes months to determine optimal stimulation parameters and some patients do not respond at all, indicating the need for a better understanding of DBS in OCD. The *Sapap3*$^{-/-}$ mutant mice are an animal model for OCD as they exhibit compulsion-like behavior (excessive grooming), increased anxiety, and abnormal cortico-striatal neurotransmission (Welch et al, 2007). In the present study, we investigated the effects of DBS in ALIC and vS on compulsion-like grooming and anxiety. **Methods:** *Sapap3*$^{-/-}$ mice were phenotyped and only mice grooming more than 13% of time were selected for further experiments. Mice were implanted with DBS electrodes (bilateral) in the ALIC or vS (Rodriguez-Romaguera et al, 2012). After recovery, mice were placed in an open field and stimulated for 60min (300µA, 80µs, 120Hz), while grooming was scored. Then, anxiety was assessed on an elevated plus maze. In this test, mice were stimulated for 20min in their home cages and then placed in the maze and stimulated for additional 10min. A few days later, mice were stimulated for 20min and perfused 90min thereafter. Brains were sectioned, and c-Fos expression was assessed using immunohistochemistry. **Results:** We demonstrate that DBS of the ALIC effectively reduces excessive self-grooming in *Sapap3*$^{-/-}$ mice, with stimulated animals showing a maximum decrease in levels of grooming to 7% of Sham-stimulated mice ($p<0.001$). In contrast, open-arm

exploration on the plus maze, a measure of anxiety, was not affected by DBS. Preliminary results suggest that DBS of the vS has no effect on grooming or plus-maze behavior. **Conclusion:** Our findings suggest that DBS in the ALIC reduces compulsivity in the *Sapap3*$^{-/-}$ mouse model of OCD, but does not affect unconditioned anxiety. Only "pathologically" increased levels of grooming were responsive to DBS, as no effect was seen in animals grooming less than 13% of the time. Together with our previous results showing that ALIC stimulation reduces conditioned anxiety in normal rats (van Dijk et al, 2013), the ALIC seems to be a good target for reducing OCD symptoms in rodents. An important future step is to clarify the network involved in the decrease of compulsivity via ALIC-stimulation to unravel the neurobiology of OCD.

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Poster

259. Dynamic Circuitry of Stress and Anxiety

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Program#/Poster#: 259.08/Y39

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Swiss National Science Foundation 31003A-141137

Title: Evidence for psychosocial stress-induced inflammation, altered dopamine status and impaired reward-directed behaviour in mice

Authors: *G. BERGAMINI[1], H. SIGRIST[1], S. AUER[1], J. MECHTERSHEIMER[1], T. SUTER[2], B. FERGER[4], E. SEIFRITZ[3], C. PRYCE[1];
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Abstract: Understanding the aetio-pathophysiology of depression is the route to identification of efficacious anti-depressant strategies. A major theory is that stress-induced inflammation is aetiological in depression and that one of its effects is to alter dopamine signaling leading to symptoms of maladaptive response to punishment and hyposensitivity to reward. In rodents and humans, stimulation of the immune system results in a number of behavioural changes which overlap both with those exhibited during infection/sickness and those that constitute symptoms of depression. They are mediated by circuitry to which the basal ganglia and dopamine (DA) function are central, suggesting that changes in the latter underlie the behavioural pathologies. Inflammatory cytokines can act in the brain to affect the monoamine neurotransmitter systems and dopamine signalling in the basal ganglia may be a primary target. The kynurenine-pathway is known to be activated by inflammatory signals as well as chronic stress and it has been

implicated in the pathophysiology of depression. It is hypothesized here that psychosocial stress in mice can induce a) peripheral and CNS inflammatory response, b) activation of the kynurenine pathway, c) attenuation of mesolimbic DA signaling and d) changes in reward-directed behaviour. Mice exposed to chronic social defeat (CSD) exhibited peripheral inflammatory responses including splenomegaly accompanied by increased splenic granulocytes, inflammatory monocytes and T helper 17 cells. CSD-induced peripheral activation of the kynurenine pathway was found in the liver. Immunohistochemical analysis in mesolimbic regions revealed microglia activation in the ventral tegmental area (VTA) in CSD mice. Regarding the effects of CSD on DA signaling, CSD mice showed decreased dopamine turnover (DOPAC/DA) in the nucleus accumbens (NAcc) and a reduced hyper-locomotor activity in response to a DA transporter inhibitor (GBR 12909). The operant behaviour tests, progressive ratio schedule (PRS) and learned non-reward (LNR), were used to assess CSD effects on reward-directed behaviour under demanding conditions. CSD mice obtained less rewards in the PRS test and more errors in the LNR test, compared to controls. Dopamine depletion in the NAcc - achieved using the neurotoxin 6-hydroxydopamine - also induced these operant effects, implicating mesolimbic DA dysfunction in CSD mice. These data support the stress-inflammation-dopamine hypothesis for depression and the model will be utilised to identify novel targets for restoring DA function as an antidepressant treatment. Funded by the Swiss National Science Foundation 31003A-141137.

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Poster

259. Dynamic Circuitry of Stress and Anxiety

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Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Support: Janssen Pharmaceutica research grant

Title: The development of a novel animal model of depression to study the implication of inflammation, stress and hippocampal neurogenesis

Authors: *K. MUSAELYAN[1], M. T. EGELAND[2], C. M. PARIANTE[2], C. FERNANDES[3], S. THURET[1];
[1]Basic and Clin. Neurosci., [2]Psychological Med., [3]MRC Social, Genet. & Developmental Psychiatry Ctr., Inst. of Psychiatry, KCL, London, United Kingdom

Abstract: A growing body of evidence supports the involvement of the immune system in depression neurobiology; however its role in the causality of depression and in the mechanisms of antidepressant response is not yet clear. To study the role of inflammation in depression and in the mechanisms of antidepressant action a valid animal model is required. To develop such a model, we combined exposure to an inflammatory stimulus modelled by peripheral lipopolysaccharide (LPS) injection with an environmental stress-based model of depression - unpredictable chronic mild stress (UCMS) to achieve a depression-like phenotype incorporating behavioural, immune and neurogenic changes seen in depression. In a pilot experiment we exposed adult male BALB/c mice to UCMS for 6 weeks. A behavioural test battery was applied at the end of UCMS. In a separate experiment mice were administered LPS (0.1 mg/kg, 0.33-0.83 mg/kg and 0.83 mg/kg treatment groups) or saline once weekly for 6 weeks via intraperitoneal injections. At the end of both experiments blood and brain tissues were collected for cytokine analysis, gene expression and immunohistochemistry (IHC) respectively. To access hippocampal neurogenesis IHC with Ki67 as a proliferation marker and doublecortin (DCX) as a neuroblasts marker were used. To detect microglial cells ionized calcium-binding adapter molecule 1 (Iba1) marker was used. UCMS exposed mice displayed significant coat state deterioration and locomotor hyperactivity in a novel arena, but did not display anxiety-like avoidance behaviour in an anxiogenic environment or behavioural despair in the forced swim test. Additionally, mice displayed altered food reward behaviour in sucrose preference, cookie and novelty suppressed feeding tests. LPS exposed mice displayed signs of sickness behaviour, such as reduced food intake and weight loss 24 hrs after injection, with the 0.33-0.83 mg/kg group showing persistent phenotypes throughout 6 weeks of treatment, as well as elevation of peripheral cytokines interleukin 6, tumour necrosis factor α and interleukin 2. These results showed that hyperlocomotion was the dominating phenotype induced by UCMS and this observed hyperactivity may have masked potential anxiety and behavioural despair responses. LPS treatment resulted in a sickness behavioural response and cytokine elevation, which persisted in the 0.33-83 mg/kg treatment group. Analysis of adult hippocampal neurogenesis, microglial activation and gene expression in the brain will further reveal the endophenotypes induced by this model and their relevance for depression.

Disclosures: **K. Musaelyan:** A. Employment/Salary (full or part-time):; PhD studentship from Janssen Pharmaceutica. **M.T. Egeland:** None. **C.M. Pariante:** 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.; Received a grant from Janssen Pharmaceutica. **C. Fernandes:** 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.; Received a grant from Janssen Pharmaceutica. **S. Thuret:** 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.; Received a grant from Janssen Pharmaceutica.

Poster

259. Dynamic Circuitry of Stress and Anxiety

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 259.10/Y41

Topic: C.16. Cognitive, Emotional, and Behavioral State Disorders

Title: Modulation of glutamatergic transmission by amphetamine in cognition related circuits of hippocampus and prefrontal cortex

Authors: *S. A. NEALE[1,2], P. H. HUTSON[3], T. E. SALT[2];
[1]Neurexpert, London, United Kingdom; [2]Visual Neurosci., UCL Inst. of Ophthalmology, London, United Kingdom; [3]Shire Pharmaceut., Wayne, PA

Abstract: An emerging body of evidence suggests the psychostimulant amphetamine (AMPH) may affect neuronal function by mechanisms additional to modulation of monoamine levels, such as modulation of glutamatergic neurotransmission1. Understanding these effects of AMPH may be of value when considering the potential therapeutic benefits of AMPH for treatment of psychiatric disorders1. The current study investigates actions of AMPH on neuronal activity in acute *in vitro* slice preparations of adult rat hippocampus and prefrontal cortex (PFC). In the hippocampus, field excitatory postsynaptic potentials (fEPSPs) were evoked by Schaffer collateral/commissural pathway stimulation and recorded from the CA1 region. In the PFC, fEPSPs were evoked by stimulation in the forceps minor and recorded in layer V/VI. In both regions the fEPSPs appeared to be predominantly AMPA receptor-mediated. In the CA1 region, AMPH (10 μM) did not significantly alter the mean fEPSP amplitude, although slice-to-slice variability suggested a heterogenous effect. AMPH (100 μM) increased the fEPSP amplitude. This effect increased during a 15 min washout period to 122 ± 2% of control (n=8; mean ± SEM; $P < 0.001$). Associated with the increase in amplitude there were significant effects on fEPSP slope and the time to peak. The effects of AMPH on the fEPSP amplitude, slope and time to peak were all significantly less in slices pre-incubated with the NMDA receptor antagonist D-AP5 (50 μM), suggesting these actions of AMPH involve, at least partially, NMDA receptor activation. In the PFC, AMPH (100 μM) produced a depression, rather than an increase, of the evoked fEPSP: following 15 minutes of AMPH application the fEPSP amplitude was reduced to 73 ± 7% of control amplitude (n = 7; mean ± SEM; $P < 0.05$). On washout of AMPH there was a small recovery in the amplitude to 80 ± 4% of baseline levels (n = 7; mean ± SEM; $P < 0.05$). FFT analysis of activity in the PFC revealed changes across biologically-relevant aspects of the power spectrum, including statistically significant increases in power in the gamma-frequency range. By contrast overall there was no significant effect of AMPH on the power spectrum in the CA1 region. In conclusion, these results show that AMPH can differentially affect glutamatergic transmission in different cortical circuits and that some of these actions appear to be via interactions with NMDA-receptor-mediated response components. Furthermore, the changes seen in the power spectrum suggest that the actions of AMPH affect neuronal circuit behaviour,

and such selective effects may be important in the effects of AMPH on cognition and attention. Hutson et al. Pharmacology & Therapeutics 143 (2014) 253-264

Disclosures: **S.A. Neale:** A. Employment/Salary (full or part-time):; Neurexpert Limited, London, UK. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-owner and share holder of Neurexpert Limited. **P.H. Hutson:** A. Employment/Salary (full or part-time):; Shire. **T.E. Salt:** A. Employment/Salary (full or part-time):; Neurexpert Limited, London, UK. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Share holder and Director of Neurexpert Limited.

Poster

259. Dynamic Circuitry of Stress and Anxiety

Location: Hall A

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Topic: F.03. Motivation and Emotion

Support: NIMH R36 MH106332

NIMH R37 MH058883

NIH 5R25NS080687

Title: Infralimbic BDNF regulates extinction of active avoidance

Authors: ***L. E. ROSAS-VIDAL**, W. A. RAMOS-GUASP, G. J. QUIRK;
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Abstract: The infralimbic prefrontal cortex (IL) is necessary for fear extinction (Do-Monte et al., 2015) as well as the extinction of platform-mediated avoidance (Bravo-Rivera et al., 2014). Brain-derived neurotrophic factor (BDNF) is necessary for synaptic plasticity underlying learning and memory processes. We recently reported that blocking extracellular BDNF in IL during fear extinction training impairs its acquisition and recall (Rosas-Vidal et al., 2014). Here we show that blocking extracellular BDNF in IL during avoidance extinction training had no effect on the acquisition of extinction (Sal, n= 12; anti-BDNF, n= 13; p= 0.609), but impaired the recall of avoidance extinction the next day ($p< 0.001$). Using immunohistochemistry to measure neuronal BDNF, we found that avoidance extinction did not increase BDNF in IL neurons (p=0.61; No-Ext., n= 5; Ext., n= 8), suggesting that BDNF in IL may be released by inputs from the ventral hippocampus (vHPC) and/or the basal amygdala (BA), both of which show increased neuronal BDNF after fear extinction (Chhatwal et al., 2006; Rosas-Vidal et al., 2014). Accordingly, avoidance extinction increased neuronal BDNF in vHPC (p= 0.004) and

mediodorsal thalamus (MD; p=0.049), but not BA (p=0.99). Our findings suggest that avoidance extinction may depend on BDNFergic inputs to IL from the vHPC and MD, rather than from BA.

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Poster

259. Dynamic Circuitry of Stress and Anxiety

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Title: Optogenetic silencing of prelimbic cortex in active avoidance

Authors: ***M. M. DIEHL**, J. RODRÍGUEZ-ROMAGUERA, P. A. PAGÁN-RIVERA, G. J. QUIRK;
Psychiatry, Univ. of Puerto Rico, Sch. of Med., San Juan, PR

Abstract: We previously showed that pharmacological inactivation of prelimbic prefrontal cortex (PL) with muscimol impairs the expression of platform-mediated avoidance in response to a conditioned tone (Bravo-Rivera, et al., 2014). Here, we used an optogenetic approach to silence PL glutamatergic neurons only during the tone period, in rats trained in our avoidance task. Silencing PL somata with archaerhodopsin (CaMKIIα-eArchT3.0) during the entire 30 sec tone did not block avoidance, as most rats had moved to the platform by the final 2 sec (n=9/9 eYFP controls, n=12/15 Arch, p=0.27). However, PL silencing decreased the amount of time rats spent on the platform (eYFP=90%, Arch=49%, p=0.002), suggesting that silencing PL delayed platform mounting. In agreement with this, Arch increased the latency to mount the platform (eYFP=4.8s, Arch=11.6s, p=0.03). This delay in mounting was not due to a decrease in fear, as freezing (p=0.96) and bar press suppression (p=0.22) were unaffected. This suggests that PL glutamatergic activity accelerates, but may not be necessary for, avoidance responses. We next wanted to assess whether silencing PL during specific time periods within the tone presentation would also affect platform mounting. We have previously reported that PL neurons exhibit responses to both tone onset and platform mounting in this task (Bravo-Rivera, et al., 2014 SFN abstract). We therefore assessed if silencing PL during platform mounting (typically 3 to 20 sec after tone onset) would also delay mounting. Our preliminary data (Arch n=7, eYFP n=3) show that silencing 3-20 sec during the tone did not delay platform mounting (eYFP=83%, Arch=79%,

p=0.45), suggesting that the initial tone response (0-3 sec) may be important for accelerating avoidance, a hypothesis we are currently testing.

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Poster

259. Dynamic Circuitry of Stress and Anxiety

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 259.13/Y44

Topic: F.03. Motivation and Emotion

Title: Inducible pharmacogenetic inhibition of protein synthesis in lateral amygdala

Authors: *P. SHRESTHA[1], P. AYATA[2], N. HEINTZ[2], E. KLANN[1];
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Abstract: Auditory fear conditioning is a widely used behavioral paradigm for studying the neural and molecular mechanisms underlying learning and memory. The first clues that de novoprotein synthesis may be necessary for long term memory formation came about in the 1960s (Flexner, 1963) when broad protein synthesis inhibitors (PSI) blocked long-term memory (LTM) leaving the short-term memory intact. Similar pharmacological methods have been applied over the years to demonstrate that protein synthesis in lateral amygdala is required for consolidation of long-term fear memory. However, methodological concerns and off target effects of broad PSIs such as anisomycin and cycloheximide have made it necessary to seek out alternative approaches to investigate the role of protein synthesis in memory processes. It was recently shown that eukaryotic initiation factor 2α (eIF2α) phosphorylation, the rate limiting step in translation, bidirectionally regulates the switch from short- to long-term synaptic plasticity and short- to long-term memory. Here we present a novel drug inducible pharmocogenetic strategy to modulate eIF2α phosphorylation in a cell type specific manner. We show that our approach is able to reversibly block protein synthesis both *in vitro* and in the lateral amygdala *in vivo*. We currently are determining the requirement for proper eIF2α phosphorylation and de novo protein synthesis in specific cell types in the lateral amygdala for the consolidation of long-term memory following auditory fear conditioning.

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Poster

259. Dynamic Circuitry of Stress and Anxiety

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Topic: F.03. Motivation and Emotion

Support: NARSAD

MH096251

MH100583

Title: Adiponectin regulates anxiety behaviors and DA neuron firing in the VTA

Authors: F. SUN, S.-Y. CHENG, *X. FANG, J. LIU, D. LODGE, X.-Y. LU;
Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: Adiponectin is a protein hormone secreted from adipocytes. Our previous studies have shown that adiponectin regulates depressive behaviors. In this study, we examined the effects of adiponectin on anxiety behaviors. We found that intracerebroventricular infusion of adiponectin produces anxiolytic effects in the elevated plus-maze and light/dark tests, whereas adiponectin haploinsufficiency causes anxiogenic-like behaviors. *In vivo* extracellular electrophysiological recordings revealed that infusion of adipoRon, a novel potent and selective adiponectin receptor agonist, into the ventral tegmental area (VTA) inhibits DA neuron firing. Furthermore, acute restraint stress increased anxiety and DA neuron firing in the VTA. The effect of acute stress on DA neuron firing was reversed by intra-VTA infusion of AdipoRon. Taken together, these results suggest that adiponectin is involved in regulating anxiety behaviors and modulating VTA DA neuron activity.

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Poster

259. Dynamic Circuitry of Stress and Anxiety

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Topic: F.03. Motivation and Emotion

Support: NIH Grant R01-MH087583

NIH Grant RO1-MH099085

Title: Antidepressant effects of ketamine in male and female rats

Authors: *A. SARKAR[1], F. JOHNSON[2], M. KABBAJ[1];
[1]Biomed. Sci., [2]Psychology, Florida State Univ., Tallahassee, FL

Abstract: Depression, one of the most common mood disorders in the world, is twice as prevalent amongst women as in men. Several studies have linked gender to the occurrence of depression and the efficacy of antidepressant treatments. Studies from our lab have shown female rats to be more sensitive to the antidepressant ketamine, an NMDA receptor antagonist, as compared to males. A single injection of 2.5 mg/kg dose of ketamine has antidepressant-like effect in females but not in males, that respond to doses of 5 mg/kg and above. We hypothesize a role for gonadal hormones in the treatment efficacy of ketamine at lower doses in females. Social isolation stress (IS) is known to evoke depression and anhedonia-like behaviour in rats. In this study we investigated the effect of ketamine on IS induced a) anhedonia and depression-like behaviour, b) changes in spine density in the mPFC, using HSV-GFP infusion and confocal microscopy and c) molecular changes at the synaptoneurosomes in the mPFC, in male rats and in female rats that received ketamine either during the diestrus or the proestrus phase of their estrus cycles. Our results showed that 8 weeks of IS gives rise to anhedonia and depression-like behaviour in the sucrose preference test and Porsolt's forced swim test in males, concomitant with a decline in spine density in the pre-limbic region of the mPFC. Analysis of spine morphology revealed a decline in the number of mushroom spines and thin spines in the proximal segment of the apical tuft of dendrites of layer V of the PL. IS also evoked a decline in the levels of pre-synaptic protein Synapsin1 and post-synaptic proteins GluR1 and PSD95 implicated in synaptic maturation, in synaptoneurosomal preparations. A single dose of ketamine (5 mg/kg) administered to animals after 8 weeks of IS completely removed the behavioural, spine density and molecular deficits in males, while the 2.5mg/kg dose rescued only a subset of these deficits partially. Additionally, we observed an interesting growth in thin spine population concomitant with a decline in stubby spine numbers, post 3 hours of ketamine treatment. Studies are currently underway to investigate the efficacy of the same doses of ketamine administered during different phases of the estrus cycle in female rats, on behaviour, spine density and synaptosomal protein levels. Upon completion, our study will provide a novel understanding of the influence of gonadal hormones on susceptibility to stress and the efficacy of antidepressant treatments in male and female animals.

Disclosures: **A. Sarkar:** None. **F. Johnson:** None. **M. Kabbaj:** None.

Poster

259. Dynamic Circuitry of Stress and Anxiety

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 259.16/Z3

Topic: F.03. Motivation and Emotion

Support: R01MH087660

Title: Neuro circuitry changes in response to unconditioned fear exposure

Authors: *A. MITCHELL[1], A. F. MOHED[1], R. E. JACOBS[2], E. L. BEARER[1];
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Abstract: In post-traumatic stress disorder (PTSD) the response to fear is damaged or changed. People with PTSD may feel fear even when they are not in danger. We hypothesize that the biological basis for this is an alteration in neurocircuitry, particularly in the mesocortical limbic system. In this study we investigate the effects of unconditioned fear, focusing on a validated PTSD model, the serotonin transporter knock-out (SERT-KO) mouse. Our hypothesis is that the experience of unconditioned fear (UF) affects the structure and functional circuitry of the brain even in wildtype mice. We theorize that these changes will resemble those found in the SERT-KO in the absence of fear exposure. By looking at UF circuitry dynamics with magnetic resonance imaging (MRI), we are analyzing mouse brain circuity in an entirely new way. Each genotype, SERT KO and WT littermates, received manganese intraperitoneal injections and then were imaged by MRI at five time points in an 11.7T Bruker magnet. Manganese is a contrast agent for MRI that detects electrical activity in the brain, as Mn^{2+} enters active neurons through voltage-gated Ca^{2+} channels and gives a hyperintense signal in T_1-weighted MRI. We used a naturally occurring UF provocateur, predator odor. We followed behavior before and after predator odor in the light-dark box. We used our automated “skullstripping” pipeline to remove the non-brain image and then aligned them. Statistical parametric mapping with SPM8 identified statistically relevant voxel-wise intensity changes. ANOVA comparisons between SERT KO and WT mice reveled statistical differences across and between genotypes and time. Lastly we conducted region of interest analyses (ROIs) on the images to pin point the degree of intensity changes in particular brain areas, and to determine overall manganese levels over time. Normalization of intensities across time points will allow more sensitivity in finding significant differences produced by UF, and to determine their persistence or resolution over time. Preliminary results show significant differences in brain activity in immediate response to unconditioned fear between SERT KO and WT littermates, and persistence of the effect in the SERT.

Disclosures: **A. Mitchell:** None. **A.F. Mohed:** None. **R.E. Jacobs:** None. **E.L. Bearer:** None.

Poster

259. Dynamic Circuitry of Stress and Anxiety

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Program#/Poster#: 259.17/Z4

Topic: F.03. Motivation and Emotion

Support: R01 HD075066

R21 HD070662-01

Title: Differential medial prefrontal cortex Egr-1 expression in variants of standard contextual fear conditioning

Authors: *T. CHAKRABORTY, A. ASOK, W. SCHREIBER, M. E. STANTON, J. B. ROSEN;
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Abstract: Contextual fear conditioning variants, such as standard contextual fear conditioning (sCFC) and the context preexposure facilitation effect (CPFE), allow the study of different aspects of fear learning. Single trial SCFC gives insight into context acquisition and context-shock association (context-US) in one trial. The CPFE temporally dissociates incidental context and context-US learning so each phase can be studied individually. Using the early immediate gene, *Egr-1*, we demonstrate different *Egr-1* expression profiles in the prefrontal cortex (PFC) for the two CFC variants in adult rats. Male Long Evans rats were trained in the CPFE or sCFC. During sCFC, rats were preexposed, then shocked in a single trial. 24h later retention of fear conditioned freezing was tested. Controls included a no shock and an immediate shock group. In the CPFE, animals were preexposed to either the training (PRE) or an alternate (ALT) context on Day 1. On Day 2, rats were given an immediate shock in the PRE context. Context conditioned freezing was tested on Day 3. *In situ* hybridization in the PFC for *Egr-1* expression was performed after sCFC training, CPFE preexposure, and CPFE training. Homecage controls were used as a baseline measure *of Egr-1* activity. Rats exposed to sCFC froze significantly more than control rats during the retention test. sCFC rats expressed higher levels of *Egr-1* in the anterior cingulate and orbitofrontal cortices compared to all controls. *Egr-1* in prelimbic and infralimbic cortices was elevated above homecage rats in sCFC, no- and immediate- shock rats, which did not differ from each other In the CPFE, PRE rats froze more than ALT rats during retrieval. PRE and ALT *Egr-1* levels in all PFC regions were elevated above HC both after preexposure and training. After training PRE rats expressed more *Egr-1* activity in the anterior cingulate, orbitofrontal, infralimbic and prelimbic cortices compared to ALT rats. These results suggest that *Egr-1* in the adult PFC exhibits different expression profiles after sCFC and CPFE, and replicate *Egr-1* patterns previously found in juvenile animals. The findings suggest the role of various prefrontal cortex regions are involved in different aspects of contextual fear conditioning at an early age that persists into adulthood.

Disclosures: T. Chakraborty: None. **A. Asok:** None. **W. Schreiber:** None. **M.E. Stanton:** None. **J.B. Rosen:** None.

Poster

259. Dynamic Circuitry of Stress and Anxiety

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 259.18/Z5

Topic: F.03. Motivation and Emotion

Support: Swiss National Foundation

Title: The Lateral Hypothalamic Parvafox-nucleus projects to emotional circuits

Authors: *A. BILELLA[1], G. ALVAREZ-BOLADO[2], M. R. CELIO[1];
[1]Med., Univ. of Fribourg, Fribourg, Switzerland; [2]Dept. of Neuroanatomy, Univ. of Heidelberg, Heidelberg, Germany

Abstract: The neural circuits underlying emotional valence and motivated behaviors involve several brain areas. Two of the most studied and discussed of them are the prefrontal cortex (PFC) and the periaqueductal gray (PAG). Moreover, several studies describe an involvement of the hypothalamus in the regulation of the emotions. Here we describe the efferent connections of a novel hypothalamic nucleus projecting to the PAG and PFC. This particular group of cells, recently discovered, is the Parvafox-nucleus. It is a cord-like structure comprised of intermingled parvalbumin-positive neurons and Foxb1-expressing neurons, and lodged within the ventrolateral hypothalamus. By using Cre-dependent viral constructs stereotactically injected in the Parvafox nucleus of *Foxb1-Cre* and *parvalbumin/Foxb1-Cre* mice we have mapped its efferent connections. These experiments have revealed a strong caudal projection to the PAG and the hindbrain and a minor rostral projection to the PFC. The caudal projection divides into two different bundles and reaches the dorsolateral and ventrolateral portions of the PAG. Labeled terminals are found also in the supraoculomotor nucleus (Su3), the cuneiform nucleus (CnF), the dorsomedial tegmental area (DMTg) and the locus coeruleus (LC). A small group of axons terminates in the gigantocellular formation (Gi) of the midbrain. These tracing results pave the way for further functional investigation of the Parvafox-nucleus. On this preliminary basis, we are tempted to speculate that the Parvafox nucleus participates in the regulation of emotional expression.

Disclosures: **A. Bilella:** None. **G. Alvarez-Bolado:** None. **M.R. Celio:** None.

Poster

260. Song Learning and Auditory Processing

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 260.01/Z6

Topic: F.04. Neuroethology

Support: NIH RO1MH070712

Title: Differential contributions of FoxP2 isoforms to vocal learning and variability

Authors: *Z. D. BURKETT[1], J. A. MORALES[2], S. A. WHITE[1];
[1]Integrative Biol. & Physiol., [2]Ecology and Evolutionary Biol., UCLA, Los Angeles, CA

Abstract: The transcription factor FoxP2 is essential for the proper development of learned vocalizations such as speech and birdsong. In humans, FOXP2 mutations that affect its ability to interact with DNA cause a severe speech and language disorder characterized by an inability to execute fine orofacial movements necessary for speech as well as difficulty in language processing. Multiple endogenous splice isoforms of FoxP2 exist in humans and the zebra finch species of songbird. The full-length isoform contains a poly-Q repeat, a zinc finger and leucine zipper domain to mediate dimerization, a forkhead box necessary for nuclear localization and DNA binding, and an acidic C-terminus domain. A second isoform, referred to as FoxP2.10+, lacks both the forkhead box and acidic C-terminus but retains the zinc finger and leucine zipper domain and is therefore capable of dimerizing with other FoxP molecules but deficient in entering the nucleus or influencing transcription on its own. Previous work indicates that knockdown or over-expression of the full-length isoform in zebra finch basal ganglia song nucleus Area X during sensorimotor learning result in similarly poor learning and elevated vocal variability. The similar deficits of these opposing interventions suggest that behaviorally regulated cycling of FoxP2 is a key component of proper vocal development. Here, we use constructs to over-express the full-length or 10+ FoxP2 isoforms in Area X of juvenile zebra finches and observe differential behavioral results for each isoform. The results replicate our previous finding that over-expression of the full-length isoform results in poor tutor song copying and blocks practice-induced changes in vocal variability. Surprisingly, over-expression of the 10+ isoform has no observable effect on tutor song copying. However, birds over-expressing the 10+ isoform show pronounced alterations in practice-induced vocal variability. Unlike birds with full-length or GFP over-expression, those over-expressing the 10+ isoform sing increasingly stable songs with practice. These novel results indicate a potentially unique function for multiple endogenous isoforms of FoxP2 in vocal learning and the acute regulation of variability.

Disclosures: **Z.D. Burkett:** None. **J.A. Morales:** None. **S.A. White:** None.

Poster

260. Song Learning and Auditory Processing

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Topic: F.04. Neuroethology

Support: NIH R01MH070712

NIH 5T32HC00722834

Title: Beyond sensorimotor learning: Striatal FoxP2 affects maintenance of learned vocalizations in adult zebra finches

Authors: *N. F. DAY, C. Y. KIM, S. A. WHITE;
Integrative Biol. & Physiol., UCLA, Los Angeles, CA

Abstract: Human mutations in the FOXP2 transcription factor result in language disorders and altered basal ganglia structure. The neural underpinnings of speech learning can be investigated in songbirds because, like speech, birdsong is learned through social interactions, relies on auditory feedback and cortico-basal ganglia circuitry, and compensates for experimentally-induced errors. In the songbird brain, most FoxP2-enriched areas (e.g. cortex, thalamus) show a static expression level, whereas Area X (a song-dedicated basal ganglia nucleus), shows dynamic regulation: FoxP2 mRNA and protein decrease when juvenile and adult males sing, after which songs are more variable. In contrast, when adult male zebra finches engage in courtship (directed) song or refrain from singing, FoxP2 is high. Song stereotypy is greater when FoxP2 is high in non-singing juvenile birds. This 'on-line' regulation during singing is critical for song learning, but the role of FoxP2 in the active maintenance of a learned vocal motor skill is poorly understood. What is known includes that auditory feedback is crucial for the learning and the maintenance of song and speech, because deafness in adulthood causes both to deteriorate. In songbirds, hearing and FoxP2 are linked: The more a bird hears itself practice, the lower its Area X FoxP2 levels; no such correlation is observed in deaf birds. We hypothesize that cycles of behavior-driven FoxP2 regulation in adult songbirds enable song maintenance. Thus, we used an adeno-associated virus (AAV) to constitutively augment levels of FoxP2 in Area X of adult zebra finches to test whether singing behavior in the mature organism is altered. Birds were injected with either GFP- or FoxP2-overexpressing AAV constructs; a subset of each group were then deafened. FoxP2-mediated song stereotypy and deafening-induced song de-crystallization were quantified. We observe that FoxP2 overexpression in deafened birds hastens deterioration of phonological and syntactical song elements. We also examined social context-associated syllable variability and investigated synaptic interactions between cortical auditory afferents and FoxP2-expressing neurons in Area X. Our findings suggest that behavior-driven regulation of FoxP2 in adult animals is important for song maintenance, indicating that it influences song beyond the sensorimotor phase of vocal learning. Post-organizational effects of FoxP2 in adult birds suggest that human cases of FoxP2 mutations involve post-developmental deficits in addition to developmental ones.

Disclosures: N.F. Day: None. **C.Y. Kim:** None. **S.A. White:** None.

Poster

260. Song Learning and Auditory Processing

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 260.03/Z8

Topic: F.04. Neuroethology

Support: DC 009975

NS 087506

Title: Neural representation of learned vocal behavior in motor cortex of juvenile songbirds

Authors: *R. C. YUAN, S. W. BOTTJER;
USC, Los Angeles, CA

Abstract: Similar to speech acquisition in humans, vocal learning in zebra finches entails a process of sensorimotor integration in which auditory feedback of juvenile birds' vocalizations guides refinement of variable immature vocal sounds into stereotyped adult vocal patterns. In zebra finches, the output of this sensorimotor processing is conveyed to RA, a region of motor cortex that drives vocal output. Neurons in RA of adult zebra finches demonstrate greater response strength to playback of the bird's own song than to playback of conspecific songs. However, little is known about the selectivity of RA neurons during the learning period: do neurons in RA of juvenile birds demonstrate selective tuning for their own immature song over other vocal stimuli? We investigated this question by making extracellular recordings in RA of anesthetized juvenile zebra finches (40-48 dph) and testing the neural response to playback of each bird's own song (OWN) against playback of two additional juvenile songs (mirror reverse of each bird's own song and age-matched conspecific song) and two adult songs (adult tutor song and adult conspecific song). All multi-unit sites demonstrated significant excitatory responses to playback of OWN and at least one other stimulus (n = 23/23), displaying an increase in mean firing rate during song playback. Across all sites, response strength to playback of each juvenile stimulus was significantly greater than the response to either adult song stimulus. Within responses to juvenile song stimuli, there was no significant difference in response strength between playback of OWN and age-matched conspecific song (n = 6). This pattern of results suggests that neurons in RA of juveniles engaged in sensorimotor learning prefer acoustic features inherent to juvenile vocalizations over adult vocal patterns, but do not prefer the bird's own song over other immature vocal sounds. Surprisingly, preliminary single-unit analysis revealed a minority of units that demonstrated an excitatory response to song playback (n = 5/46); a large number of units showed response suppression (n = 23/46), and many units did not change their baseline firing rate in response to song (n = 18/46). Qualitative inspection suggested that single RA neurons in juveniles (as in singing adult birds) shift from tonic firing during baseline to phasic bursting separated by periods of suppression during song playback, such that units excited by song show no significant change in mean firing rate during playback compared to baseline. Further analysis will test this observation, which predicts an increase in mean burst fraction and peak instantaneous firing rate of single units during song playback.

Disclosures: R.C. Yuan: None. **S.W. Bottjer:** None.

Poster

260. Song Learning and Auditory Processing

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 260.04/Z9

Topic: F.04. Neuroethology

Support: R01 DC004722

Title: Timing vs. sequencing in song learning

Authors: *J. HYLAND BRUNO, O. TCHERNICHOVSKI;
Psychology, Hunter Col., New York, NY

Abstract: What sort of central pattern generator gives rise to birdsong? Male zebra finch song is a complex learned vocal sequence, but it is also highly rhythmic: how is the learning of phonology, syntax, and rhythm orchestrated during song development? One possibility is that song units are learned and then chained together by some sequence generator. Alternatively, if the CPG is a rhythm generator, then song changes (such as adding a syllable) must comply with a metronomic framework. To test this question, we trained juvenile zebra finches to change their songs by incorporating a new syllable which either fit or violated the prior song rhythm. Preliminary results indicate that birds were more likely to accomplish this task when the rhythmic framework was preserved. In contrast, when the duration of the new syllable was not a small integer multiplier of the prior song meter, pupils' endpoint songs remained unstable and off-target, both in terms of temporal regularity and syntactical stereotypy. These findings provide motivation for investigating where and how rhythm may be generated in the songbird brain.

Disclosures: J. Hyland Bruno: None. **O. Tchernichovski:** None.

Poster

260. Song Learning and Auditory Processing

Location: Hall A

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Topic: F.04. Neuroethology

Support: Amabel Boyce James '74 Fund for Summer Research in the Sciences

Brachman Hoffman Fund

Title: Sequentially tutored zebra finches exhibit mirror patterns of lateralized neuronal activation in the avian auditory cortex

Authors: E. M. OLSON, R. K. MAEDA, *S. M. H. GOBES;
Neurosci., Wellesley Col., Wellesley, MA

Abstract: In monolingual humans, language-related brain activation shows a distinct lateralized pattern, in which the left hemisphere is often dominant. Studies are not as conclusive regarding the localization of the underlying neural substrate for language in bilinguals. Lateralization of the neural substrate for first and second language appears to depend on a number of factors including proficiency and early experience with each language. Similar to humans learning speech, songbirds learn their vocalizations from a conspecific tutor early in development. In the wild, zebra finches (*Taeniopygia guttata)* are exposed to several adult conspecific tutors at the same time and tutoring by two adult conspecifics is also possible in the laboratory (Yasaki-Sugiyama, et al. 2004). Here, we used a similar dual tutor paradigm to investigate memory-related neuronal activation (measured as the expression of the immediate early gene *ZENK*) in sequentially tutored male zebra finches. We measured the number of Zenk positive neurons and calculated lateralization ratios in response to exposure to the first tutor song, learned early in development (before 33dph), and to the second tutor song, learned later in development (61dph-90dph), in the caudomedial nidopallium (NCM) and in the caudomedial mesopallium (CMM). There were no differences in the absolute number of Zenk positive neurons between sequentially tutored birds exposed to songs from their first or their second tutor. However, when comparing the lateralization ratios in the NCM, we found mirror patterns of lateralization in the two song exposure groups. The more the birds had retained from their first tutor (% similarity), the more right-lateralized they were when exposed to that song (Pearson's $r = -0.91$, $p = 0.001$); however, the more birds had learned from their second tutor, the more left-lateralized they were when exposed to that song (Pearson's $r = 0.89$, $p = 0.003$). Our results suggest that in sequentially tutored songbirds, memory of song learned later in development is encoded predominantly in left-hemispheric circuits. In humans, perceptual traces of memories from the first language remain in the left hemisphere even when a second language subsequently replaces the first (Pierce, et al. 2014). Whether learning from a second tutor in songbirds results in overwriting of the original memory engram or generates parallel traces of two tutor song memories remains to be elucidated, but if song learning resembles language learning, traces of the first memory may remain in the left hemisphere.

Disclosures: E.M. Olson: None. **R.K. Maeda:** None. **S.M.H. Gobes:** None.

Poster

260. Song Learning and Auditory Processing

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 260.06/Z11

Topic: F.04. Neuroethology

Title: mTOR cascade signaling in the songbird auditory cortex is required for developmental song learning

Authors: *S. AHMADIANTEHRANI[1], S. E. LONDON[2];
[2]Psychology, [1]Univ. of Chicago, Chicago, IL

Abstract: The mechanistic Target of Rapamycin (mTOR) cascade regulates both transcription and translation, processes required for learning and memory. Young male zebra finches (*Taeniopygia gutatta*) learn to sing by memorizing a song from an adult "tutor" bird during a sensitive period (days 30-65 post-hatch; P30-65). We set out to determine if the mTOR cascade is activated in the auditory cortex, a region necessary for tutor song memorization, and contributes to tutor song memorization. First, we confirmed the presence of this cascade in the juvenile auditory cortex using Western blot analysis for mTOR and two of its downstream targets. We next tested if the mTOR cascade is activated in young males and females after hearing song playbacks. We used immunohistochemistry to quantify mTOR activation in the higher order auditory areas, the caudomedial nidopallium (NCM) and the caudomedial mesopallium (CMM). To ascertain if playback-induced mTOR activation coincides with the age of onset for tutor song memorization, we tested P23 and P30 birds. In P23 birds, song playbacks did not result in mTOR cascade activation in either the NCM or CMM. In contrast, song playbacks led to the activation of mTOR signaling in the NCM and CMM of P30 males, but not P30 females. Because we observed playback-mediated mTOR activation in P30 males, we hypothesized that this cascade is necessary for tutor song memorization. To test this possibility, we used a controlled tutoring procedure with intra-auditory cortex infusions of either rapamycin, which specifically inhibits mTOR activation, or SC79, which constitutively activates mTOR signaling. When we quantitatively compared the pupils' songs to the tutor's song, we found that birds who received rapamycin or SC79 copied significantly less of the tutor song compared with birds in the control vehicle group. This demonstrates that finely tuned mTOR signaling in the auditory cortex during tutoring experiences is required for song copying. Our behavioral data are consistent with a growing body of molecular evidence that suggests that both inhibition and excessive activation of mTOR signaling are equally detrimental. In *ex vivo* and *in vivo* models, mTOR acts as a molecular hub, integrating incoming synaptic signals and directing multiple outputs to form an appropriate neuronal response. Thus, our results provide a foundation for the use of developmental song learning in the zebra finch as a powerful *in vivo* model of how precisely modulated experience-dependent activation of mTOR during a specific developmental stage encodes meaningful information to shape behavior.

Disclosures: S. Ahmadiantehrani: None. **S.E. London:** None.

Poster

260. Song Learning and Auditory Processing

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Topic: F.04. Neuroethology

Support: Whitehall Foundation 2013-08-71

Title: Epigenetic histone modifications correlate with learning potential in the auditory forebrain of juvenile songbirds

Authors: *S. E. LONDON[1], T. K. KELLY[2];
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Abstract: Sensitive periods are phases in life when experience has maximal influence on brain organization. One hallmark of a sensitive period is that experience itself is the dominant signal ending the phase. Juvenile male zebra finches have a sensitive period for learning a complex natural behavior, song. The sensitive period is likely primarily represented in the sensory component of developmental song learning; preventing a young male from hearing song extends his ability to copy song from another bird but does not upset the normal onset or progression of vocal motor production. Notably, the experience required to close the sensitive period for sensory song learning (tutor song memorization) is song - other auditory stimuli such as calls is not sufficient. This suggests that identifying the mechanisms by which song experience is encoded during the sensitive period for tutor song memorization can provide novel insight into sensitive periods for cognition. Epigenetics is a powerful mediator of brain function, as modifications to DNA and histone proteins can be added and removed based on experience and can coordinate downstream molecular and cellular processes by regulating transcription of sets of genes. Here, we assessed if histone modifications are regulated by tutor experience in a neural locus essential for tutor song memorization, the auditory forebrain. We compared Posthatch day 65 (P65) males exposed to tutor song (low learning potential) to those prevented from hearing song during the normal learning phase (high learning potential) to examine epigenomic differences of age-matched birds in different learning potential states. We performed genome-wide DNA sequencing after chromatin immunoprecipitation (ChIP-seq) for histone modifications (H3K9me3, H3K27me3, H3K4me3) associated with silent, repressed, and active chromatin respectively, as well as for RNA Polymerase II (PolII), a direct measure of transcription. Results indicated that tutor experience globally reduces transcription in the auditory forebrain. Further, functional enrichment analysis suggested that genes involved in cellular differentiation, stabilizing neural circuits, and learning and memory are regulated by tutor experience-dependent histone modifications. Thus, tutor experience is encoded in the epigenome, with clear potential to regulate molecular and cellular processes that may underlie neural circuit learning potential. As tutor song memorization is a tractable model for sensitive periods for learning, this line of inquiry has implications to define cognitive sensitive periods for normal and disordered neural development.

Disclosures: S.E. London: None. **T.K. Kelly:** None.

Poster

260. Song Learning and Auditory Processing

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Topic: F.04. Neuroethology

Support: NIH Grant R01MH105519

Title: miR-9 regulates vocal learning and performance in the zebra finch

Authors: *Z. SHI, A. WEBER, X. LI;
Neurosci. Center, Louisiana State Univ. He, New Orleans, LA

Abstract: Mutations in the FOXP2 gene cause speech and language impairments in humans. Dysregulation of FoxP2 expression in Area X of the zebra finch, a basal ganglia nucleus required for vocal learning, leads to impairments of vocal communication behavior. Thus proper function and precise regulation of the FOXP2 is critically required for vocal communication. We recently identified microRNA miR-9 as a potential regulator of the FOXP2 gene by *in vitro* experiment. We also showed that miR-9 is abundantly expressed in Area X, and miR-9 expression is regulated during development, suggesting its roles in vocal development and learning. To understand the *in vivo* functions of miR-9, we used a lentiviral system to manipulate miR-9 expression in Area X in male juvenile zebra finches, and examined vocal learning of the virally injected animals. We found that comparing to non-injected animals and animals injected with a control virus, miR-9 overexpression in Area X in juvenile zebra finches resulted in abnormal songs when these juveniles reach adulthood. Their songs are less similar to their tutor's song at both the syllable and motif levels. Following the developmental trajectory of vocal learning in these animals, we found that impairments in vocal learning are already apparent at post hatching day 60 and persist through adulthood. Moreover, the acoustic features of the songs of these animals are more variable comparing to controls, suggesting that vocal performance is also impaired in these animals. In addition to FOXP2, miR-9 is known to regulate the expression of FOXP1, a paralog gene of the FOXP2. FOXP1 is thought to function as a transcription factors by forming a heterodimer with FOXP2, and dysfunctions of FOXP1 have been implicated in neurodevelopmental disorders such as autisms. Here we show that overexpression of miR-9 in Area X downregulates the expression of both FoxP1 and FoxP2 *in vivo*; and several FOXP2 downstream genes, which have important functions in neural development and plasticity, are also regulated. Taken together, our results provide evidence to support the roles of miR-9 in vocal development and learning via regulating complex gene expression network, which includes the FoxP1 and FoxP2 and their respective downstream genes.

Disclosures: Z. Shi: None. **A. Weber:** None. **X. Li:** None.

Poster

260. Song Learning and Auditory Processing

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Topic: F.04. Neuroethology

Support: JSPS KAKENHI 25640097

JSPS KAKENHI 25290063

Title: Familial genetic bias in vocal babbling pattern at early song development

Authors: D. SATO[1], *K. WADA[2];
[1]Grad. Sch. of Life Sci., [2]Hokkaido Univ., Sapporo, Hokkaido, Japan

Abstract: Learned vocalization is used as one of the crucial acoustic biosignals representing individual traits for mating and territorial defense. Songbirds learn song patterns by listening to a tutor song and performing self-motivated vocal practice during the sensitive developmental period. However, when and how individual differences in song patterns develop during learning remains unelucidated. Here we provide evidence indicating that an individual difference exists in the vocal output even at the earliest stage of song learning. Zebra finch male juveniles start singing a subsong, which is not merely disordered a vocalization but is regulated with biased variability at the temporal regulation of syllables. Epigenetic developmental factors, parental care before fledging and song tutoring, do not affect the generation of differences in subsong patterns among juveniles. In contrast, parental pair combination produces a familial bias of the distribution of syllable duration in the subsongs of their juveniles. These results indicate a potential genetic mechanism for generating individual differences in vocal outputs at the starting point of vocal development, suggesting an inherited trait of learning bias for the acquisition of vocal patterns.

Disclosures: D. Sato: None. **K. Wada:** None.

Poster

260. Song Learning and Auditory Processing

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Topic: F.04. Neuroethology

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Sandler Family Foundation

Title: Genetic constraints on the learning of a complex song phenotype

Authors: *D. G. METS, M. S. BRAINARD;
UCSF/HHMI, San Francisco, CA

Abstract: Learning reflects the influence of experience on genetically determined circuitry. Both the ways in which experience shapes behavior during learning and the ways in which genetics shape non-learned phenotypes have been widely studied. However, little is known about how experience and genetics interact to determine complex learned phenotypes. Vocal learning in songbirds provides a rich system for investigating experiential and genetic contributions to learning; the output of learning (song) is quantifiable and we can manipulate both the experiential contributions (through computer tutoring) and genetic contributions (through breeding). Here we examine the relationship between experiential and genetic contributions to learning of an ethologically relevant phenotype, the tempo of song production (quantified in syllables produced per second). Distinct genetic lines of the Bengalese finch (Lonchura striata domesitca) were bred from parents with different song tempos. When juvenile birds of a given line were tutored with synthetic songs that varied only in tempo, they developed adult songs with tempos that varied with the tutor song. Hence, as expected, the structure of song was shaped by experience. However, when the tempo of the tutor song was held constant, juveniles from different genetic lines developed song tempos that strongly correlated with those of their fathers, even though these juveniles had never heard their fathers sing. Thus, under controlled tutoring conditions, we found an unexpectedly strong genetic contribution to the mean song tempo. We further investigated the interaction between genetics and experience by tutoring several genetic lines (that expressed slow, medium and fast songs) on a set of synthetic songs that differed only in tempo. We again found that, across all lines, tutoring experience influenced song tempo. However, each line learned different amounts of the stimulus tempo, revealing a significant gene by environment interaction (GXE; a non-linear interaction between genetic and experiential contributions to song tempo). Taken together, these findings demonstrate a strong genetic contribution both to the mean tempo of song and to the degree to which tempo is influenced by experience. Our results provide a striking demonstration of how genetics can both shape and constrain the influence of experience on a complex learned phenotype.

Disclosures: D.G. Mets: None. **M.S. Brainard:** None.

Poster

260. Song Learning and Auditory Processing

Location: Hall A

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Program#/Poster#: 260.11/Z16

Topic: F.04. Neuroethology

Support: ERC-2010 Votecom AdG 268911

SNF grant 31003A_127024

Title: Rapid learning of auditory discrimination via observation and its limited generalization

Authors: *G. NARULA, R. H. R. HAHNLOSER;
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Abstract: Learning to imitate members of your own species in order to survive, prosper, or procreate has been identified in social animals such as humans, non-human primates, and several avian species. Typically, successful imitation is reported when observing animals selectively mimic complex motor behaviors performed by expert demonstrators. For example, songbirds such as the Zebra Finch (Taeniopygia guttata) have the capability of vocal learning by imitating the songs of a tutor experienced early in life. However, little is known about the ability of zebra finches, a highly social species, to learn simple stimulus-response mappings from demonstrating conspecifics. Here, we investigate whether adult zebra finches have the capacity for observational learning of a difficult auditory discrimination task. Briefly, pairs of adult zebra finches were placed in adjacent cages in sound isolation chambers, with one bird acting as a 'Demonstrator' for the other, the 'Observer'. In the first phase of the experiment, the Demonstrator is trained to discriminate between short and long renditions of a typical zebra finch song syllable. We use a Go/No-Go operant conditioning protocol (Tokarev & Tchernichovski 2014, Canopoli et al 2014), where the reinforcing agent associated with one of the two stimulus classes is a strong puff of air applied one second after stimulus offset. A special perch is used by the Demonstrator to trigger stimuli and/or air-puffs, as well as to interact with the Observer. Demonstrators eventually learn to stay or leave the perch before the onset of the air-puff, providing the Observer with a behavioral response to the stimulus. After reaching a given performance criterion, we replace the Demonstrator with the Observer and subject it to the same task. We show that Observers are significantly faster than Demonstrators at achieving the performance criterion. A control group that was exposed to several stimulus and reward cue (sound of air-puff) pairings prior to the actual training period did not show a similar increase in learning rate, allowing us to reject perceptual learning as the mechanism of accelerated learning in observers. We also show that prior knowledge of stimulus value does not provide observers the ability to rapidly learn stimulus-reward associations, allowing us to reject observational conditioning as well. Furthermore, we show that observers are slower than demonstrators at generalizing their learned behavior to new instances of the same stimulus. Finally, we present preliminary findings which suggest that vocal communication between the birds plays an important role in producing the benefits of observation.

Disclosures: G. Narula: None. **R.H.R. Hahnloser:** None.

Poster

260. Song Learning and Auditory Processing

Location: Hall A

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Program#/Poster#: 260.12/Z17

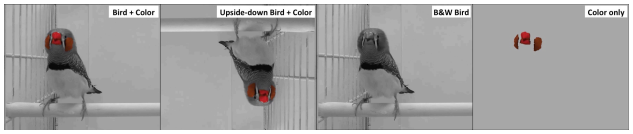
Topic: F.04. Neuroethology

Support: NSF, Behavioral systems

Title: What information can zebra finches extract from video clips?

Authors: *I. LJUBICIC[1,2], J. GORDON[1], O. TCHERNICHOVSKI[1];
[1]Psychology Dept., Hunter College, CUNY, New York, NY; [2]Biol., Grad. Center, CUNY, New York, NY

Abstract: Video playbacks can be a powerful research tool to study avian visual communication, species recognition, mate choice, and song learning. We are developing a system for studying how controlled sensory and social scenarios may affect song imitation in zebra finches. A serious challenge is that the color output of a video monitor does not match the color vision of zebra finches. To test if this would hinder identification of birds in a video, we automatically segmented the colorful beak and cheeks of a singing male and varied the context they were presented in. We can also rotate the color space in the videos to more closely match the reflectance spectra of the birds' feathers and stimulate the correct photoreceptors in zebra finches. Even without such manipulations, preliminary results show that video colors have a positive effect in attracting the birds compared to B&W videos. Furthermore, presenting a moving, colored beak and cheeks (without the bird) makes the video less attractive. The birds perceive the colors in the context of the singing male. Surprisingly, rotating the video image by 180 degrees to show the male upside-down, did not reduce preference for the video. This suggests that, in contrast to many mammals, zebra finches can perceive rotated images with relative ease. Our results indicate that zebra finches are attending broadly to the information in the videos, and are not just responding to the patterns of colors and lights. We can now design a "zebra finch TV" that will allow us to test which social contexts are important for tutor choice and song learning.



Disclosures: I. Ljubicic: None. **J. Gordon:** None. **O. Tchernichovski:** None.

Poster

260. Song Learning and Auditory Processing

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 260.13/Z18

Topic: F.04. Neuroethology

Support: DC08854

Title: The brain in flux: transient changes in hemispheric lateralization accompany prolonged exposure to a novel auditory environment

Authors: *L. YANG, D. S. VICARIO;
Rutgers Univ., New Brunswick, NJ

Abstract: Over the course of a lifetime, an individual is exposed to a wide and varying range of acoustic stimuli. While developmental auditory exposure may establish the groundwork for perceptual processing, exposure to novel acoustic stimuli can modify perceptual filters in the mature individual, e.g., during acquisition of a second language. Songbirds provide a model for studying adult plasticity in auditory cortex as a function of recent auditory experience, due to many similarities with the human auditory system, e.g. a critical period for vocal learning, and hemispheric lateralization for complex acoustic stimuli. Lateralization of auditory processing has been shown in electrophysiological and IEG studies of the songbird caudo-medial nidopallium (NCM), a higher auditory area. Zebra finches passively exposed to heterospecific (HET) aviary sounds for 4 or 9d showed a narrowing of tuning width and a reversal in the normal pattern of lateralization compared to controls that heard playback of a conspecific (CON) aviary. In the present study we continued these playbacks to later time points to assess changes in lateralization after prolonged exposure to a novel HET environment. Adult male zebra finches were exposed to either CON or HET auditory environments via playbacks for a period of 14 or 30d. Then, multi-unit activity was recorded using electrodes placed bilaterally in NCM of awake restrained zebra finches during presentation of novel zebra finch and canary songs. Hemispheric differences in absolute response magnitude (ARMs) and stimulus-specific adaptation (SSA) were measured. Subjects in the 14 and 30d groups showed the normal pattern of right side higher ARMs and right side faster SSA, regardless of recent auditory experience (CON or HET). Thus, the pattern of lateralization in the 14 and 30d HET groups was reversed from the reversed pattern seen at 4 and 9d groups (Yang & Vicario, 2014), but was similar to the baseline pattern of lateralization (Phan & Vicario, 2010). These data suggest that exposure to a novel acoustic environment initially challenges the system to modify its perceptual filters, accompanied by a reversal of lateralization after 4 and 9d. However, after prolonged exposure, the HET environment becomes familiar and no longer poses a challenge, leading to a reversion back to the normal pattern of lateralization. We will also test whether patterns of lateralization at various time points correspond to an enhancement in behavioral discrimination. In conclusion, hemispheric lateralization changes transiently during auditory perceptual learning, perhaps as part of an underlying process by which perceptual filters are modified to process new sounds.

Disclosures: L. Yang: None. **D.S. Vicario:** None.

Poster

260. Song Learning and Auditory Processing

Location: Hall A

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Program#/Poster#: 260.14/Z19

Topic: F.04. Neuroethology

Support: NSERC

Title: Differential effects of experience on immediate early gene response in black-capped chickadees

Authors: *A. H. HAHN, L. M. GUILLETTE, D. LEE, N. MCMILLAN, J. HOANG, C. B. STURDY;
Psychology, Univ. of Alberta, Edmonton, AB, Canada

Abstract: Black-capped chickadees produce a chick-a-dee call containing four note types (A, B, C, and D). D notes are a biologically important component of the call that is used to identify flock, species, food availability, and predator threat level. In a recent study, using adult wild-caught black-capped chickadees we found that the immediate early gene (IEG) response in the caudomedial mesopallium (CMM) and caudomedial nidopallium (NCM) was similar following presentation of heterospecific vocalizations with acoustic structure similar to D notes. In the current study, we examined how rearing environment affects IEG response to conspecific D notes. Black-capped chickadees were reared with adult conspecifics, with adult heterospecific mountain chickadees, or without adults. All hand-reared birds and a group of field-reared black-capped chickadees were presented with conspecific D notes and we quantified IEG expression in CMM and NCM. We found that chickadees reared with conspecifics had an IEG response similar to field-reared birds, while birds reared without adults had significantly less IEG expression. Birds reared with heterospecifics had an IEG response that was intermediate between the other two hand-reared groups. We also examined the vocalizations produced by all of the hand-reared birds, and we found that birds reared with heterospecifics and birds reared without adults were not producing species-typical D notes. Taken together, these results suggest that having prior experience with conspecific D notes was not the only factor driving the IEG response. But, our results suggest that experience with adults or adult vocalizations (i.e., conspecifics or closely-related heterospecifics) also influences the IEG response in chickadees.

Disclosures: **A.H. Hahn:** None. **L.M. Guillette:** None. **D. Lee:** None. **N. McMillan:** None. **J. Hoang:** None. **C.B. Sturdy:** None.

Poster

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Topic: F.04. Neuroethology

Support: NIH R01-MH096705

MSU RAIND Grant

Title: Rhythm does not influence ZENK expression in NCM of zebra finches up to day 45

Authors: *J. A. LAMPEN[1], K. JONES[2], J. MCAULEY[2], S.-E. CHANG[3], J. WADE[2]; [1]Neurosci., [2]Psychology, Michigan State Univ., East Lansing, MI; [3]Psychiatry, Univ. of Michigan, Ann Arbor, MI

Abstract: Rhythm is important in language processing and learning. Many human disorders involve deficits in rhythm perception and production, including stuttering, autism, and Parkinson's disease. Zebra finches present a strong model for investigating the neural responses to rhythm because they are a vocal learning species with a naturally rhythmic song. Adult zebra finches exhibit increased expression of the immediate early gene ZENK following exposure to arrhythmic compared to rhythmic song in the caudomedial nidopallium (NCM), caudomedial mesopallium (CMM), and nucleus taeniae (Tn). NCM and CMM are homologous to auditory association cortex in humans and Tn is the avian homolog of the amygdala. To understand development of rhythm discrimination, we exposed male and female juvenile zebra finches at three stages to sets of natural zebra finch songs (rhythmic) or songs in which the duration of the silences between notes was modified to disrupt the natural timing (arrhythmic). We hypothesized that rhythm discrimination develops following template formation, which is complete by day 45. Juveniles were exposed to one of the stimulus types at day 15 post hatching, prior to song template memorization, day 25, during template formation but prior to sensorimotor integration, or at day 45, during sensorimotor integration in males. We expected a greater ZENK response to arrhythmic vs. rhythmic songs in birds at day 45. Immunohistochemistry for ZENK revealed a significant effect of age in NCM ($F=10.7$, $p<0.001$), with 15-day-old birds having greater density of ZENK expressing cells than birds at both days 25 and 45 (Tukey HSD $p<0.05$). Effects of sex and rhythmicity were not detected. These results are consistent with findings of high levels of constitutive ZENK expression within NCM at day 20, and that ZENK expression is not inducible by song exposure until later ages (Stripling et al., 2001). The lack of sex differences in this brain region parallels the pattern found in adults. The lack of an effect of rhythm condition at any juvenile age, in contrast with our findings in adults, suggests that NCM function related to song quality discrimination might occur in both sexes later in development than we anticipated. The data also suggest that early sensorimotor integration in males is not sufficient to allow rhythm discrimination within this brain region. In NCM, the ability to compare the song with a template or to detect temporal regularity through another mechanism seems to develop closer to adulthood. Additional brain regions relevant to auditory-motor integration for song learning are under investigation.

Disclosures: **J.A. Lampen:** None. **K. Jones:** None. **J. McAuley:** None. **S. Chang:** None. **J. Wade:** None.

Poster

260. Song Learning and Auditory Processing

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Support: JSPS KAKENHI 26430027 to SY

JSPS KAKENHI 26115526 to Y Y-S

Title: State-dependent auditory selectivity for familiar songs in the auditory association cortex of juvenile songbirds

Authors: ***S. YANAGIHARA**, Y. YAZAKI-SUGIYAMA;
Okinawa Inst. of Sci. and Technol., Onna-Son, Japan

Abstract: Sensory information processing can be modulated depending on behavioral states of animals, such as asleep, awake, or attentive. The latter states regulate higher cognitive function, like learning. As in human speech acquisition, songbirds learn to sing through social interaction with adult birds. Juvenile zebra finches scarcely copy recorded songs just by passive hearing. However, when a recorded song is presented during operant conditioning (key peck triggers song playback), juveniles successfully learn from it (Tchernichovski et al. 2001). This raises the possibility that bird's behavioral state modulates auditory information processing, which regulates song learning. Recently, we found that subsets of neurons in the zebra finch auditory association cortex, the caudomedial nidopallium (NCM), exhibit highly selective responses to tutor song (TUT) or the juvenile bird's own developing song (BOS) following tutor song experience (Yanagihara & Yazaki-Sugiyama 2014 SFN abstract). These neurons are thought to encode memories of songs that the birds have experienced. Here, we examined whether experience-dependent auditory activity in NCM is modulated by behavioral states or social context. Previous studies show that neurons in the song system nuclei robustly respond to BOS during sleep, while they do not during wakefulness (Dave et al. 1998, Schmidt & Konish 1998, Nick & Konishi 2001, Rauske et al. 2003, Cardin & Schmidt 2003, 2004). Unlike song system nuclei, we found that song-selective neurons in NCM of juvenile zebra finches, greatly decreased selectivity to familiar songs while birds were asleep. Furthermore, we found that social context markedly modulates auditory responses of song-selective neurons. Presence of a tutor enhanced auditory responses in those neurons to both TUT and BOS, but not to other songs. In preliminary experiments, we also found that infusion of noradrenaline, a neuromodulator involved in arousal/attention, similarly enhanced auditory responses only to TUT and BOS, but not to other

songs. Taken together, auditory response selectivity is modulated by both behavioral state (awake or asleep) and social context (presence of a tutor) in NCM. Presumably these state-dependent modulations of auditory response selectivity are partially mediated by the noradrenergic system. These elements are suggested to underlie behavioral state-dependent memory formation of tutor song.

Disclosures: S. Yanagihara: None. **Y. Yazaki-Sugiyama:** None.

Poster

260. Song Learning and Auditory Processing

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Topic: F.04. Neuroethology

Support: NSERC

NSERC USRA

Title: Courtship song preferences in female zebra finches arise independently of early auditory experience

Authors: *Y. CHEN[1], O. CLARK[2], V. NG[2], S. C. WOOLLEY[2];
[2]Biol., [1]McGill Univ., Montreal, QC, Canada

Abstract: Early social and sensory experiences are critical in shaping perception and social behavior; consequently, such developmental experiences can also profoundly influence adult preferences for particular stimuli. Female songbirds use song to identify male conspecifics and choose preferred mates. However, we know little about how early social and sensory experience affect female song preferences and the neural circuits underlying female preferences. We have previously found that female zebra finches prefer a male's courtship ('directed') song over his non-courtship ('undirected') song, regardless of the familiarity of the male. To test whether this preference is dependent on a female's early auditory experience, we compared song preferences and neural response to song between female zebra finches raised without exposure to adult male song ('isolate females') and normally reared females. Using a callback assay, we quantified adult female responses to directed and undirected songs from multiple different males and found that isolate females preferred the courtship songs to non-courtship songs. In particular, they significantly increased their calling in response to the courtship songs and decreased calling in response to non-courtship songs when compared to baseline calling. Moreover, their responses were indistinguishable from normally reared females, suggesting preferences for courtship song are not significantly affected by developmental exposure to song. In a separate group of birds, we investigated the degree to which developmental song exposure affected auditory responses. Our preliminary analysis of immediate early gene (EGR-1) expression indicates that auditory

responses in higher-level auditory areas, including the caudomedial mesopallium (CMM) and caudomedial nidopallium (NCM), are not significantly different between normally and isolate reared birds. These data indicate that the strong preference for female-directed courtship song is not dependent on early auditory exposure to song but may instead reflect an inherent bias in the auditory system.

Disclosures: **Y. Chen:** None. **O. Clark:** None. **V. Ng:** None. **S.C. Woolley:** None.

Poster

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Topic: F.04. Neuroethology

Support: R03HD068960

Title: Experimental manipulations in the developing songbird affect both vocal imitation and auditory memories

Authors: ***D. S. VICARIO**[1], B. BELL[1], M. L. PHAN[1], K. L. BUCHANAN[2];
[1]Rutgers Univ., Piscataway, NJ; [2]Sch. of Life and Envrn. Sci., Deakin Univ., Geelong, Australia

Abstract: Songbirds provide a translational model for two forms of learning that occur during a critical period in development: 1) sensory-motor learning that subserves vocal imitation; and 2) the formation of long term sensory memories. Normal vocal development requires auditory and social experience with an adult tutor and extensive vocal practice, guided by a memory of the tutor song. In the songbird, the outcome of this imitation process can be quantified as the similarity of the bird's song to the tutor model. We have previously documented a neuronal memory for the tutor song, formed in the juvenile period, that can be measured in the adult caudomedial nidopallidum (NCM); the strength of this memory is correlated with imitation quality. In addition to storage of the tutor's song, NCM contributes to discrimination and memory for species-specific vocalizations heard in adulthood. However, this system for vocal learning and memory is sensitive to perturbations. Various studies have shown that pharmacological inhibition of NCM produces decrements in song learning and that bilateral NCM lesions impair song discrimination. Dosing with oral atorvastatin, beginning in the critical period for song learning, degrades song copying, reduces memory for the tutor song, and impairs the formation of memories for novel auditory stimuli in NCM in adulthood. The present study tested for the effects of environmental stressors (e.g. handling and an impoverished diet) on vocal learning and memory. We reduced the quality of food available to parents (who regurgitate food to their young) and handled the birds daily to weigh them during a limited early portion of the critical period for vocal learning (days 5-30phd). After an initial lag in weight gain, birds

achieved normal weight by 30phd, at which times all subjects received a normal diet ad libitum and handling was minimized. In adulthood (110phd), song imitation fidelity and electrophysiological responses to conspecific songs were assessed. The data suggest that mild environmental stress during the early critical period of development (n=10; 4 females; 6 males) can result in deficits in the quality of song imitation and in the neuronal memory for conspecific songs. Taken together, these results show that the quality of sensorimotor learning can be affected not only by direct experimental insults like pharmacological lesions, but also by side effects from commonly prescribed medicines and enhanced stress during the early stages of development.

Disclosures: **D.S. Vicario:** None. **B. Bell:** None. **M.L. Phan:** None. **K.L. Buchanan:** None.

Poster

260. Song Learning and Auditory Processing

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Hercules Grant AUHA0012

FWO Grant G044311N

Title: Longitudinal *in vitro* diffusion tensor imaging study maps structural plasticity in the brain of juvenile male zebra finches throughout the critical period for vocal learning

Authors: J. HAMAIDE, G. DE GROOF, *A.-M. VAN DER LINDEN;
Univ. of Antwerp, Antwerp, Belgium

Abstract: Structural development of the zebra finch (ZF) brain has been investigated mainly by invasive methods such as histology. These *ex vivo* imaging techniques are highly sensitive and specific to particular biological phenomena but do not allow repeated measures or correlations with behavioral changes. Here we present a longitudinal Diffusion Tensor Imaging (DTI) study aimed at mapping structural development of the male ZF brain related to the process of vocal learning. DTI has proven to be a non-invasive Magnetic Resonance Imaging (MRI) tool sensitive to changing myelin contents and microstructural reorganization of the brain[1]. Juvenile male ZFs (Taeniopygia guttata; n=16) underwent repeated imaging sessions at seven time points throughout the sensory and sensorimotor phase of vocal learning and after song crystallization. A whole-brain voxel-based analysis (repeated-measures ANOVA) was performed on the DTI parameter maps i.e. fractional anisotropy (FA), mean diffusion (MD) and the eigenvalues. Most significant microstructural rearrangements take place in two waves situated within the sensory

and sensorimotor phase i.e. between resp. 20 - 30 days post hatching (dph) and 40 - 65 dph. From the early sensory phase (20 dph) until song crystallization (90-120 dph), MD and FA gradually decrease and increase resp. and this happens in a caudal-to-rostral wave mostly involving pallial and subpallial brain areas. Interestingly, from 40 dph onwards, the rostral border of Field L appears to delineate the caudal border of the area that still displays a change in MD values compared to adulthood. These observations are in line with studies investigating human brain development using DTI, where it has been shown that sensorimotor cortices mature prior to higher-order associative brain areas[2]. When zooming in on the main effect of age for FA, a DTI parameter sensitive to fiber- or myelin- containing structures, specific subparts of the lamina mesopallialis, the lamina frontalis superior and the tractus occipitomesencephalicus can be identified and, interestingly, their establishment can be situated in time. Songs recorded during the sensorimotor phase and after song crystallization will be analyzed and correlated to the DTI data of the same individual. Voxel-wise correlation of DTI findings to song output and to the outcome of a parallel auditory fMRI study targeting the neural substrate of tutor song selectivity in ontogeny performed at the lab will shed further light onto structural and functional neuronal changes during the critical period of vocal learning. [1] Mori and Zhang, 2006 Neuron; [2] Yoshida et al., 2013 Pediatr Radiol

Disclosures: **J. Hamaide:** None. **G. De Groof:** None. **A. Van Der Linden:** None.

Poster

260. Song Learning and Auditory Processing

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Topic: F.04. Neuroethology

Title: Sensory expectation in male HVC for call communication in zebra finches

Authors: ***S. MA**[1,2], A. TER MAAT[1], M. GAHR[1,2];
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Abstract: Zebra finches are social animals that communicate with each other using calls and songs. While only the males are singing, female and male share a similar repertoire of calls to communicate within their social group and in particular with their mates. To facilitate these social interactions, it requires brain mechanisms for call communication. Forebrain nucleus HVC is a cortex-like sensorimotor area essential for song production and learning. In order to study the role of the HVC in call communication, we used a wireless telemetric system for simultaneous measurement of neural activity and vocalizations in animals that freely interacted with each other. HVC displayed stereotypic activity patterns for call production and processing of those female calls that were uttered in bouts of male-female vocal exchange. The premotor activity in

HVC did not necessarily produce a call but fictive motions to compare between sensory states of self and external cues to predict upcoming female calls. The prediction with fictive motions could not be done independently without sensory cues. The male HVC also displayed a neural activity to sensory inputs of incoming female calls, which may be responsible for evaluating the precision of call production with incoming sensory inputs of female calls. Such HVC based response to female calls depends on a fictive premotor activity that equals sensory expectation of female calling.

Disclosures: **S. Ma:** None. **A. ter Maat:** None. **M. Gahr:** None.

Poster

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Topic: F.04. Neuroethology

Support: People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme (FP7/2007-2013) under REA grant agreement n° 302549.

Title: Vocal sequence predictability modulates traveling wave activity in songbird auditory forebrain

Authors: ***G. J. BECKERS**[1], J. A. BROEKHUIZEN[1], N. C. RATTENBORG[2], J. J. BOLHUIS[1];
[1]Utrecht Univ., Utrecht, Netherlands; [2]Max Planck Inst. for Ornithology, Seewiesen, Germany

Abstract: Bird songs arguably have the most complex structure among all animal vocalizations, except human speech, as they may consist of long and variable sequences of different vocal element types. How such sequences are perceived remains poorly understood. Of particular interest is whether their recognition depends mainly on memory of (generalized) recurring sound objects consisting of element chunks, or, perhaps additionally, involves analysis of regularities in the sequence per se (sequence rule learning). Previously we have shown that secondary regions in the auditory forebrain of anesthetized zebra finches, Taeniopygia guttata, are sensitive to short-term stimulus history statistics, as they exhibit synchronized activity of action potential firing preferably in response to calls that are deviant within a series of calls that are standard (i.e. statistically expected). Such activity could reflect a stimulus-specific form of short-term auditory memory, but it may also reflect a violation of predicted auditory input. We recorded action and local field potentials at 64 sites in parallel in the auditory forebrain of isoflurane anesthetized zebra finches, and played sequences of song elements in random order, interspersed with episodes where elements followed a simple sequence rule (repetitions, bigrams). In addition to temporally and spatially stereotypic responses that are confined to the primary thalamorecipient

area L2, we found slow-oscillations that travel in complex temporospatial patterns across secondary auditory areas. These propagating waves appear to be similar to those that have been described earlier in other zebra finch brain regions and that have been linked to NREM sleep. We found that in the auditory forebrain such traveling slow oscillation activity is stronger during episodes with random sequences than it is during episodes where vocal elements are repeated. Further, random vocal elements that immediately followed a regular sequence elicited stronger slow-wave activity than the same elements situated in a random context. Taken together, these data suggest that traveling slow-wave activity in the songbird auditory forebrain is modulated by short-term predictability of elements in a vocal sequence.

Disclosures: G.J. Beckers: None. **J.A. Broekhuizen:** None. **N.C. Rattenborg:** None. **J.J. Bolhuis:** None.

Poster

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New York Stem Cell Foundation

DFG (Va 742/1-1/2)

Title: Inhibition systematically silences an instructive signal during motor learning

Authors: *D. VALLENTIN[1], G. KOSCHE[1], D. LIPKIND[2], M. A. LONG[1];
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Abstract: Zebra finches learn their songs by listening to and imitating a tutor. During development, sensory input is necessary in order to effectively imitate the tutor's song. Recent experiments have demonstrated that disrupting neuronal activity in the premotor nucleus HVC during tutor song exposure can significantly block song imitation (Roberts et al. 2012), suggesting that the passive engagement of the motor pathway may be necessary to establish proper singing behavior. Here, we directly measure the impact of the tutor song on HVC neurons during and after learning. We recorded intracellularly from premotor cells and found that the tutor song has the potential to drive precise spiking responses in the awake juvenile zebra finch, but not in the adult bird. What is the mechanism that can affect a developmental decrease in the premotor neuron's response to tutor song input during song learning? To measure developmental changes in the strength of auditory representation, we performed voltage clamp recordings of

HVC premotor neurons in awake zebra finches. We found that the amplitude, frequency and precision of identified excitatory events evoked by the tutor song did not decrease during learning. However, the firing of local inhibitory interneurons as well as inhibitory synaptic events onto premotor neurons in HVC became significantly more precisely timed to the tutor song motif depending on the learning stage of the bird. To investigate whether the emergence of precise inhibition during learning is sufficient to prevent premotor neurons from spiking in the adult zebra finch, we pharmacologically minimized the impact of inhibition. Local application of a low concentration of the GABAA antagonist gabazine led to a reemergence of tutor song-driven spiking responses in premotor neurons. We then asked whether the precise inhibition can accurately target the portions of the song that have been learned. We trained zebra finches with a simple tutor song consisting of four identical syllables (AAAA). After the birds gained proficiency in the performance of syllable A, we extended the tutor song by adding syllable B (ABAB) (Lipkind & Tchernichovski 2011). We recorded interneuron activity and inhibitory currents onto premotor neurons in zebra finches that copied A well and copied B poorly, and found that inhibition was selectively stronger during syllable A versus syllable B. Taken together, these data suggest that inhibition within HVC progressively blocks an instructive signal in an accurate manner during motor learning, potentially to 'write protect' that motor skill.

Disclosures: **D. Vallentin:** None. **G. Kosche:** None. **D. Lipkind:** None. **M.A. Long:** None.

Poster

260. Song Learning and Auditory Processing

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: F.04. Neuroethology

Support: Supported in part by Israel Science Foundation Grant No. 1978/13

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Title: High order interactions between synapses affect the average dynamics of the synaptic efficacy in recurrent neural networks

Authors: *N. RAVID[1], Y. BURAK[2,3];
[1]ELSC, Hebrew Univ., Jerusalem, Israel; [2]Edmond and Lily Safra Ctr. for Brain Sci., [3]Racah Inst. of Physics, Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: The structure and plasticity of synaptic connectivity underlies much of our ability to process and store information. In the past two decades, spike timing dependent plasticity (STDP) has become one of the main foundations of theoretical research on plasticity and learning. Previous works that explored theoretically the change in the synaptic efficacy according to STDP mainly focused on local approximated terms that depend on the firing rate of the pre- and the

post-synaptic neurons and the synaptic efficacies between them (for example: Babadi and Abbott 2013). We recently developed an analytical framework in which the average change in the synaptic efficacy, due to STDP, can be evaluated precisely in recurrent networks of Poisson neurons with arbitrary connectivity. In this framework, the local learning plasticity rule of Babadi and Abbott arises in a systematic manner as the first order term of an expansion in the strength of synaptic efficacies. We show that higher order terms lead to an effective interaction between synapses of different neurons that can significantly alter the dynamics, and affect the global structure of the neural network in steady state. As an example, we consider the spontaneous formation of wide synfire chains, in which distinct groups of neurons share similar connectivity, and sequentially project to each other. It has been hypothesized that this architecture underlies the synchronous neural activity observed in the premotor nucleus of songbirds HVC (Hahnloser et al. 2002 , Long et al. 2010). We show that the high order terms in our theory can promote the formation of wide synfire chains without the need to introduce structural constraints or correlated external input.

Disclosures: **N. Ravid:** None. **Y. Burak:** None.

Poster

260. Song Learning and Auditory Processing

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Topic: F.04. Neuroethology

Support: ANR Acoustics 700602

Fyssen Foundation Research Grant

Title: Sequence processing in a songbird secondary auditory area

Authors: ***N. GIRET**, A. CAZALA, C. DEL NEGRO;
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Abstract: The complexity of the human language is thought to be unique in the animal kingdom, given several human specific features such as the recursive aspects of syntax, which consists in embedding phrases within phrases. However, songbirds can be trained to behaviorally distinguish sequences of syllables according to their recursive structure. As humans, songbirds are able to learn and produce vocalizations containing sequences of syllables with specific transition probabilities. How sequences are processed into the songbird brain remains to be characterized. Songbirds offer a great opportunity to address this issue: they have a set of interconnected telencephalic auditory brain areas including an analog of the mammalian secondary order auditory cortex, the nidopallium caudo-medial (NCM). In this area, repeated exposure to a conspecific vocalization induces a decrement of the elicited spiking response or

gene expression. In order to investigate whether the NCM is involved in syntax processing, we played back sequences of syllables to anaesthetized birds while recording the neuronal activity of NCM neurons. The sequences contained one introductory note followed by sequences with (AnBn) or without ((AB)n, (CD)n, ABCD or CDEF) a recursive structure (where A, B, C, D, E, F are song syllables and n the number of repetition). During an exposure, we broadcasted a given sequence 50 times and then either the same sequence or a different one (thus differing either in syllable identity or order) 10 times. Preliminary results show habituation of the spiking responses to the repeated exposure of a sequence. Changing the syllable identity but not the syllable order induces an increase of the spiking response, thus suggesting a sensitivity of NCM neurons to acoustic features of the syllables but not to the sequence structure.

Disclosures: **N. Giret:** None. **A. Cazala:** None. **C. Del Negro:** None.

Poster

260. Song Learning and Auditory Processing

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Program#/Poster#: 260.25/Z30

Topic: F.04. Neuroethology

Title: Neuritin (CPG-15) may contribute to synaptic plasticity in brain areas for sensory song learning during development

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[1]Inst. For Mind and Biol., Chicago, IL; [2]Dept of Psychology, Inst. for Mind and Biology, Committee on Neurobio., Univ. of Chicago, Chicago, IL

Abstract: Young male zebra finches learn to sing by memorizing the structure of a "tutor's" song. Tutor song memorization normally occurs during one sensitive period from posthatch day 30-65 (P30-65). Birds constantly experience song in their social environment, thus the limited learning window suggests that there are neural properties that permit tutor song memorization, and that these characteristics change across the sensitive period. Balancing synapse plasticity and stabilization is an essential processes in neural development and learning and memory. Neuritin (also called CPG-15) promotes synapse stabilization and is developmentally regulated, activity dependent, and implicated in cognitive function in other systems. We therefore assessed neuritin expression in the zebra finch to determine its potential role in developmental song learning. We focused on P25, P45, and P65 males and females who were either raised normally (i.e. in a social environment and exposed to song) or with one adult female (in a social environment, but isolated from song) to assess the effect of age, sex, and song experience on neuritin expression just prior to, during, and at the close of, the sensitive period for tutor song memorization. *In situ* hybridization revealed that all three ages, neuritin is present in the secondary processing regions of the auditory forebrain, CMM and NCM, and in HVC. We did not detect neuritin mRNA in

RA, LMAN, or Area X, suggesting a greater role for neuritin in auditory processing than in motor production. Preliminary results also suggest that the levels of neuritin mRNA are higher in male HVC than female HVC. Surprisingly, song playbacks in adults does not seem to induce neuritin expression in the auditory forebrain, likely because acoustic isolation for up to two weeks does not lower baseline mRNA levels. It may be therefore that neuronal activity regulates neuritin in the songbird differently than in rodents, and/or neuritin is regulated differently in secondary sensory cortex than other regions. As neuritin contributes to synaptic plasticity during development, after neural activity, and in adult cognitive disorders, these data provide a foundation for future studies to tie neuritin with the neural processes that underlie normal and disordered cognitive function during development.

Disclosures: B. Borden: None. **S.E. London:** None.

Poster

261. Molecular Techniques

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Title: Rapid molecular profiling of genetically defined cell types using viral TRAP

Authors: *E. F. SCHMIDT[1], A. R. NECTOW[2], M. I. EKSTRAND[2], A. MOUSA[1], K. L. MCGUIRE[1], C. E. SFERRAZZA[1], B. C. FIELD[2], Y. LIANG[3], G. S. RABINOWITZ[4], K. SAWICKA[4], N. HEINTZ[1,5];
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Abstract: Recent advances in translational profiling methods have enabled the systematic characterization of the molecular properties of specific cell types in complex tissues. These methods have proven particularly useful for studies of the mammalian central nervous system, where hundreds of heterogeneous cell types are intermixed, making neuronal subtype isolation exceptionally difficult. The translating ribosome affinity purification (TRAP) technique is a direct, rapid method for isolating polysomal RNA from genetically defined cell populations *in*

vivo. It requires the targeted expression of EGFP-tagged ribosomal subunit L10a (EGFPL10a) in identified cell populations. Traditionally, this has been achieved through the generation of novel BAC transgenic mouse lines that label cell types of interest (bacTRAP), or crossing Cre recombinase expressing lines to Cre-dependent EGFPL10a reporter mice, thus requiring the expression of two independent alleles. Here, we report the development of a viral strategy for the rapid profiling of CNS cell types that can be used in combination with any existing Cre driver line. This strategy allows for TRAP profiling of genetically and anatomically defined cell types within a few weeks of virus injection and circumvents the need to create new transgenic strains or undergo lengthy breeding strategies. We engineered an adeno-associated viral (AAV) vector to express EGFPL10a in a Cre-dependent manner (AAV-FLEX-EGFPL10a). We demonstrate the incorporation of the EGFPL10a transgene into functional polysomes *in vivo*, and validate its utility as a tool to access translating mRNAs from various defined neural populations in the midbrain and hypothalamus. A direct comparison of high throughput RNA sequencing results from layer 6 corticothalamic projection neurons revealed a high correlation between the viral TRAP (vTRAP) and bacTRAP approaches and demonstrated the ability of vTRAP to distinguish regional differences in gene expression within the same cell type. Taken together, these results establish utility and broad applicability of the vTRAP strategy for profiling cell types within or outside of the CNS in a Cre-dependent manner.

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Poster

261. Molecular Techniques

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Program#/Poster#: 261.02/Z32

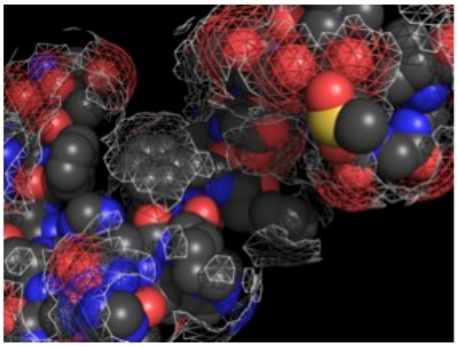
Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Sequence specific radiolytic footprinting study of monomer, oligomeric and fibrillar Aβ42

Authors: *A. KLINGER[1], J. KISELAR[2], A. PARAVASTU[3], T. ROSENBERRY[4]; [1]Dechiperbio, Wyndmoor, PA; [2]Case Western Reserve Univ., Cleveland, IL; [3]Florida State Univ., Tallahassee, FL; [4]Mayo Clin., Jacksonville, FL

Abstract: Increasing evidence suggests that soluble aggregates of amyloid-β (Aβ) are the pathogenic species in Alzheimer's disease (AD). However, detailed structural information on these species remains scarce due to low levels of endogenous Aβ oligomers and uncertainties surrounding current *in vitro* model systems. Herein, we describe a hydroxyl radical footprinting (HRF) study of Aβ42 monomers, small aggregates, homogenous and stable oligomers, and fibrils. Specific side chain solvent accessibilities of individual residues in the aggregated and

fibril forms of Aβ42 are measured with respect to the same residues of Aβ42 in a fully exposed reference state. These data provide residue specific side chain solvent accessibility protection factors and are used in complement with biophysical characterizations and ss-NMR analyses of the same systems. Results are discussed in the context of proposed NMR models of Aβ oligomers with implications towards further development of therapeutic and diagnostic strategies.



Disclosures: **A. Klinger:** None. **J. Kiselar:** None. **A. Paravastu:** None. **T. Rosenberry:** None.

Poster

261. Molecular Techniques

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 261.03/Z33

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: MojoSort™, a versatile nanoparticle for magnetic isolation of CX3CR1+ cells with high purity, yield, and preserved functionality

Authors: H. ZHANG[1], M. TAM[1], D. GOHEL[1], J. KOURY[1], T. OIDA[1], X. ZHAO[1], S. GARCIA MOJICA[1], X. YANG[1], *M. TAYLOR[2];
[1]BioLegend, San Diego, CA; [2]Biolegend, Dedham, MA

Abstract: Isolation of a defined population from a complex mixture of cells is a frequent technical challenge. Strategies have developed over recent decades, including gradient centrifugation, Fluorescent Activated Cell Sorting (FACS) and Magnetic Cell Isolation. Magnetic labeling is the most commonly used approach, as it is a fast, reliable and convenient method to obtain discrete populations with high purity and yield, and the cells are readily available for downstream applications. Here we present a new type of magnetic nanoparticle that can be used in most commercially available separation systems, including magnetic separation columns. To illustrate the speed, ease and utility of the system for obtaining fully functional CX3CR1+ cells, a major marker for glial cells, bone marrow precursors were isolated from C57Bl/6 mice using CX3CR1-conjugated MojoSort™ particles. Immunophenotype of the >10-

fold enriched cells was confirmed by flow cytometry. The CX3CR1+ cells were then differentiated towards a macrophage-like phenotype *in vitro*. After culture, LPS and CpG were used to activate the CX3CR1 derivative cells and the activated phenotype was compared to non-stimulated cells. Surface markers analyzed include CD80, CD86 and MHC II. The supernatant was collected and screened for the presence of 24 inflammatory cytokines, such as IFN-β, GM-CSF, IL-1, IL-12 and MIP. The isolated cells show a differential activation phenotype, as well as a defined cytokine profile, in accordance with the stimulation used when compared to non-stimulated cells. Thus, we establish the high quality performance of BioLegend's new low-cost magnetic cell separation system and its relevance for neuroscience research.

Disclosures: **H. Zhang:** A. Employment/Salary (full or part-time):; BioLegend. **M. Tam:** A. Employment/Salary (full or part-time):; BioLegend. **D. Gohel:** A. Employment/Salary (full or part-time):; BioLegend. **J. Koury:** A. Employment/Salary (full or part-time):; BioLegend. **T. Oida:** A. Employment/Salary (full or part-time):; BioLegend. **X. Zhao:** A. Employment/Salary (full or part-time):; BioLegend. **S. Garcia Mojica:** A. Employment/Salary (full or part-time):; BioLegend. **X. Yang:** A. Employment/Salary (full or part-time):; BioLegend. **M. Taylor:** A. Employment/Salary (full or part-time):; BioLegend.

Poster

261. Molecular Techniques

Location: Hall A

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Program#/Poster#: 261.04/Z34

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Pre-sequencing quality control metrics predicting success of sequencing single-cell transcriptomes

Authors: *M. VAN DEN HURK[1,2], C. BARDY[2], J. ERWIN[2], B. P. F. RUTTEN[1], G. KENIS[1], H. W. M. STEINBUSCH[1], F. H. GAGE[2];
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Abstract: Single-cell sequencing has emerged as a powerful tool for the quantification and characterization of transcripts in individual cells, including neurons. Although several methods are available to assess the quality of RNA or cDNA starting material prior to sequencing, there have only been limited investigations into which quality control (QC) parameters predict the success of sequencing single-cell transcriptomes. By correlating various pre-sequencing QC metrics with actual HiSeq sequencing data of over a hundred single neurons, we define a set of quantitative variables that identify single-neuron cDNA preps yielding good sequencing success. Our data reveals that several quantitative metrics, obtained from cDNA fragment profiles (size distribution) and quantitative real-time PCR measurements of expression of housekeeping genes and RNA spike-in controls, represent relevant predictors of read mapping and detectability of

genes and transcripts. The results and suggestions presented here may prevent redundant sequencing of low-quality cDNA preps and improve the quality of sequencing data.

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Poster

261. Molecular Techniques

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the Funding Program for Next Generation World-Leading Researchers, Grant No. LS081

the Uehara Memorial Foundation

Title: Practical optimization of *in situ* hybridization procedure for the detection of microRNAs and mRNA expression in brain tissues

Authors: ***A. KASAI**, S. KAKIHARA, R. OKADA, K. HAZAMA, T. NAKAZAWA, K. NAGAYASU, A. HAYATA-TAKANO, N. SHINTANI, H. HASHIMOTO;
Osaka Univ., Suita, Japan

Abstract: MicroRNAs (miRNAs) are endogenous small noncoding RNA transcripts that individually fine-tune the expression of target genes. Recent evidence supports fundamental roles for miRNA dysregulation in brain diseases as well as brain development. Since understanding spatial-temporal expressions of miRNAs is important to examine miRNA function, a fast and effective miRNA *in situ* hybridization (ISH) protocol using locked nucleic acid (LNA) probes has been developed. However, detection of low-abundance miRNAs by ISH remains to considerably depend on each laboratory technique to obtain reproducible results particularly in heterogeneous brain tissues. Here, we aim to optimize the procedures for *in situ* detection of miR-34a which is specifically expressed in parvalbumin positive γ-aminobutyric acid neuron in the brain. First, we investigated the effect of probe accessibility on the ISH signal intensity and pattern. In thalamic reticular nucleus (TRN) which is a thin layer of parvalbumin positive neuron, the ISH signal intensity of miR-34a in fresh brain samples was stronger than that in fixed brain samples. Proteinase K treatment was dose-dependently enhanced the ISH signals in both fixed and fresh samples. Second, since stringent hybridization condition is also important for intensity and specificity of ISH signals, we examined the conditions for hybridization temperature and stringency wash to get miRNA ISH signals. Although the ISH signals were

hardly detected at 30°C below the Tm of the probe, the signals could be obtained at 37°C below the Tm. In addition, the signals were a high signal-to-noise ratio at 50% formamide concentration. Third, the specificity of the miRNA ISH signal under optimized procedures was confirmed by comparing ISH and TaqMan RT-PCR for a miR-34 family member, miR-34c. miR-34c signals at 37°C below the Tm were predominantly detected in choroidal plexus unlike in the case of miR-34a. In accord with the ISH signal pattern, TaqMan RT-PCR showed that miR-34c was abundantly expressed in brain region including choroidal plexus and miR-34a in TRN. These data suggest that ISH at 37°C below the Tm detects miRNAs reliably. Moreover, we examined if the optimized protocol could have broad versatility. All miRNA ISH signals we tested showed each own expression patterns. Finally, by combining with mRNA fluorescence ISH, we also identified the type of the miRNA-expressing cells. Thus, the present study provides a comprehensive solution for detecting *in vivo* localization of a wide variety of miRNAs and contributes to the understanding of miRNA function.

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Poster

261. Molecular Techniques

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Support: NIH Grant R01 AG041250

NIH Grant R01 CA163640

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Title: Improved gene delivery to adult mouse spinal cord through the use of hybrid adeno-associated viral (aav) vectors

Authors: ***J. J. SIU**[1], C. WANG[2], L. CAO[2];
[1]Neurosci., [2]Mol. Virology, Immunology, and Mol. Genet., Ohio State Univ., Columbus, OH

Abstract: Gene therapy is characterized by the delivery of nucleic acids into cells to treat or modify disease. Viral vectors are often used as vehicles to deliver genes to target cells. Of the possible candidates, adeno-associated virus (AAV) is ideal for neurological disorders because of its safety profile & promising results in current clinical trials. One challenge in AAV gene therapy is effective transduction of large numbers of the appropriate cell type, which can be overcome by modulating the viral capsid. This is accomplished through DNA shuffling, or the

random assembly of capsid genes from various AAV serotypes to produce new chimeric variants. Previously, some of our novel hybrid AAV vectors (Rec2, Rec3, Rec4) demonstrated higher viral yield, gene expression, and transduction volumes compared to naturally occurring serotypes such as AAV9, when injected directly into brain. We therefore hypothesized that direct injection of these new vectors into spinal cord would result in similar observations. To test this, green fluorescent protein (GFP) was first cloned into vectors AAV9 and Rec2-4, all of which drive gene expression through the CAG (hybrid CMV-chicken beta-actin) promoter. Next, 2x10^9 viral particles of each AAV vector was injected unilaterally into the T9 vertebral level of spinal cord in 9-week-old mice. Mice were sacrificed at 3 weeks post-injection, and brain and spinal cord were collected to evaluate cellular tropism, gene expression, and transduction volume compared to AAV9 control. Through immunohistochemistry, we demonstrated that Rec3 and Rec4 were able to transduce a broader region of spinal cord compared to AAV9 while Rec2 seemed to display higher GFP transgene expression than AAV9. All Rec vectors were able to transduce NeuN+ neurons. Further analysis will characterize the tropism towards glia and the extent to which these hybrid vectors are superior to natural AAV. Future work with these vectors will explore other methods of viral delivery and the expression of neurotrophic factors to protect against neurodegenerative diseases affecting brain and/or spinal cord.

Disclosures: **J.J. Siu:** None. **C. Wang:** None. **L. Cao:** None.

Poster

261. Molecular Techniques

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Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: DARPA RCI N66001-12-C-4025

NIH R01 HL111598

Title: Exposure to blue light alters gene expression in murine microglia

Authors: ***K. P. CHENG**[1], S. K. BRODNICK[1], J. WILLIAMS[1], J. J. WATTERS[2];
[1]Biomed. Engin., [2]Comparative Biol., Univ. of Wisconsin Madison, Madison, WI

Abstract: The advent of optogenetics along with the prevalence of fluorescent assisted studies have led to a dramatic increase in the exposure of blue light to living tissues *in vitro* and *in vivo*. However, the potential effects of blue light alone on non-transgenic, native cellular behavior has been little reported. Here we studied the effect of repetitive low-level blue light (450nm) exposure on primary murine microglial gene expression in both *in vitro* and *in vivo* models. All experiments were performed using the C57BL/6 mouse strain with *in vitro* experiments using primary microglia isolated from the postnatal day 4-7 pup brain, and *in vivo* experiments used

10- 11 week old adult male mice. Illumination with 450nm light was provided using either CREE XT-E Royal Blue LED's or a Sutter Lambda DG-4 with appropriate 450nm bandpass filters. Following blue light exposure, microglial gene expression was immediately assessed via qRT-PCR. We found that blue light altered basal microglial gene expression in both a gene- and dose- dependent manner. Blue light delivered in this manner had a potent anti-inflammatory effect on the microglial LPS-response *in vitro*. Preliminary data *in vivo* suggest that blue light similarly reduces inflammatory gene expression in isolated microglia. These results demonstrate that long-term optogenetic and fluorescence-based studies in living tissues may have previously unreported off-target cellular effects, and also suggest the possibility of using blue light as a therapeutic agent. (Supported by DARPA RCI N66001-12-C-4025 and R01 HL111598).

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Poster

261. Molecular Techniques

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Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: Walter V. and Idun Berry Postdoctoral Fellowship

Title: A convenient real-time neuronal molecule tracking system using a novel bidirectional-expressing system

Authors: *Y. YANG[1], Y. GENG[1], J. CHU[2], M. Z. LIN[1];
[1]Pediatrics, Stanford Univ., Stanford, CA; [2]Shenzhen Inst. of Advanced Technology, Chinese Acad. of Sci., Shenzhen, China

Abstract: Dendritic spines and synapses are plastic and play critical roles in neuronal functions. The dynamics of synaptic molecules contribute to synaptic plasticity and have been typically studied by co-expressing a fluorescent protein(FP)-tagged protein of interest together with a single fluorescent protein in a different color to trace the fine structure of neurons. However, low co-transfection efficiency, fast decay in fluorescent signal intensity in transient transfections, and poor properties of available red fluorescent protens (RFPs) severely place burdens to these studies. In addition, tracking and analysis of punctuated, dynamic synaptic molecules are often hindered by heave labor, potential bias and inconsistency due to manual analysis. Here we report an optimized neuronal long-term expression system, which allows concurrent expression of the protein of interest and a new RFP, mCrimsonT, as the cell-tracing marker. This system uses a novel bidirectional expression plasmid where the FP-tagged protein of interest is encoded in the forward direction and mCrimsonT is encoded in the reverse direction. mCrimsonT is a newly

developed RFP featuring high brightness and photostability. More importantly, mCrimsonT does not form aggregations in neurons, serving as an ideal cell morphological marker. We also report a MATLAB script that enables automatic outlining of neuronal dendritic structures and quantification of fluorescent signals. With this fluorescent labeling system and Matlab scripts, we are able to automatically trace neuronal dendritic tree through live time-lapse recording and analysis of punctuated, dynamic signal changes of specific synaptic molecules with much improved efficiency.

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Poster

261. Molecular Techniques

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Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: MEXT/JSPS KAKENHI 24500408

MEXT/JSPS KAKENHI 25123709

MEXT/JSPS KAKENHI 15K14333

MEXT/JSPS KAKENHI 15H01430

Title: A single vector platform for high-level gene transduction of central neurons with Tet-Off adeno-associated virus

Authors: ***H. HIOKI**, J. SOHN, M. TAKAHASHI, S. OKAMOTO, T. KANEKO;
Kyoto Univ. Grad. Sch. of Med., Kyoto, Japan

Abstract: To achieve high-level transgene expression in neurons, we developed new single vector platform with adeno-associated virus (AAV). The platform, AAV SynTetOff, is mainly composed of two parts: 1) the regulator part expresses tetracycline-controlled transactivator (tTA) under the control of human synapsin I promoter (SYN; Hioki et al, 2007); and 2) the response part produces transgene under the control of Tet-Response Element composite promoter (TRE; Hioki et al, 2009). We first assessed the expression level of GFP *in vitro* with Neuro-2A cells. One week after the infection with AAV2/1 SynTetOff-GFP, SYN-GFP-pA, or CMV-GFP-pA, expression levels of GFP mRNA were examined by quantitative real-time reverse transcription PCR (qRT-PCR). The expression level with SynTetOff-GFP was 15.6- or 1.8-fold higher than with SYN-GFP-pA or CMV-GFP-pA, respectively. We also measured average intensity per pixel of GFP-native fluorescence (GFP-NF) in the infected cells. With SynTetOff-GFP, the intensity exhibited 14.5- or 1.8-fold increase as compared with SYN-GFP-pA or CMV-GFP-pA. We then injected the AAV2/1 vectors into the mouse neostriatum. One

week after the injection with SynTetOff-GFP, GFP-NF was clearly observed not only in the injection site but also in the projection targets such as globus pallidus and substantia nigra. On the other hand, GFP expression with SYN-GFP-pA and CMV-GFP-pA was very low so that the slight signals were detected only in the injection site. Actually, GFP-NF with SynTetOff-GFP was markedly higher than with SYN-GFP-pA or CMV-GFP-pA, 34.3- or 43.3-fold higher. In addition, GFP expression with CMV-GFP-pA was not neuron-specific (around 75.5%), whereas the expression with SynTetOff-GFP and SYN-GFP-pA was almost specific for neuronal cells. Furthermore, we added the palmitoylation site (pal) sequence, one of the plasma membrane-targeting signals, to the N-terminal of GFP, and injected AAV2/1 SynTetOff-palGFP into the mouse neostriatum. Neuronal processes of the infected neurons were more clearly visualized as compared with SynTetOff-GFP. These results indicate that newly developed single vector platform, AAV SynTetOff, is promising genetic tool for strong gene transduction and efficient labeling of neurons.

Disclosures: H. Hioki: None. **J. Sohn:** None. **M. Takahashi:** None. **S. Okamoto:** None. **T. Kaneko:** None.

Poster

261. Molecular Techniques

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 261.10/Z40

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Generation of human iN cell line

Authors: *S. LAU[1,2], N. AVALIANI[3], A. HEUER[2], M. KOKAIA[3], M. PARMAR[2]; [2]Developmental and Regenerative Neurobio., [3]Exptl. Epilepsy, [1]Lund Univ., Lund, Sweden

Abstract: Human fibroblast can be directly reprogrammed to induced neurons (iNs) using lineage specific transcription factors. However, only a limited number of iNs can be generated from each conversion. Because the resulting iNs are post-mitotic, the low conversion could limit their use in biomedical applications and regenerative medicine. In this study, we have designed a dual promoter system that expresses two reprogramming factors from the same vector. The resulting iNs exhibit functional properties of neurons *in vitro* and in slice cultures. The 2-factor iN cells survive and innervate the host brain after transplantation to the adult rat brain. We discovered that by changing the order of transcription factors in the constructs, conversion efficiency was greatly altered, suggesting that protein expression levels and stoichiometry has a great impact on reprogramming efficiency. By further modifying the dual promoter system we were able to generate fibroblasts that carried the construct and that could be stably expanded whilst not loosing their potential to be efficiently converted to neurons when conversion was initiated, allowing for expansion and cryo-preservation. The high conversion efficiency, in

combination with the possibility to passage and bank cells expressing the dual promoter construct, points to the possibility that human iNs can be used for large scale studies such as drug screening and disease modeling.

Disclosures: S. Lau: None. **N. Avaliani:** None. **A. Heuer:** None. **M. Kokaia:** None. **M. Parmar:** None.

Poster

261. Molecular Techniques

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 261.11/Z41

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Support: NIH NRSA Postdoctoral Fellowship F32NS090722 (Fink)

NIH Director's transformative award 1R01GM099688 (Nolta)

California Institute for Regenerative Medicine (CIRM) DR2-05415 (Wheelock/Nolta)

Dake Foundation (Fink)

Donations from Roberson Family

Donations from TeamKJ

Title: Patient centric gene targets for silencing the Huntington's disease mutation in adult and juvenile HD

Authors: *P. DENG[1,2], K. FINK[2], A. KOMARLA[1,2], J. HALMAI[1], A. TORREST[2], J. GUTIERREZ[2], T. TEMPKIN[3], V. WHEELOCK[3], L. CARVAJAL-CARMONA[1], D. SEGAL[1], J. NOLTA[2];
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Abstract: Huntington's disease (HD) is an autosomal dominant disorder characterized by an abnormally long CAG expansion. Although the gene causing HD has been well characterized in adults, the exact function of the wild-type and mutant proteins are still unknown. Wild-type huntingtin protein is essential for development, while mutant huntingtin accumulates in the cells of HD patients. Nuclear aggregates cause alterations in gene transcription resulting in cellular impairment including dysfunction in RNA synthesis, protein quality control, impaired mitochondrial activity, cellular inclusions, and activation of apoptotic pathways. While the mutant protein is toxic it normally takes multiple decades before the resulting cell death leads to overt symptomology and clinical manifestation of the disease. In the juvenile form of HD (jHD),

disease onset occurs before the age of 20 and in many cases prior to the age of 10 and the functional decline is more rapid. While this early onset and severe progression has been attributed to a larger CAG expansion, this correlation does not always hold true. Since its discovery in 1993, there has been little headway in producing therapeutics for the disorder. Our lab has shown preliminary data suggesting selective transcriptional repression of the mutant huntingtin allele by targeting SNPs solely present in the HD population. The identification of novel SNPs in the HD population has allowed a host of research groups to produce various methods to silence expression of the mutant huntingtin protein such as antisense oligonucleotides, siRNA treatments and DNA nucleases. SNPs for the adult HD population are well characterized; but little research has been conducted to characterize SNPs in jHD. This is a current deficit in the field as a whole as jHD individuals would likely benefit from early intervention due to the severity and early disease onset compared to adult HD. We have isolated novel regions of interest through Sanger sequencing the promoter and exon 1 of the mutant huntingtin allele in a jHD patient population. A combination of conventional molecular biology techniques and bioinformatic analysis of HD haplogroups have been used to identify unique allele-signatures for jHD. This analysis will provide a rich and robust data set for other groups, including our own, to continue developing potential gene silencing techniques for a currently underserved subpopulation in HD. Furthermore, comparison analysis between adult and jHD SNPs provides insight into the unique mutations present in both populations and may be further studied in order to better understand causes for disease onset beyond CAG length.

Disclosures: **P. Deng:** None. **K. Fink:** None. **A. Komarla:** None. **J. Halmai:** None. **A. Torrest:** None. **J. Gutierrez:** None. **T. Tempkin:** None. **V. Wheelock:** None. **L. Carvajal-Carmona:** None. **D. Segal:** None. **J. Nolta:** None.

Poster

261. Molecular Techniques

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 261.12/Z42

Topic: G.01. Molecular, Biochemical, and Genetic Techniques

Title: Inhibition of microRNA-128 promotes excitability of dissociated cortical networks in a multiwell microelectrode array assay *in vitro*

Authors: *K. MCSWEENEY[1,2], A. B. GUSSOW[1,3], S. BRADRICK[4], S. PETROVSKI[1], D. MILLARD[5], W. N. FRANKEL[6], D. B. GOLDSTEIN[1];
[1]Columbia Univ., New York, NY; [2]Duke Univ., Durham, NC, NC; [3]Duke Univ., Durham, NC; [4]Univ. of Texas Med. Br., Galveston, TX; [5]Axion Biosystems, Atlanta, GA; [6]The Jackson Lab., Bar Harbor, ME

Abstract: Recent studies have indicated the involvement of microRNAs in the development of epilepsy phenotypes. For instance, mice deficient in microRNA-128 are prone to fatal seizures. Here, we describe the development of an *in vitro* assay to screen the functional impact of microRNAs on seizurogenic risk. We utilized multiwell microelectrode array (MEA) technology to record the electrophysiological development of dissociated neural networks *in vitro*. Specifically, excitability phenotypes, such as the synchronization of network activity, were evaluated in response to the down-regulation of microRNA-128. We used primary cortical neurons from wild-type post-natal day zero C57BL/6J mice and a lentivirus delivered microRNA sponge to target the mature microRNA-128. Sponges are competitive microRNA inhibitors which express tandem repeats that are partially complementary to the microRNA sequence. The non-invasive and label-free nature of the MEA allowed chronic measurements of network activity throughout culture development, and excitability was quantified through a combination of spike, burst, and synchrony metrics describing the organization of the network activity. Our data show that down regulation of microRNA-128 by inhibition with a sponge results in significantly increased neuronal activity throughout network development. These results are consistent with the previous observations that microRNA-128 deficiency promotes neuronal excitability and illustrate the utility of the MEA platform in evaluation of the role microRNAs play in the regulation of functional network phenotypes.

Disclosures: K. McSweeney: None. **A.B. Gussow:** None. **S. Bradrick:** None. **S. Petrovski:** None. **D. Millard:** None. **W.N. Frankel:** None. **D.B. Goldstein:** None.

Poster

262. Genomics, Proteomics, and Systems Neurobiology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 262.01/Z43

Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: NIH Grant R01NS031609

NIH Grant P30DA08310

Title: L-to-D-amino acid isomerization of peptides in the nervous system

Authors: *H.-C. TAI, I. LIVNAT, E. T. JANSSON, S. S. RUBAKHIN, J. V. SWEEDLER; Univ. of Illinois At Urbana-Champaign, Urbana, IL

Abstract: The study of cell-to-cell signaling peptides contributes to the fundamental understanding of physiological processes and improves the effectiveness of pharmaceutical drugs. Neuropeptides undergo a variety of post-translational modifications (PTMs) that are often crucial for their signaling properties. Importantly, conversion of an L-amino acid to a D-amino acid in a neuropeptide can substantially alter the three dimensional structure and modify the

bioactivity and metabolism of the D-amino acid-containing peptide (DAACP). However, peptide isomerization is rarely characterized because this PTM is undetectable in current mass spectrometry-based peptidomics due to the lack of an associated mass shift. To uncover both the extent and functional role of peptide isomerization in the nervous system, we have developed a series of techniques that enable the identification of DAACPs from the neuropeptidome of *Aplysia* californica and other species. The first step in our multi-stage method is to assay endogenous neuropeptides using aminopeptidase M (APM), which selectively cleaves off L-amino acids but not D-amino acids. Resistance to digestion indicates the possibility of a D-amino acid, and peptides that degrade slowly on exposure to APM are considered candidate DAACPs. Subsequent confirmation is achieved by isolating candidate peptides with liquid chromatography and analyzing the chirality of their component amino acids. The presence of specific D-amino acids is determined through acid hydrolysis of the purified peptide, derivatization of its amino acids with Marfey's reagent for chiral separation, and analysis with a triple quadrupole mass spectrometer. We have previously reported the characterization and function of GdFFD in A. californica [Bai, J. Biol. Chem., 2013]; here we report a novel D-tyrosine-containing peptide, GdYFD, from the same achatin-like neuropeptide prohormone (ALNP). Furthermore, a third and longer peptide (amino acid sequence SYADSKDEESNAALDSFAED) from ALNP also appears to be a DAACP, suggesting that the isomerizing enzyme is capable of acting on a wide range of peptides varying from three to twenty amino acids long. While previously reported peptide isomerases in other species have been found to be somewhat flexible in the sequences they recognize, our results indicate that the isomerase in A. californica may be highly promiscuous in terms of peptide length as well as sequence. To advance understanding of this fascinating enzyme, an assay for detecting peptide isomerase activity is being developed using ALNP-expressing neurons and will complement the discovery of functional D-amino acid-containing neuropeptides.

Disclosures: **H. Tai:** None. **I. Livnat:** None. **E.T. Jansson:** None. **S.S. Rubakhin:** None. **J.V. Sweedler:** None.

Poster

262. Genomics, Proteomics, and Systems Neurobiology

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Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: NSF-0744649

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NIH grant 1R01GM097502

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NASA grant NNX13AJ31G

McKnight Brain Research Foundation

Title: Neurosystematics and genealogy of neurons: Single-cell sequencing and computational approaches to reconstructing the natural classification of neural systems across species

Authors: *L. L. MOROZ[1,2,4], A. B. KOHN[3], C. BOSTWICK[2], E. DABE[2], G. WINTERS[2], Q. YANG[5], R. D. HAWKINS[5,8], K. KOKOT[9], N. WHELAN[9], K. HALANYCH[9], J. RUSSO[6], I. MOROZOVA[6], S. KALACHIKOV[6], J. JU[7,6];
[1]The Whitney laboratory for Marine Biosci., Univ. of Florida, Saint Augustine, FL; [2]Neurosci., [3]The Whitney laboratory for Marine Biosci., Univ. of Florida, Gaineswille, FL; [4]McKnight Brain Inst., Gaineswille, FL; [5]Neurosci., [6]Ctr. for Genome Technol. & Biomolecular Engin., [7]Chem. Engin. and Pharmacol., Columbia Univ., New York, NY; [8]New York State Psychiatric Inst., New York, NY; [9]Biol., Auburn Univ., Auburn, AL

Abstract: Neurons are different not only because they have different functions but also because they might have different genealogies. However, the enormous diversity of neurons both within the same nervous system and across species presents tremendous challenges for their unbiased classification. Here, we developed novel approaches and algorithms toward establishing the natural classification of neurons. Our research strategy is based upon (1) high-throughput single-cell RNA-seq and single-cell epigenomic analyses of entire neuronal circuits, and (2) implementation of stochastic approaches from information theory and phylogenomics to incorporate the inherent statistical uncertainty in the operation of the genome, as well as natural selection within complex systems. These approaches are less developed in neuroscience, mostly due to the lack of necessary comparative information from the majority of animal phyla. First, we performed single-cell transcriptome and methylome analyses of hundreds of individually identified neurons in the defensive and feeding circuits of *Aplysia* californica under control conditions and during neuroplasticity (e.g. capture of nascent RNAs during behavioral learning or transmitter applications). As a result, we reconstructed an initial classification of neurons within the same nervous system, with the surprising finding that some neuronal subtypes are more different compared to each other than to selected non-neuronal cells. Second, we expanded this approach to homologous neurons in other related molluscan species (gastropods and cephalopods), and screened various cell phenotypes in mammalian nervous systems as well as in selected basal metazoans (ctenophores, sponges, placozoans and cnidarians). The results suggest that different classes of neurons and synapses might have evolved more than once (convergent evolution) and allow us to reconstruct the genealogy of neurons, trace ancestral cell lineages, and establish the natural classification of neurons within neural circuits across the majority of animal phyla. The field of Neurosystematics is emerging. This might be an analog of the periodic table for neurons, with the predictive power to delineate novel neuronal phenotypes and fundamental constraints on the origins and parallel evolution of neural systems.

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Poster

262. Genomics, Proteomics, and Systems Neurobiology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 262.03/AA1

Topic: G.02. Genomics, Proteomics, and Systems Biology

Title: Development of a novel tool for the assessment of health outcomes and sickness behavior in rodents

Authors: *E. B. ENGLER-CHIURAZZI[1,2], D. N. DOLL[2,3], A. E. KERR[2,3], B. L. UNDERWOOD[4], J. H. WIMSATT[4], J. W. SIMPKINS[2,2];
[1]Physiol. and Pharmacol., [2]Ctr. for Basic and Translational Stroke Res., [3]Ctr. for Neurosci., [4]Office of Lab. Animal Resources, West Virginia Univ., Morgantown, WV

Abstract: In preclinical experimental neuroscience research using rodent models, animal subjects are commonly administered pharmacological agents and/or undergo a diverse range of surgical and non-surgical procedures for the evaluation of impacts on cognitive, affective and locomotor behaviors. Many of these procedures/agents can have a negative impact on overall health and may induce sickness behaviors in treated animals. These sickness behaviors can include poor self-care, disrupted locomotion, altered sensitivity to pain, aphagia, adispia, etc. The presence of these sickness behaviors could have a profound impact on the functional outcomes of interest, possibly confounding the interpretation of behavioral results. Until recently, there was no standard scale for determining the presence of these health alterations among experimental subjects. Here, we describe a newly-developed, multi-dimensional scale for the assessment of health outcomes and sickness behaviors in rodents. This tool has the benefit of 1) detecting a wide range of health outcomes including self-care measures, respiratory rate, posture changes, hydration and nourishment status, locomotion and social interaction, and weight and temperature changes, 2) being rapid to administer (approximately one minute per subject), 3) being adaptable so that specific phenotypes observed following unique insults may be added to the scale at the user's discretion, 4) having high inter- and intra-rater reliability due to its simple scoring system, 5) being repeatable at multiple timepoints during the recovery period, and 6) involving limited animal handling given that most scale outcomes can be evaluated while the subject is in its home cage. We have optimized this tool in several rodent models of brain disease and insult, including lipopolysaccharide-induced inflammation and transient middle cerebral artery occlusion. Scores on this scale can range from 0-20, with lower score representing healthy animals, mid-range scores representing mild-moderate sickness, and higher scores representing severe sickness/poor

health. Use of this tool will aid in the identification and control of health states and behaviors that can impact performance on subsequent behavioral tests. As well, this tool can serve as a triage mechanism to rapidly and consistently identify experimental animals in need of veterinary attention and intervention.

Disclosures: E.B. Engler-Chiurazzi: None. **D.N. Doll:** None. **A.E. Kerr:** None. **B.L. Underwood:** None. **J.H. Wimsatt:** None. **J.W. Simpkins:** None.

Poster

262. Genomics, Proteomics, and Systems Neurobiology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 262.04/AA2

Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: NSF EAGER Award IOS-1255695

Title: Strategy for achieving a complete cns transcriptome for *Aplysia*, a model system in learning and memory

Authors: *P. SHRESTHA[1], J. ORVIS[2], L. J. TALLON[2], A. MAHURKAR[2], C. M. FRASER[2], T. W. ABRAMS[3];
[2]Inst. of Genome Sci., [3]Pharmacol., [1]Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: The feasibility of molecular approaches to studying learning and memory in *Aplysia* californica was substantially enhanced by release of an RNAseq assembly from Broad Institute in 2012. This assembly was achieved using Illumina HiSeq reads with the Trinity assembler, developed collaboratively by Broad Institute and Hebrew University for de novo reconstruction of transcriptomes from large-volume RNAseq read data sets. Despite read sets of >50 million for the Broad Institute RNAseq assembly, analysis of specific predicted transcripts revealed that many were fragmented or missing substantial regions. Some neurobiologically important transcripts had only ~25% coverage in the assembled contigs, whereas other contigs were complete. The present project was initiated in an effort to improve the RNAseq assembly for *Aplysia* CNS. We focused first on improving quality of RNA preps and increasing total read numbers. With >10x increase in read numbers, the coverage of the assembled transcripts improved. However, some of the same specific gaps in the 2012 assembly remained, and fragmentation of transcripts persisted. The consistency of the gaps suggested RNA degradation in Illumina library preparation step; this was confirmed for specific transcripts by PCR screening of the original RNA prep. Comparing a range of times for the chemical shearing step revealed that briefer shearing reduced the incidence of very short fragments below the minimum length for Illumina sequencing, which in turn yielded more complete assemblies. Although the transcriptome assembly improved substantially, for some transcripts with near complete

coverage, fragmentation persisted. We are now exploring bioinformatic approaches to link remaining fragments. We have also used a statistical approach to improving orientation correction of contigs with strand-specific reads, for which a small percentage are found to be unreliable in each library. To produce a useful annotated transcriptome, ORFs were called using TransDecoder and predicted proteins were searched using a multiple tools. HMM3 searches were performed against a custom HMM collection that includes TIGRFams and PFam, then BlastP was conducted against SWISSPROT, as well as Human and Mouse genome builds. The annotated assembly and assembled contigs are available at: http://*aplysia*genetools.org/

Disclosures: P. Shrestha: None. **J. Orvis:** None. **L.J. Tallon:** None. **A. Mahurkar:** None. **C.M. Fraser:** None. **T.W. Abrams:** None.

Poster

262. Genomics, Proteomics, and Systems Neurobiology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 262.05/AA3

Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: NIH Grant F32 NS083196

Title: A novel method for cell specific labeling of the nascent proteome

Authors: *R. M. BARRETT[1], H.-W. LIU[1], R. H. GOODMAN[1], M. S. COHEN[2];
[1]Vollum Inst., [2]Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Translational regulation is necessary for many biological processes, a fact highlighted by the large discrepancy between mRNA and protein abundance. Although genetic methods have been developed for monitoring cell-specific transcriptional regulation within heterogeneous tissues, no such methods exist for determining the translatome, which can be equally important for cellular identity and function. We describe here a novel method to label the nascent proteome with cell-type resolution in heterogeneous tissue. This method uses a chemical genetic strategy based on de-blocking of a click-tagged puromycin analogue that can be activated by expression of penicillin G acylase in genetically targeted cell populations. The puromycin analogue incorporates into nascent peptides, which can be coupled to an azide reporter via click chemistry. Using our method, we demonstrate cell-specific labeling and identification of newly-synthesized proteins in cells and tissues. This unique strategy can be applied to many tissues, including the brain, and will allow us to answer questions about neural functions that require time sensitive gene expression including learning, drug addiction, and circadian rhythms.

Disclosures: R.M. Barrett: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Takeda Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds);

US Provisional Patent Application 62/096,364 entitled CELL SPECIFIC LABELING OF NEWLY SYNTHESIZED PROTEINS. **H. Liu:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Takeda Pharmaceuticals. **R.H. Goodman:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Takeda Pharmaceuticals. **M.S. Cohen:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Takeda Pharmaceuticals.

Poster

262. Genomics, Proteomics, and Systems Neurobiology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 262.06/AA4

Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: NIMH 5R44MH091909 (SLK)

Title: UnipicK+ for efficient collection of single cells from cell cultures and microdissection of complex tissues with subsequent dispensing of acquired material

Authors: *S. L. KARSTEN, A. ZAVALA, Z. MA, L. C. KUDO;
NeuroInDx, Inc., Signal Hill, CA

Abstract: Today cell specific studies are indispensable for basic and translational research in neuroscience. Yet collecting single or individual cells directly from cell cultures for downstream single cell analysis or recultivation (e.g. clonal expansion) remain a challenge. Tissue heterogeneity also poses a difficulty in retrieving cell and region specific molecular information. Current instrumentation, such as fluorescence assisted cell sorting (FACS) and laser assisted acquisition such as laser capture microdissection (LCM), are costly, complicated and often methodologically limited. We have developed a versatile capillary-based vacuum-assisted cell and tissue acquisition instrument, UnipicK+ with which individual fluorescently labeled and/or morphologically distinct live cells can be acquired from adherent, suspended, and 3D cultures grown in standard cell culture dishes. The instrument is suitable for individual cell collection and region specific acquisition of cell clusters and subanatomical areas from 10 to 500 µm thick slices of complex heterogeneous tissues such as the brain - native, fresh frozen, or sucrose treated. The instrument may be integrated with a fluorescence compatible inverted microscope or used as a free standing instrument mounted over most inverted microscopes, providing flexibility in the laboratory. UnipicK+ demonstrates a wide range of cell and tissue acquisition parameters. It collects individual cells from adherent cell cultures in as small as 15nl volume, compatible with various downstream single cell analyses and Next generation Sequencing (NGS). Here, individual cells were collected based on morphology from human neuroblastoma SH-SY5Y, CHO, and 3T3 cell cultures, immediately dispensed into individual wells, and successfully recultured for clonal expansion, demonstrating the minimal effect the process has on cellular

viability. Further single Purkinje cells, individual motor neurons and subanatomical regions (e.g. CA1-3, dentate gyrus) were efficiently collected from rat and mouse brain tissue sections. Dissociation protocols for hard to dissect tissues have been developed as well. High quality RNA and proteins were isolated from all collected samples. UnipicK+'s compatibility with most inverted microscopes, low cost, ease of use, single cell resolution, and minimal impact on cell viability, make it is a unique and vital instrument for neuroscience research.

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Poster

262. Genomics, Proteomics, and Systems Neurobiology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 262.07/AA5

Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: NS45260

Title: Single-neuron lipidomics analysis of hippocampal granule cells

Authors: ***C. B. MERRILL**[1], A. ARMIROTTI[2], A. BASIT[2], Y. JIA[1], C. M. GALL[1], G. LYNCH[1], D. PIOMELLI[1];
[1]Anat. and Neurobio., Univ. of California-Irvine, Irvine, CA; [2]Inst. Italiano di Tecnologia, Genoa, Italy

Abstract: The human brain contains as many as 100 billion neurons. Shape, electrical properties and gene expression differentiate them into a large number of distinct types - possibly more than any other organ system. This cellular diversity underpins the brain's unique capacity to form complex networks that allow for the processing of huge amounts of sensory information coming from the external and internal environment. Moreover, electrical activity and chemical signals can cause profound long-lasting changes in the properties of individual neuronal types, which in turn influence brain network activity. Understanding the molecular basis for this diversity and its dynamics is a key goal of neuroscience. The application of reverse transcriptase-polymerase chain reaction (RT-PCR) to study gene expression in individual neurons (Lambolez *et al.*, 1992) was a critical step toward achieving this goal. The insights provided by this technique are invaluable, but only illuminate differences in the transcriptome. Post-transcriptional changes in the metabolome are beyond its reach. Lipids are key constituents of the neuronal metabolome. They control protein traffic, facilitate cell-cell recognition and give rise to hundreds of molecules that carry information both within and across brain cells. Understanding how lipids work is the primary objective of lipidomics (Piomelli *et al.*, 2007). Here, we combined single-cell patch clamp electrophysiology and high-resolution nano-liquid chromatography/ tandem mass spectroscopy (LC/MS/MS) to profile the lipidome of individual granule cells in the mouse

dentate gyrus. Granule cells are among the smallest (≈5 μm diameter) and most plastic neurons found in the mammalian brain. Individual granule cells were isolated from acutely dissected slices using whole-cell patch clamp and their lipidome was probed by nano-LC/MS/MS. Signals for multiple lipid classes involved in neuronal structure and function were distinguished from background noise and unambiguously identified. High-frequency stimulation of the medial perforant path, a major excitatory afferent to the granule cells, resulted in substantial and reproducible changes in the levels of several of these lipids. While still preliminary, the results suggest that single-neuron lipidomics is feasible, and can be used to unmask activity-dependent fluctuations in the lipid composition of small neurons in mammalian brains. References Lambolez, B. et al. AMPA receptor subunits expressed by single Purkinje cells. **Neuron,** v. 9, n. 2, p. 247-58, Aug 1992. Piomelli, D.; Astarita, G.; Rapaka, R. A neuroscientist's guide to lipidomics. **Nat Rev Neurosci,** v. 8, n. 10, p. 743-54, Oct 2007.

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Poster

262. Genomics, Proteomics, and Systems Neurobiology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 262.08/AA6

Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: NCCAM, ODS, and NCI Grant P50AT006273

Title: Proteomic quantification and site-mapping of s-nitrosylated proteins using isobaric iodotmt reagents

Authors: Z. QU[1], R. BOMGARDEN[3], R. VINER[4], J. LI[5], J. ROGERS[3], J. CHENG[5], C. GREENLIEF[6], J. CUI[1], D. LUBAHN[2], G. SUN[2], *Z. GU[1];
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Abstract: S-Nitrosylation, a redox-based protein post-translational modification, has been increasingly recognized as a key molecular mechanism for Nitric Oxide (NO) signaling. It plays an important role in physiological conditions and in diseases such as diabetes, asthma, heart failure, cancer, and neurodegenerative disorders. S-Nitrosylation of critical cysteine residues on specific proteins regulates their activities and has been demonstrated in protein misfolding, transcriptional regulation and aberrant enzymatic activation. Detection and quantification of

protein S-nitrosylation have been challenging tasks due to instability and low abundance of the modification. Many studies have used mass spectrometry (MS)-based methods with different thiol-reactive reagents to label and identify proteins with S-nitrosylated cysteine (SNO-Cys). Here, we present a novel iodoTMT switch assay (ISA) using an isobaric set of thiol-reactive iodoTMTsixplex reagents (126-131) to specifically detect and quantify protein S-nitrosylation. Irreversible labeling of SNO-Cys with the iodoTMTsixplex reagents enables immune-affinity detection of S-nitrosylated proteins, enrichment of iodoTMT-labeled peptides by anti-TMT resin, and importantly, unambiguous modification site-mapping and multiplex quantification by liquid chromatography−tandem MS. In this study, the ISA approach was applied to investigate protein S-nitrosylation in a cellular neuroinflammation model—endotoxin lipopolysaccharide (LPS)-stimulated murine BV-2 microglial cells. We further evaluated the effects of S-allyl cysteine (SAC), an active nutritional compound of aged garlic extract, on S-nitrosylation in LPS-stimulated BV-2 cells and revealed that SAC acts in antioxidant signaling and mitochondrial metabolic pathways. ISA proved to be an effective proteomic approach for quantitative analysis of S-nitrosylation in complex samples and will facilitate the elucidation of molecular mechanisms of NO signaling under physiological conditions and nitrosative stress in disease.

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Poster

262. Genomics, Proteomics, and Systems Neurobiology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 262.09/AA7

Topic: G.02. Genomics, Proteomics, and Systems Biology

Title: Western blot optimization using BlotCycler, automated western blot processor

Authors: A. MARGULIS[1], D. P. CHIMENTO[2], A. ZAORSKI[2], *R. YUKHANANOV[1]; [1]Precision Biosystems, Mansfield, MA; [2]Rockland Immunochemicals, Inc., Limerick, PA

Abstract: Western blot assays provide information about the presence and levels of protein expression in cell and tissue. The immunodetection protocol consists of multiple steps including blocking of the nonspecific binding sites, incubation using primary and secondary antibodies and extensive washing between steps. The resulting complex is detected by fluorescence or using appropriate chromogenic or luminescent substrates, depending on the type of detection agent or enzyme conjugated to the secondary antibodies. Immunodetection of proteins is often performed manually and involves multiple steps that easily introduce bias and errors. We have compared the traditional manual western blotting and a novel type of automatic western blot by using BlotCycler™ processor developed by Precision Biosystems. Manual western blotting is a labor-

intensive, time-consuming process. The quality of results is dependent on multiple subjective and objective factors such as the qualification and technical skills of the personnel performing the assay and the accuracy of temporal and temperature control, especially during the immunodetection step. Blotcycler™, automated western blot processor, uses fluidic control system that allows to eliminate the variability associated with immunodetection and to achieve higher sensitivity using optimized washing procedure. In this study we have compare protein detection using different incubation and washing temperature and have shown that incubation and washing at 4°C yields using automated western blot procedure yields significantly higher signal that allows reproducible detection of low expression proteins.

Disclosures: **A. Margulis:** A. Employment/Salary (full or part-time):; Precision Biosystems. **D.P. Chimento:** A. Employment/Salary (full or part-time):; Rockland Immunochemicals, Inc. **A. Zaorski:** A. Employment/Salary (full or part-time):; Rockland Immunochemicals, Inc. **R. Yukhananov:** A. Employment/Salary (full or part-time):; Precision Biosystems.

Poster

262. Genomics, Proteomics, and Systems Neurobiology

Location: Hall A

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Program#/Poster#: 262.10/AA8

Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: NIH Grant DA018310

NIH Grant GM067193

Title: Top down proteomics enables quantitative analysis of mouse brain tissue proteoforms

Authors: ***P. THOMAS**[1], K. KIM[1], R. T. FELLERS[1], R. D. LEDUC[1], B. P. EARLY[1], S. S. RUBAKHIN[2], E. V. ROMANOVA[2], J. A. ZOMBECK[2], J. S. RHODES[2], J. V. SWEEDLER[2], N. L. KELLEHER[1];
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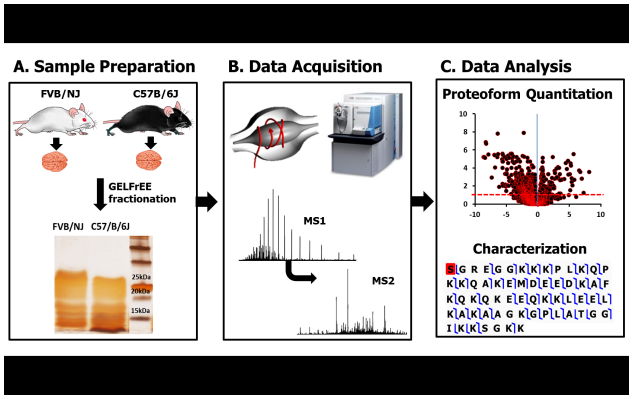
Abstract: Development of top-down proteomics technologies has facilitated brain proteome research. State-of-art high resolution mass spectrometry and bioinformatics data analysis enable us to simultaneously identify and quantify intact proteoforms. Here, we employ a quantitative Top Down proteomics approach in discovery mode to investigate the intact proteome of brain tissues for two strains of inbred mice, C57BL/6J and FVB/NJ. Whole mouse brain tissue was lysed and proteins below 30kDa were fractionated using Gel-Eluted Liquid FRaction Entrapment Electrophoresis (GELFrEE) technology. Global qualitative top down data analysis identified 983 unique gene products (710 for C57BL/6J and 546 for FVB/NJ) arising from 3,772 proteoforms (2,940 for C57BL/6J and 2,096 for FVB/NJ, respectively). A proteoform is defined as a single protein molecule arising from all combinatorial sources of variation (e.g. genetic variation,

alternatively spliced RNA, transcripts, and post-translational modifications). Comparative quantitative analysis between C57BL/6J and FVB/NJ revealed 422 confidently identified Quantitation Mass Targets (QMTs) showing expression differences ($\alpha<0.05$, see Figure Panel C) and 241 proteoforms found in only one strain. A similar analysis between two mice of the same strain showed no significant difference, indicating that the differences observed are real. Gene ontology (GO) analysis for differentially expressed proteoforms further clarified the dissimilarities in neurobiology between two inbred mouse strains. This study sets the stage for the comprehensive characterization of the mouse brain intact proteome < 30kDa and will soon be applied to the analysis of drug addiction in mouse brains.



Disclosures: **P. Thomas:** None. **K. Kim:** None. **R.T. Fellers:** None. **R.D. LeDuc:** None. **B.P. Early:** None. **S.S. Rubakhin:** None. **E.V. Romanova:** None. **J.A. Zombeck:** None. **J.S. Rhodes:** None. **J.V. Sweedler:** 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.; NIDA P30 DA018310. **N.L. Kelleher:** 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.; NIDA P30 DA018310, NIGMS R01 GM067193.

Poster

262. Genomics, Proteomics, and Systems Neurobiology

Location: Hall A

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Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: NSF grant NSF-0744649

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Title: Single-neuron methylomes and transcriptomes: determining the epigenomic basis of neuronal identity

Authors: *E. C. DABE[1,2], R. S. SANFORD[1,2], A. B. KOHN[1], L. L. MOROZ[1,2];
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Abstract: On the genomic level, neuronal identity is encoded by complex epigenetic mechanisms leading to coordinated expression of about 10,000 protein-coding genes per neuron. To address these mechanisms, we performed single-cell bisulfite sequencing from single identified neurons using *Aplysia californica* as an experimental model. Single-cell DNA methylation profiling was complemented by single-cell RNA-seq analysis. *Aplysia* have lower overall genome-wide methylation (0.75-1%) than humans (1-4%), but both have more CpG methylation in their gene bodies compared to promoter regions, suggesting a common role for DNA methylation in gene regulation across phyla. *Aplysia* also showed evidence of non-canonical CHG (.05-.1%) and CHH (.25-.5%) methylation. Genome-wide methylation percentages are consistent across neuronal phenotypes and peripheral tissues. Quantal-type analysis of all individual CpG sites in single polyploidy neurons, whole ganglia, and diploid non-neuronal tissue yielded similar methylation distribution regardless of sequencing coverage, suggesting that each copy of the genome in a polyploid neuron has the same methylation pattern like diploid cells from peripheral tissues. Specifically, we produced methylome profiles (n=3 per cell type) for two symmetrical serotonergic (5-HT) interneurons (Metacerebral cells) and two homologous cholinergic (ACh) motor neurons (LPL1 and R2) with an average sequencing depth of 25X coverage per single cell. We also made single-neuron transcriptomes (n=3 per cell type) that mapped to 75% or more of the 26,299 *Aplysia* gene models. We used these data sets to discern what transcription factor (TFs) could control neurotransmitter phenotypes and performed *in situ* hybridization to map their expression across the CNS. *Pitx*, which has been shown to be a regulator of 5-HT neuronal identity in planarians, is expressed in *Aplysia* serotonergic cells. We also correlated DNA methylation patterns with expression of TFs and 5-HT or ACh biosynthesis genes. Though methylation patterning for each gene was unique, we found that genes for 5-HT biosynthesis machinery had lower levels of CHG methylation in 5-HT neurons than in outgroup neurons. ACh synthesis and signaling genes had higher CpG gene body methylation in cholinergic neurons. Non-neuronal tissue (salivary gland) showed no transcript expression of neurotransmitter biosynthesis genes and had lower levels of both CpG and CHG methylation of respective genes compare to the identified neurons studied. Our study suggests that DNA methylation contributes to the maintenance of overall neuronal phenotypes, but additional epigenetic mechanisms might also be involved.

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Poster

262. Genomics, Proteomics, and Systems Neurobiology

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Program#/Poster#: 262.12/AA10

Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: The Health and Medical Research Fund (HMRF), Food and Health Bureau, Hong Kong Special Administrative Region Government (Ref. No.: 01122016)

Title: Neurometabolomic profiling of neurotransmitters after repeated exposure of Pacific ciguatoxin-1

Authors: *C. H. E. MA[1,2], E. N. Y. LEI[3], N. P. B. AU[1], G. KUMAR[1], Y. L. MAK[2,4], L. L. CHAN[1,2,4], P. K. S. LAM[2,3,4], M. H. W. LAM[3];
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Abstract: Ciguatera fish poisoning (CFP) remains one of the most challenging foodborne illness in humans caused by the consumption of ciguatoxin (CTX)-contaminated tropical fish. CFP has become a global health issue due to the increasing international trading of tropical fish species. CFP cases have been reported around the world not limited to the tropical regions and its incidence has been increasing in the past decades. CFP patients usually result in gastrointestinal and neurological disorders. Gastrointestinal disorders usually last for two weeks only after the onset of symptoms; however, severe neurological manifestations can persist for months to years in about 20% of patients. The chronic neurological effects of CTXs on motor function remain largely unknown. Our initial study indicated that the motor function was affected significantly after repeated exposure of pacific ciguatoxin-1 (P-CTX-1). Electroencephalography recordings suggested that P-CTX-1 exert a direct effect on motor cortex electrical activity in brain. In the present study, we aim to understand if reduction in brain activity after repeated exposure of P-CTX-1 correlates with the perturbation of the neurotransmitter expression profile of in the motor cortex. Sub-lethal doses of P-CTX-1 were administrated intraperitoneally in male adult C57BL/6 mice at day 0 and 3, and the motor cortexes of the exposed mice were harvested two hours after second administration. 48 neurotransmitters were quantified using liquid chromatography tandem-mass spectrometry, and normalized with the protein content of the brain tissue for multivariate analysis. Preliminary expression profile study revealed that several neurotransmitters were altered significantly in P-CTX-1 exposed mice. By combining with metabolic pathway analysis, the neurometabolomic profile will provide an insight into the

mechanistic action of P-CTX-1 on brain activity which accounts for the reduced electrical activity in motor cortex after P-CTX-1 exposure.

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Poster

262. Genomics, Proteomics, and Systems Neurobiology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 262.13/AA11

Topic: G.02. Genomics, Proteomics, and Systems Biology

Title: Post-sampling changes in opioid peptide levels and the effect of tissue heat stabilization

Authors: ***M. SÖDERQUIST**[1], L. SEGERSTRÖM[2], M. BORÉN[1], I. NYLANDER[2]; [1]Denator AB, Uppsala, Sweden; [2]Uppsala Univ., Uppsala, Sweden

Abstract: Aim of Investigation: The present project aims to evaluate the use of conductive heat transfer for the preservation of sample composition in a routine radioimmunoassay protocol for measurement of opioid peptides in rat brain. This is done to investigate the effects of sampling and handling during the analytical process in order to evaluate how the analytical results are influenced. Methods: Different extraction procedures were tested on heat stabilized and non-stabilized tissue and the effect was evaluated on the levels of opioid peptides in rat hypothalamus (HT), striatum (Str), and cingulate cortex (Cg). Dynorphin A (Dyn A), Dynorphin B (Dyn B), Leu-enkephalin-Arg6 (Leu-Arg), and Met-enkephalin-Arg6Phe7 (MEAP) was analysed using radioimmuneassay (RIA). Results: Levels of Dyn A, Dyn B, and MEAP in HT were higher in stabilized tissue than in non-stabilized. Leu-Arg, a degradation product from the dynorphins, showed an opposite pattern. Stabilization before freezing resulted in higher Dyn A, Dyn B, and MEAP compared to samples which were stabilized in a frozen state. Conclusions: This work proves that samples enzymatically stabilized with heat can be analyzed using standard RIA protocols and keep the sample integrity. All of the peptide levels were stable two hours post-stabilization in room temperature, proving the instrument's application in the clinic. Extraction protocols without boiling and with non-denaturing buffers was made possible with heat stabilized samples extending the range of possible extraction buffers for extracting peptides from biological samples. Acknowledgments: Funding was provided from the Swedish Medical Research Council and ERAB

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Poster

262. Genomics, Proteomics, and Systems Neurobiology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 262.14/AA12

Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: FSU College of Medicine

Title: Fractionation dependent improvements in proteome resolution in the mouse hippocampus by isoelectric focusing and mass spectrometry

Authors: *J. L. BUNDY, R. S. NOWAKOWSKI;
Biomed. Sci., Florida State Univ. Col. of Med., Tallahassee, FL

Abstract: Liquid-chromatography mass-spectrometry (LC-MS/MS) has become the method of choice to identify and quantify simultaneously hundreds of proteins from a single biological sample. Here, we show that from a typical brain region, such as mouse hippocampus, the number of proteins identified in an LC-MS/MS experiment can be increased from hundreds to thousands by fractionating the protein sample prior to the LC-MS/MS analysis. To achieve this improvement we exploited fractionation of complex samples upstream of LC-MS/MS by isoelectric focusing (IEF) which has been shown to improve proteome resolution by increasing the number of spectral counts. However, LC-MS/MS experiments can become impractically large when paired with methodologies such as IEF, which increase the number of necessary LC-MS/MS runs. To optimize both the benefits and drawbacks of IEF, an experiment was conducted in which the proteome of mouse hippocampal peptides was surveyed using IEF fractions varying in number and complexity. Protein extract from a single mouse hippocampus was digested and isoelectrically focused into 12 fractions. Aliquots of fractionated peptides were then pooled to make more complex samples containing six (6F), four (4F), or a single fraction (1F). The original unfractionated sample (UF), the pooled samples, and the 12 unpooled fractions (12F) were subjected to LC-MS/MS analysis. Samples consisting of multiple fractions (12F, 6F, and 4F) surveyed the hippocampal proteome with greater breadth (protein identifications), depth (spectral counts), and technical precision than samples consisting of a single LC-MS/MS sample (1F and UF). In 1F, the most complex LC-MS/MS sample, 745 proteins were identified. Fractionation of the hippocampal peptides resulted in additional protein identifications with 1761, 2601, and 3390 protein identifications in 4F, 6F, and 12F respectively. For proteins detected in all samples (740 proteins), the number of associated spectral counts per protein increased 2.66, 3.68, and 6.14 fold in 4F, 6F, and 12F relative to 1F. Finally, by using regression-interpolated synthetic datasets, quantitative precision and statistical power to discover differentially abundant proteins were found to be improved by sample fractionation. This investigation shows that isoelectrically focusing tryptic peptides prior to LC-MS/MS can increase the number of protein identifications over 4-fold, and the number of spectral counts over

6-fold. The rationale presented here can be generalized to other investigations of other complex samples, and can be used to alter experimental designs to meet specific experimental goals while minimizing cost and analysis time.

Disclosures: J.L. Bundy: None. **R.S. Nowakowski:** None.

Poster

262. Genomics, Proteomics, and Systems Neurobiology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 262.15/AA13

Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: NIH Grant CA183671

Title: The effect of elevated aSyn expression on the phosphoproteome in neuronal cells

Authors: *R. J. SCHUSTER[1], L. L. PARKER[3], J.-C. ROCHET[2];
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Abstract: Parkinson's disease (PD) is characterized by a loss of dopaminergic neurons in the substantia nigra and the formation of cytoplasmic inclusions called Lewy bodies. Lewy bodies are enriched with fibrillar forms of the presynaptic protein, alpha-synuclein (aSyn). aSyn contains four tyrosine residues that can be phosphorylated by various kinases. However, the impact of phosphorylation of these residues on the protein's propensity to aggregate and elicit neurotoxicity remains poorly understood. Evidence suggests that phosphorylation of alpha-synucleinaSyn tyrosine residues decreases with age in human brain, and this decrease is more pronounced in patients diagnosed with dementia with Lewy bodies. Here we carried out a study aimed at identifying tyrosine kinases that phosphorylate aSyn, thus regulating the protein's neurotoxicity, in a cellular model of PD. Mass spectrometric (MS) analysis of cell-permeable, kinase-specific biosensor peptides by multiple reaction monitoring (MRM) data acquisition was used to determine the effects of aSyn expression levels on the activation of tyrosine kinases in the SH-SY5Y dopaminergic neuronal cell line. SWATH-MS, a recently developed data acquisition method which combines the breadth of shotgun proteomic analysis and the depth of tandem mass spectrometric analysis, was employed to analyze the effects of aSyn expression on the proteome of SH-SY5Y cells, (including protein expression and post-translational modification profiles). We identified the specific tyrosine residues of the monomeric form of recombinant human alpha-synucleinaSyn phosphorylated by Abl, Lyn and Fyn kinases. Phosphorylation of the recombinant protein by Lyn and Fyn interfered with nitration of aSyn tyrosine residues and with aSyn oligomerization induced by incubating the protein with tetranitromethane. Current MS analysis is underway to determine the effect of increased aSyn

expression on the activity of Abl, Src family and Syk kinases using cell-permeable tyrosine kinase biosensors. These data advance our understanding of the role of aSyn post-translational modifications in PD pathogenesis and suggest modulation of these modifications could be a strategy to slow neurodegeneration in PD.

Disclosures: R.J. Schuster: None. **L.L. Parker:** None. **J. Rochet:** None.

Poster

262. Genomics, Proteomics, and Systems Neurobiology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 262.16/AA14

Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: NCCAM, ODS, and NCI Grant P50AT006273

Title: Quantitative proteomic analysis of brain protein levels in mice after transient cerebral ischemic injury using isobaric DiART sixplex reagents with tandem mass spectrometry

Authors: *H. SONG[1,2,3], H. ZHOU[1,2,3], Z. QU[1,2], D. Y. CHUANG[1,4,2,3], S. CHEN[1,2,3], S. LI[5], J. LI[2,6], J. CHENG[2,6], M. C. GREENLIEF[3,7], D. B. LUBAHN[2,4], J. CUI[1,2,3], A. SIMONYI[1,4,2], G. Y. SUN[1,4,2,3], Z. GU[1,2,3];
[1]Pathology and Anatom. sciences, Univ. of Missouri, Columbia, Columbia, MO; [2]Ctr. for Translational Neuroscience, Univ. of Missouri Sch. of Med., Columbia, MO; [3]Ctr. for Botanical Interaction Studies, Univ. of Missouri, Columbia, MO; [4]Biochem., Univ. of Missouri Sch. of Med., Columbia, MO; [5]Chem. and Biochem., Univ. of Maryland, Maryland, MD; [6]Computer Sci., Informatics Institute, Univ. of Missouri, Columbia, MO; [7]Chem., Univ. of Missouri, Columbia, MO

Abstract: Stroke is the fifth leading cause of death and a major cause of disability in the United States; ischemic stroke accounts for approximately 87 percent of all cases. Quantitative proteomic approach and advance bioinformatics tools have been implemented in translational ischemic stroke research to identify biological pathways, to understand cerebrovascular pathophysiology, and to develop novel therapeutics and diagnostics. New perspectives are needed to evaluate the complexity of ischemic stroke and the variability of outcomes. The purpose of the present study is to analyze possible factors and signaling events influencing different functional outcomes following injury by ischemic stroke. We used deuterium isobaric amine reactive tagging (DiART) sixplex reagents followed by liquid chromatography tandem mass spectrometry to globally investigate the differential levels of proteins in cortex, striatum and hippocampus affected by bilateral common carotid artery occlusion (BCCAo) for 30 min followed by a 3-day reperfusion in male C57Bl/6J mice. Results include measurement of sensorimotor deficits ranking either moderate or severe compared to sham surgery defined by the

rotarod test. Time of falling (s) is used to define the motor function: >80 is the sham group, between 80 and 20 is the moderate group, and <20 is the severe group. Our protocol detected 1828 proteins in cortex, 1806 in hippocampus, and 1716 in striatum in total. Among these, 44 proteins in cortex, 26 proteins in striatum, 42 proteins in hippocampus were differentially expressed, with more than 1.3-fold change, in moderate behavioral deficit group affected by BCCAo. Additionally, there were 91, 85 and 90 proteins with significant changes in level in cortex, striatum and hippocampus, respectively, in severe behavioral deficit group compared to sham group. By using Ingenuity Pathway Analysis, we found that some of the differentially expressed proteins, either in moderate or in severe group, play potential roles in inflammatory disease and response, mitochondrial dysfunction, Nrf2-mediated oxidative response, and neurodegeneration pathways. In addition, we demonstrated the role of NADPH oxidase and activation of the mitogen-activated protein kinase pathways associated with production of reactive oxygen species and signaling events in microglial cells after transient BCCAo in mice. To summarize, quantitative proteomic analysis identified proteins/pathways associated with oxidative and inflammatory responses correlated with neurobehavioral dysfunctions after ischemic stroke, and thus offer promising targets for therapeutic preventions and interventions.

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Poster

262. Genomics, Proteomics, and Systems Neurobiology

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Program#/Poster#: 262.17/AA15

Topic: G.02. Genomics, Proteomics, and Systems Biology

Title: Sample preparation of brain tissue for extraction of β-amyloid and other neuronal markers

Authors: *S. GUTIERREZ, I. STRUG, J. L. SMITH, T. NADLER;
EMD Millipore, Danvers, MA

Abstract: The key to effective analysis of brain proteins starts with the disruption of the tissue in the appropriate buffer, which not only allows the complete extraction of proteins but is also compatible with the desired downstream analysis. While there are many protocols reported for the isolation and purification of neuronal biomarkers, most of these methods involve sequential extractions with aqueous buffers to remove the hydrophilic proteins followed by application of organic solvents to suspend the hydrophobic fraction. The isolation is often complemented by a delipidation step. The current protocols are lengthy, and usually fail to provide complete sample analysis. Here we present an alternative protocol for efficient solubilization of membrane-associated proteins like β-amyloid that results in three fractions each of which are fully

compatible with many downstream analyses like Western blotting, ELISA or mass spectrometry. During the extraction process, the total protein and lipid content was monitored by mid-infrared (MIR) spectrometry and by a fast immunodetection method. Each fraction was digested in a centrifugal ultrafiltration device (3 kDa) and analyzed by RP-UPLC-MS^{e} using Xevo G2-S mass spectrometer. The data from each was evaluated in regards to confidence of neuronal biomarkers detection, protein yield and number of identified proteins. The merits of analyzing multiple fractions over a single preparation will be elucidated.

Disclosures: **S. Gutierrez:** A. Employment/Salary (full or part-time):; EMD Millipore. **I. Strug:** A. Employment/Salary (full or part-time):; EMD Millipore. **J.L. Smith:** A. Employment/Salary (full or part-time):; EMD Millipore. **T. Nadler:** A. Employment/Salary (full or part-time):; EMD Millipore.

Poster

262. Genomics, Proteomics, and Systems Neurobiology

Location: Hall A

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Program#/Poster#: 262.18/AA16

Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: MH103361

NS045758

NS066345

Title: 4-Thiouracil tagging of RNA for transcriptional characterization of excitatory forebrain neurons

Authors: *D. F. ALZATE-CORREA[1,2], K. SAKAMOTO[3], K. KARELINA[1], K. F. HANSEN[1], K. HOYT[1,2], K. OBRIETAN[1];
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Abstract: One major challenge when performing transcriptional profiling of Central Nervous System (CNS) tissue is that a typical sample represents a wide range of cell types, and thus, without the use of technically challenging cell-sorting techniques, the interpretation of the data can be problematic. To address this complication we developed a Uracil Phosphoribosyltransferase (UPRT) transgenic mouse line in which RNA can be tagged and isolated in a temporally- and cell-type specific manner. UPRT is a protozoan nucleotide salvage enzyme that synthesizes Uridine Monophosphate (UMP) from Uracil, which, can then be used for RNA transcription. Importantly, UPRT can also use the Uracil analog 4-Thiouracil (4-TU) for RNA synthesis (via 4-Thiouridine generation) producing a biosynthetic tagged RNA. 4-TU-

tagged RNA can be purified from non-tagged RNA by using a very well characterized reversible reaction that couples biotin to sulfhydryl (-SH) groups, followed by streptavidin-magnetic bead precipitation. Previously, 4-TU tagging has been used in cultured cells to monitor RNA production and decay, also UPRT-expressing transgenic flies and mice have been made and tagged RNA has been successfully isolated and profiled (Cleary, Meiering et al. 2005, Miller, Robinson et al. 2009, Gay, Miller et al. 2013). To generate our UPRT transgenic line, a UPRT construct provided by Dr. Chris Doe (University of Oregon) was cloned into a bidirectional Tet-regulated vector thus allowing for forebrain excitatory neuron-specific expression when our line was crossed with the αCaMKII-tTA line. Here we provide an overview of the mouse lines that were generated, the robust expression pattern of UPRT within excitatory neurons of the cortex, hippocampus and amygdala, and our initial set of experiments in which mice were exposed to 4-TU, and tagged RNA was effectively pulled down from forebrain tissues. We foresee this mouse line becoming a useful tool for the selective profiling of gene expression following a wide array of experimental conditions.

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Poster

262. Genomics, Proteomics, and Systems Neurobiology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 262.19/AA17

Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: Polish National Science Centre Grant SONATA 2011/03/D/NZ3/01686

Title: Selective induction of alternate gene transcripts by cocaine treatment

Authors: ***M. A. ZYGMUNT**, J. RODRIGUEZ PARKITNA, S. GOLDA, M. PIECHOTA, J. FICEK, M. KOROSTYNSKI;
Inst. of Pharmacology, PAS, Kraków, Poland

Abstract: Impaired function of the brain's reward system is associated with several psychopathologies, including psychotic, affective and addictive disorders. It was observed that the pathological states correlate with long-term alterations in signaling in the mesolimbic system. Investigation of cellular mechanisms underlying the altered neuronal activity, gene expression in particular, may reveal molecular triggers for the development of the pathologies. Therefore, in this study we used next-generation sequencing (RNA-seq) to comprehensively map expression of cocaine-induced transcripts in the mouse striatum. Total RNA and small RNA sequencing was performed in samples collected 1h after acute cocaine treatment (25 mg/kg, i.p.). To identify transcripts responsive to psychostimulant treatment we used Tophat read-mapper and Cufflinks

algorithm for FPKM quantification. List of 35 expressed transcripts contained well-known activity-dependent genes e.g. Arc, Dusp1, Fosb as well as a set of novel transcripts e.g. Sik1, Dusp5, Bhlhe40 . Furthermore, in several cases we found that the induction is restricted to specific splicing variants or biotypes: alternative first exon (e.g. Stxbp1), alternative last exon (Hsph1), intron retention (Dnajb5), long non-coding RNA (Gm13889) and small RNA (Mir92b and Mir130a). The modules of transcriptional factors (e.g. SRF/CREB1 and E2F1/ETS1) that control inducible alternative transcription of genes in the striatum were identified using the seqinspector.cremag.org online tool. To compare cocaine-induced gene expression patterns between the subpopulations of striatal neurons we used fluorescence-activated cell sorting and genetically labeled dopamine receptor expressing cells. Further experiments will explore differences in activity-regulated gene expression in the D1 and D2 expressing medium spiny neurons of the striatum. Our results provide a comprehensive assessment of neuronal activity-induced gene expression at the level of individual transcriptional units.

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Poster

262. Genomics, Proteomics, and Systems Neurobiology

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Topic: G.02. Genomics, Proteomics, and Systems Biology

Support: FAPESP 2012/15660-3

FAPESP 2011/02263-3

CIHR

Title: Involvement of long non-coding RNAs in cerebral changes associated with acute-liver failure

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Abstract: Acute liver failure (ALF) leads to major pathological changes in brain that involve the development of brain edema due to profound astrocyte swelling and (Type A) hepatic encephalopathy. However, the underlying pathophysiology responsible for these effect remains unclear. Long non-coding RNAs (lncRNAs) are emerging as major regulators of cellular phenotypes. However their precise role in terms of the regulation of gene expression at the genomic level are only now beginning to be studied in detail. In order to gain new insight

regarding the involvement of lncRNAs in ALF, we utilized the established azoxymethane (AOM) model of this disorder to precipitate liver pathology. Groups of C57/BL6 mice (25-30 g) were treated with AOM (100 μg/g body weight, i.p.) and allowed to progress to the coma stage, at which time the mice were sacrificed and the frontal cortex dissected out. Cerebral tissue was then profiled at the genomic level for changes in the levels of lncRNAs. Our results show that the glutamatergic synapse, JAK-STAT pathway, NFkB, MAPK, Ras, and mTOR, are some of the major pathways targeted by this category of RNA species. Altered expression of these pathways may have significant consequences in terms of development of the RNA phenotype. Based on these findings, we conclude that lncRNAs participate in AOM- induced ALF and may play an important role in the pathophysiology of this often life-threatening illness. This study was supported by FAPESP (Brazil) and CIHR (Canada).

Disclosures: V.R. Silva: None. **A.S. Hazell:** None.

Poster

262. Genomics, Proteomics, and Systems Neurobiology

Location: Hall A

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Program#/Poster#: 262.21/AA19

Topic: G.02. Genomics, Proteomics, and Systems Biology

Title: The neurobehavioral phenotypes of the C57BL/6NJ substrain are not altered by genetic rescue of the Crb1^{rd8} retinal degeneration mutation

Authors: *S. J. SUKOFF RIZZO, G. WELLS, S. BURRILL, J. RYAN, L. C. ANDERSON, M. P. KREBS, P. M. NISHINA, M. V. WILES;
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Abstract: The C57BL/6J (B6J) mouse and its C57BL/6N (B6N) substrain are inbred strains used for genetics and pharmacology studies, and their divergent phenotypic differences across behavioral, physiological, and biochemical measures are well established (Simon et al 2013). Aside from phenotypic differences, >10,000 putative and 279 confirmed variants have been reported between the substrains (Keane et al 2011, Simon et al 2013) including a mutation in the Crb1 gene, which is expressed in retina and brain (den Hollander et al 2002), and in the B6N substrain carries the *rd8* allele. This mutation results in retinal dysplasia, degeneration and related eye phenotypes (Chang et al 2002). While it has recently been reported that rescue of this mutation by TALEN-mediated homology-directed repair corrects the eye phenotypes (Low et al 2014), the effect on the divergent neurobehavioral phenotypes is unknown. In the present studies, C57BL/6NJ mice (WT) demonstrate the expected divergent behavioral phenotypes relative to B6J mice in the open field, rotarod, pre-pulse inhibition assay, electroconvulsive seizure threshold test, and pharmacological challenge with MK-801 or ethanol. Importantly, mice homozygous for the *rd8* correction (HOM) demonstrate behavioral phenotypes in these tests

identical to the WT non-corrected controls. We also confirm rescue of the dysplastic phenotype in HOM mice as determined by fundus imaging and optical coherence tomography. Despite genetic rescue, electroretinography and optokinetic reflex assays indicate identical visual performance of HOM, WT and B6J mice, suggesting that any functional effect of the *rd8* allele in the WT genetic background is below the limit of detection at the ages studied. Taken together, these data demonstrate that rescue of the *rd8* mutation in the C57BL/6NJ substrain does not alter the neurobehavioral phenotypes or ocular function and further suggest that the genetic underpinnings of the behavioral divergence of the B6N and B6J substrains are independent of the *Crb1*^{rd8} allele.

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Poster

262. Genomics, Proteomics, and Systems Neurobiology

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Topic: A.01. Neurogenesis and Gliogenesis

Support: NIMH Contract HHSN-271-2008-0047-C

Title: Comprehensive transcriptional atlas of primate brain development

Authors: *J. A. MILLER[1], T. BAKKEN[1], S.-L. DING[1], S. SUNKIN[1], K. SMITH[1], L. NG[1], A. SZAFER[1], J. GOLDY[1], C.-K. LEE[1], A. EBBERT[1], R. DALLEY[1], N. DEE[1], J. ROYALL[1], P. D. PARKER[1], Z. RILEY[1], Z. MOLNAR[2], R. HEVNER[3], D. AMARAL[4], M. HAWRYLYCZ[1], J. HOHMANN[1], A. JONES[1], J. PHILLIPS[1], P. WOHNOUTKA[1], C. DANG[1], A. BERNARD[1], E. LEIN[1];
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Abstract: Existing gene expression atlases of the developing human brain include either fine spatial resolution at few time points or more coarse sampling of structures across development, largely due to tissue limitations. Non-human primates make ideal model systems for studying human brain development due to their evolutionary proximity to humans, protracted development and maturation, and availability of multiple specimens from precisely specified ages. Here we present a high spatial and temporal resolution transcriptional map of the rhesus macaque brain to examine the genetic underpinnings of primate cortical development. Individual layers from several cortical areas (including V1) and associated subcortical structures (including dorsal lateral geniculate nucleus) were isolated by laser microdissection and profiled with DNA microarrays at ten time points across rhesus development. Six prenatal time points correspond to

ages of peak neurogenesis for different layers in V1, and four postnatal time points correspond to key phases of postnatal development: infancy, childhood, adolescence and early adulthood. We found extensive variation between cortical layers and across developmental stages, with dramatic rates of gene expression change in all assayed regions throughout prenatal and early postnatal development. These changes reflected the progressive generation and maturation of distinct cell types, which tended to either occur synchronously across regions or with an earlier onset in early-developed subcortical structures, with very few genes peaking at a single intermediate time point. Consistent with anatomical and birthdating studies, genetic signatures of neurogenesis and gliogenesis appeared later in V1 relative to prefrontal cortex. Strikingly, post-mitotic neuron expression patterns continued to change throughout development, well after their initial generation, such that only a few genes marking adult cortical layers were similarly patterned in perinatal cortex. Finally, we found that expression trajectories were significantly more conserved between rhesus and human than between mouse and human, supporting the value of rhesus as a model organism for studying the molecular basis of human cortical development. This dataset is publicly accessible as part of the NIH Blueprint NHP Atlas at www.blueprintnhpatlas.org.

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Poster

263. G.04. Physiological Methods; G.04.a. Optical methods

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Topic: G.04. Physiological Methods

Support: NIH R56 Grant NS087249

DOD FA9550-14-1-0303

Title: Modeling of inhibition of action potentials due to infrared laser heating

Authors: ***M. GANGULY**[1], K. M. SHAW[2], M. W. JENKINS[3], H. J. CHIEL[2], E. D. JANSEN[1]; [1]Vanderbilt Univ., Nashville, TN; [2]Dept. of Biol., [3]Dept. of Pediatrics, Case Western Reserve Univ., Cleveland, OH

Abstract: Elevated temperatures are known to inhibit nerve action potentials in myelinated and unmyelinated nerves. It has been shown that infrared lasers (1.87 μm) that heat the tissue due to water absorption can be used to block action potential propagation in *Aplysia* and in the rat

sciatic nerve. Optical nerve block was shown to be spatially precise, reversible and safe, and can be used to block action potential propagation in both sensory and motor neurons. However, questions remain about the underlying mechanisms. A comprehensive computational model simulating laser-induced conduction block will be helpful in deciphering the biophysical mechanisms responsible for infrared block and optimizing parameter space to deliver more efficient/safe block for a variety different tissues. Our novel approach combines an optical-thermal model of laser-induced thermal transients with NEURON to obtain a single comprehensive model to evaluate the effects of laser light on nerve signal propagation. The optical-thermal model simulates the spatio-temporal thermal transients induced by laser light by utilizing a Monte Carlo simulation for light distribution in tissue coupled to a finite difference heat transfer model. Conduction block in the nerve section as a result of these temperature transients was simulated using NEURON combined with Python. The simulation makes it possible to explore the effect of axon diameter, the longitudinal extent of the applied heat block, and the existence of myelination on inhibition threshold. Preliminary studies demonstrate that decreasing axon diameter lowers the radiant exposure threshold for block, suggesting that smaller diameter fibers may be selectively subject to inhibition prior to large diameter fibers. This finding implies that this technology could be used to selectively block pain conducting fibers in a new approach we have coined Photonic Analgesia by Inhibition of Nerves (PAIN). This model can be used in the future to explore the large parameter space in silica and optimize the effects of laser light on the induction or inhibition of neural signals in a controlled manner.

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Poster

263. G.04. Physiological Methods; G.04.a. Optical methods

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Topic: G.04. Physiological Methods

Support: NIH/NINDS Grant R56-NS087249

DOD/AFOSR Grant FA9550-14-1-0303

Title: Infrared inhibition of capsaicin evoked afferent potentials

Authors: ***J. FORD**[1], M. JENKINS[2], H. CHIEL[2], E. D. JANSEN[1];
[1]Vanderbilt Univ., Nashville, TN; [2]Case Western Reserve Univ., Cleveland, OH

Abstract: As of yet, the role of infrared neural stimulation (INS) in the activation or inhibition of afferent neural signals is unknown. Previous work with INS has shown the ability of infrared light to evoke contact-free, highly spatially selective neural activation. More recently, infrared

irradiation of neural tissue was shown to reliably inhibit neural conduction. Generally, either compound neural action potentials (CNAPs) or motor output are used as a measure of neural inhibition. In our lab, Duke et al. (Nature Scientific Reports, Sept. 2013) showed partial and total block in both the *Aplysia* buccal nerve and rat sciatic nerve using both CNAPs and motor unit recruitment as endpoints. While indicative of efferent signals, inhibition of motor output may be an inaccurate means by which to characterize sensory fiber inhibition, since the endpoint is motor neuron recruitment and muscle fiber activation. Furthermore, there is a lack of information demonstrating the ability of infrared light to block ascending sensory fibers. To view afferent specific signals, an *ex vivo* setup is preferable because it allows for more complete control of the system. Environmental parameters may be monitored, noise and spontaneous activity reduced, and drugs may be applied more precisely. Sensory information is mediated through small, unmyelinated neurons (C fibers). Capsaicin, a vanilloid, acts as an agonist for TRPV1 channels, found in C fibers. Application of small amounts of capsaicin (~0.1-1 uM) has been shown to preferentially excite C fibers in the rat sciatic nerve. We have created an *ex vivo* setup for C fiber specific chemical activation and optical inhibition. Adult male Sprague-Dawley rats will be used. Rats will be anesthetized with isoflurane inhalation and the sciatic nerves dissected out. Nerves will be maintained in a custom flow channel and superfused with oxygenated Krebs solution. CNAPs will be recorded using suction electrodes with silver wire leads. Capsaicin will be introduced into the solution to provide excitation of the C fibers, and conduction block will be attempted with optical irradiation. Identification of the ability to inhibit neural afferent signals is necessary to more fully characterize infrared modulation of neural conduction. The described *ex vivo* approach will provide a reliable, repeatable model that allows investigation into the parameter space and optimization of infrared conduction block, which may be adapted in future studies for more precise recording techniques if necessary. A greater understanding of the parameters necessary to inhibit afferent neurons will lead to better utilization of the advantages of infrared light for neural control.

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Poster

263. G.04. Physiological Methods; G.04.a. Optical methods

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Topic: G.04. Physiological Methods

Support: NIH Grant 1R56NS087249

NIH Grant HL115373

Title: Optical inhibition for selective block of unmyelinated axonal subpopulations within nerves

Authors: *E. LOTHET[1], H. LU[2], Y. WANG[3], C. HORN[4], E. D. JANSEN[5], H. CHIEL[2], M. JENKINS[3];
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Abstract: Controlling sub-populations of unmyelinated axons within peripheral nerves would make it possible to treat a very wide variety of clinical syndromes. Within the last few years, implanted neural devices have been developed to control peripheral nerve activity (the new area of neuroceuticals). Another promising modality for control of neural activity that has recently been described has been infrared laser (IR) light. Previous studies have shown that brief pulses of IR can induce excitation of neurons. Recent studies have shown that IR can inhibit neurons with high spatial specificity. The inhibitory block is likely to be due to local temperature increases that may reduce the conductance of ion channels responsible for action potentials. We have modeled the response of unmyelinated fibers of different diameters (see Ganguly et al., this meeting) to the external application of changes in temperature. Axons of smaller diameter have lower thresholds for externally applied inhibitory influences than larger diameter axons. To test whether these modeling predictions were correct, we first studied large and small diameter individual identified axons in the marine mollusk *Aplysia* californica. Because axon size scales with soma diameter, by intracellularly controlling the activity of a large neuron (B3) and a small neuron (B43), we were able to simultaneously examine the effects of externally applied IR light to a single large and small diameter axon. We found that we could rapidly and repeatedly inhibit action potential propagation in the smaller axon before inhibiting the larger axon, and that this inhibition was also readily reversible. The functional physiology of the axons remained unchanged throughout many hours even after repeated exposure to the IR light, as action potential amplitudes and thresholds remained unchanged. To determine whether this phenomenon would also be observed in a vertebrate nerve, we applied laser light to the vagus nerve of the musk shrew. To understand the functional role of the vagus in the musk shrew, a novel preparation has been developed that makes it possible to examine activity of small sub-populations of axons in the vagus within a semi-intact preparation, during which respiration, blood pressure, EKG and body temperature can all be measured. We found that the application of IR light selectively blocked slow conducting fibers before blocking fast conducting fibers in the vagus. These results suggest that it may be possible to use IR light as a selective blocker of unmyelinated C fibers. In future studies, it may be possible to establish whether this could serve as a novel treatment for significant clinical syndromes such as chronic pain.

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Poster

263. G.04. Physiological Methods; G.04.a. Optical methods

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Title: Cortex-wide cellular-resolution imaging access in head-fixed behaving mice using chronically implanted glass cranial windows covering the entire dorsal cortical surface

Authors: *B. S. HUANG, S. VRONTOU, A. BELLAFARD, P. GOLSHANI;
Dept of Neurol., UCLA Sch. of Med., Los Angeles, CA

Abstract: *In vivo* two-photon calcium imaging in head-fixed behaving mice has enabled studies of real-time neural circuit dynamics at cellular resolution. However, one limitation of the current approach is the need to restrict imaging to a small pre-selected cortical region of interest. While this targeted approach has produced valuable insights into circuit mechanisms in more functionally circumscribed regions, it could be limiting for studies of higher-order cognitive functions, such as attention and decision-making, which may involve large-scale circuits spanning multiple regions across the cortex. To overcome this limitation, we have developed a novel cranial window preparation that provides long-term cellular-resolution optical access to the entire dorsal cortical surface of adult mice. Our new window prep entails a large craniotomy to remove the skull over an approximately 6 x 8 mm area, uncovering cortical surfaces extending from the anterior tip of frontal cortex all the way to visual cortex at the posterior end, and extending across both hemispheres. After the craniotomy, we implant a large custom-shaped glass coverslip window, which enables long-term optical access for repeated *in vivo* two-photon imaging. These chronically implanted cortex-spanning windows have been able to remain optically clear for over 3 months, with minimal bone or dural regrowth and no observed detrimental effects on the animals' health. We have implanted these windows in transgenic mice expressing GCaMP6 (the most sensitive genetically-encoded calcium indicators to-date) and have been able to image large-scale neuronal population activity from visual to parietal to frontal cortices, bilaterally, within single animals. This cortex-wide unrestricted imaging access has allowed us to measure and compare neural circuit dynamics among various cortical regions during resting state and behavioral engagement. To further extend the imaging access to cortical surfaces buried within the midline fissure, such as mPFC (medial prefrontal cortex) and ACC (anterior cingulate cortex), we have also incorporated right-angle microprisms into our new window prep. This microprism add-on is especially useful for studying circuit functions of frontal/prefrontal cortex. Overall, our new cortex-spanning window approach enables the unbiased sampling of neural circuit activity from multiple cortical regions without regard to predefined anatomical borders, opening the door to searches for critical cortical circuits mediating higher-order cognitive behaviors.

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Poster

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EMBO Long Term Fellowship

Swartz Foundation postdoctoral fellowship

Title: *In vivo* patterned photo-stimulation and imaging in independent axial planes

Authors: M. S. KOH, *F. ANSELMI, A. BANERJEE, M. B. DAVIS, D. F. ALBEANU; Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Understanding the function of neural circuits requires monitoring large populations of neurons, while simultaneously perturbing specific circuit elements. Patterned illumination techniques (1) allow the generation of flexible spatial and temporal photo-stimulation profiles, which together with multiphoton imaging (2) and optogenetic manipulations (3, 4) provide an ideal framework towards achieving this goal. We describe here a platform combining one photon patterned photo-stimulation via a digital micro-mirror device (DMD) with two photon imaging (5). Since neural circuits are often arranged in three dimensions, we developed a simple method that allows decoupling of the imaging and the photo-stimulation planes. Briefly, the pulsed infrared laser beam for scanning two-photon imaging and a blue laser (488 nm) for photo-stimulation are coupled through the same objective. The blue light intensity is modulated by the DMD chip to form arbitrary spatial-temporal patterns on the brain surface. By introducing a movable holographic diffuser in a plane conjugated with the desired photo-stimulation plane in the sample, we decouple the photo-stimulation and the imaging planes, up to an axial shift of 500 μm. Additionally, this allows axial confinement of the photo-stimulation pattern. To target large populations of neurons, we set the photo-stimulation field to 1.5 x 1.2 mm2 with a lateral resolution of ~20 μm. Two fast shutters in front of the blue laser and the PMT are used in anti-phase to rapidly alternate between photo-stimulation and imaging (10 Hz). When this technique is applied to the olfactory bulb (OB), we are able to optogenetically photo-stimulate specific neural populations within individual glomeruli while simultaneously monitoring GCaMP3 & GCamP6f signals from populations of bulb interneurons, or output neurons (mitral and tufted cells). We use the two-photon imaging to obtain anatomical and functional information to target particular structures of interest. Within the OB, our strategy offers exciting possibilities for understanding broadcasting of signals by single glomeruli and their combinations, as well as information integration rules at the level of individual neurons during olfactory behaviors. (1) Packer et al. Nat Neuroscience 2013; (2) Tian et al. Nat Methods 2010; (3) Boyden et al. Nat

Neuroscience 2005; (4) Aravanis et al. J. Neural Eng. 2007; (5) Dhawale et al. Nat Neuroscience 2010

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Poster

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Title: Effective optical neural stimulation using localized surface plasmon resonance of gold nanoparticles

Authors: *K. EOM[1], S. HWANG[2], T. KANG[2], S. YUN[1], S. SHIM[1], G. CHOI[1], K. BYUN[4], S. JUN[2,3], S. KIM[1];
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Abstract: Modulating neural cell by light has received increasing attention due to its merits such as non-invasiveness, high spatial selectivity, and contact free method. Among light modulation techniques, we previously demonstrated gold nanoparticle mediated infrared neural stimulation (INS) which could evoke neural activation with safer and more enhanced manner compared to conventional INS. However, gold nanoparticles are easily washed out under the convective

media and it is hard to induce neuronal depolarization. In this research, we demonstrated cell targeted effective neural stimulation method using cell targeted gold nanoparticles. *In vitro* experiments were performed to verify that cell targeted gold nanoparticle are effective for optical neural stimulation. Pulsed infrared was irradiated and light evoked neural response were measured. The evoked neural responses were compared with light stimulation with and without cell targeted gold nanoparticles. When stimulating the neuron with cell targeted gold nanoparticles, stimulation threshold was lowered in the cell targeted gold nanoparticles suggesting that local heating of gold nanoparticles efficiently triggers neural activation. In summary, we successfully showed cell targeted gold nanoparticles could elicit neuron activation with low threshold.

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Poster

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Program#/Poster#: 263.07/AA27

Topic: G.04. Physiological Methods

Title: Micro-point photothermal stimulation for inhibition of single neuron activity

Authors: S. YOO, J.-H. PARK, *Y. NAM;
KAIST, Daejeon, Korea, Republic of

Abstract: Optical stimulation of nerves has great attracted attention due to their minimum invasiveness and high spatiotemporal resolution. Previously we demonstrated that gold nanorods (GNRs)-mediated photothermal stimulation could inhibit the electrical activity of neural networks, which was advantageous than current optical technique in terms of reduction of laser power consumption and improvement of the reliability. However inhibition ability of the photothermal stimulation at single cell resolution is still yet investigated. Here we demonstrated that GNRs-mediated photothermal stimulation can inhibit the activity of single neuron without thermal damage. Laser beam was focused into tens of microns in diameter for micro-point photothermal stimulation (μPops), and changes of network activities were simultaneously measured for mapping of functional connections. GNRs that can absorb the near-infrared laser (NIR, 785 nm) were synthesized by seed-mediated method. Surfaces of the GNRs were modified with polyethylene glycol (SH-PEG-NH2) that has a bifunctional group for covalent bonding to gold surfaces and providing the positive charge, respectively. The negatively charged radicals were introduced to MEA substrate by brief treatment of air plasma. And the GNRs were self-assembled on MEA substrate by electrostatic forces. Hippocampal neurons were cultured on the GNRs coated MEAs and the matured networks that showed the spontaneous firing were used as

a model network. NIR was focused to 20 μm in diameter by using a high- magnitude objective for single cell stimulation. Change of the neural activity during the optical stimulation was recorded and analyzed. The PEGylated GNRs were self-assembled and immobilized on the MEA substrate, which yielded the monolayered nanostructure. The concentration of the GNRs on MEA was controllable by changing the assembly time, and the nanostructure generated the heat during the NIR irradiation. Firing of single neuron was inhibited by the μPops, which showed the high reproducibility. The spontaneous activity of neural networks began to inhibit by 12.4 W/mm2 of power density, and most activities were completely inhibited by 32 W/mm2. And thermal threshold of the photothermal stimulation at single cell level was measured to 51 W/mm2. Furthermore it was demonstrated that inhibition of the single cell firing had influence on network activities, which was utilized for mapping of excitatory or inhibitory connections within the network. Together our approach could be a novel platform to explore the network functions and connections as well as treatment of brain disorders.

Disclosures: **S. Yoo:** None. **J. Park:** None. **Y. Nam:** None.

Poster

263. G.04. Physiological Methods; G.04.a. Optical methods

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Program#/Poster#: 263.08/AA28

Topic: G.04. Physiological Methods

Support: NIH Grant R43 NS086181

Title: ScanImage for closed-loop cellular resolution brain imaging and stimulation with two-photon laser scanning microscopy

Authors: N. CLACK, E. KANG, G. JAINDL, J. KING, J. ROCAMORA, B. KIMMEL, *V. IYER;
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Abstract: ScanImage is widely used software (scanimage.org) for two-photon laser scanning microscopy (TPLSM), a powerful technique for cellular resolution imaging in the intact brain. TPLSM was recognized in 2015 by The Brain Prize for its transformative role in the ‘development, plasticity, and functional circuitry of the brain.’ In recent years, TPLSM has become increasingly used for comprehensive volumetric imaging of neural populations in awake animals, allowing fine-scale correlation of cellular activity to behavior. We present research towards extending ScanImage towards the next generation of experiments unraveling causal relations between neuronal activity and behavior. Three approaches are pursued towards this end of ‘closed loop’ TPLSM experiments. First, we have developed photostimulation controls designed to allow light-activation of targeted cells via TPLSM using optogenetics tools during

simultaneous high-speed resonant TPLSM imaging. Simultaneous photostimulation and imaging is achieved via parallel control of two independent scanner pathways and allows the study of direct causal links within living neural networks. Photoactivation of individual cells using TPLSM is shown to be feasible via very fast resonant raster scans across a cell body of interest, opening a sufficient number of light-activated channels to achieve cellular firing. Image correlation based alignment of the two scanner paths in software is shown, allowing precise targeting of photostimulation cells identified from an image acquired by the parallel imaging scanner pathway. Second, we present work towards the development of a parallel processing extensibility interface enabling custom analysis and control scripts to be developed for execution during live high-speed TPLSM imaging. Such an interface will allow experimenters to design and implement closed-loop experimental paradigms. For example, behavioral control signals could be linked to the live detection of cellular signaling, to perhaps achieve single neuron operant conditioning. Alternatively, control of targeted photostimulation could be programmably driven to linked to behavioral readouts. Third, we present work towards implementing such closed-loop experiments within a single frame (of < 30 ms) using similar analysis and control approaches implemented on commodity programmable hardware boards incorporated field-programmable gate arrays (FPGAs).

Disclosures: **N. Clack:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vidrio Technologies, LLC. **E. Kang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vidrio Technologies, LLC. **G. Jaindl:** None. **J. King:** None. **J. Rocamora:** None. **B. Kimmel:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vidrio Technologies, LLC. **V. Iyer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vidrio Technologies, LLC.

Poster

263. G.04. Physiological Methods; G.04.a. Optical methods

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Program#/Poster#: 263.09/AA29

Topic: G.04. Physiological Methods

Support: Howard Hughes Medical Institute

Title: Wavesurfer: A flexible application for neurophysiology data acquisition

Authors: ***A. L. TAYLOR**[1], B. J. ARTHUR[2], H. INAGAKI[2], J. YU[2], X. ZHAO[2], C. GRIENBERGER[2], S. WEGENER[2], D. HUNT[2], M. KOYAMA[2], V. JAYARAMAN[2], J.

MAGEE[2], N. SPRUSTON[2], K. SVOBODA[2];
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Abstract: There are many applications for neurophysiology data acquisition, but often a user is forced to choose between a turn-key solution with limited flexibility and an open-ended solution that requires up-front programming before data can be acquired. Furthermore, performing coordinated optical and electrophysiological recordings can be a challenge. Here we present Wavesurfer (wavesurfer.janelia.org), the successor to the Ephus electrophysiology data acquisition package, which enables turn-key operation for basic electrophysiology, but also allows for extensive user customization. It is designed to integrate tightly with ScanImage, the well-known laser scanning microscopy software. Like ScanImage, Wavesurfer is implemented in Matlab, and allows the user to write custom analysis, visualization, and control scripts that extend its native capabilities. It works with any National Instruments X series data acquisition board and any patch-clamp or sharp electrode amplifier. Furthermore, it features tight integration with Heka and Axon patch-clamp amplifiers. Wavesurfer saves data in HDF5 format, an open standard for scientific data. It is open-source software, released under a permissive license.

Disclosures: **A.L. Taylor:** None. **B.J. Arthur:** None. **H. Inagaki:** None. **J. Yu:** None. **X. Zhao:** None. **C. Grienberger:** None. **S. Wegener:** None. **D. Hunt:** None. **M. Koyama:** None. **V. Jayaraman:** None. **J. Magee:** None. **N. Spruston:** None. **K. Svoboda:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Vidrio Technologies, LLC.

Poster

263. G.04. Physiological Methods; G.04.a. Optical methods

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 263.10/AA30

Topic: G.04. Physiological Methods

Title: A novel wearable NIRS sensor module detects optical signal from every positions of head

Authors: *H. EDA[1], M. YAMAZAKI[2], K. SOETA[3], Y. OHTAKI[3], M. SAKURAI[3], T. YAMAUCHI[4], T. FUJITA[4];
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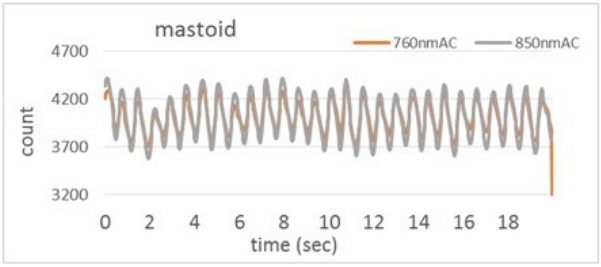
Abstract: Near infrared spectroscopy (NIRS) calculates hemoglobin parameters, such as changes in oxygenated hemoglobin (oxyHb) and deoxygenated hemoglobin (deoxyHb), using the near infrared lights around the 800 nm wavelength. We reported the portable NIRS system (Eda et.al., SfN2007) and the wearable NIRS system. The wearable NIRS system can capture

physiological information from everywhere in the body (Eda et.al., SfN2014). We have developed the system for health care at the first. For the brain science, we need to examine the brain measurement. The purpose of this study is to validate the wearable NIRS system for the brain science. NIRS sensor module (ALPS ELECTRIC CO., LTD, JAPAN) was newly developed for acquiring AC signals and DC signals separately for each wavelength. AC signals are used for measuring pulsation and DC signals are used for conventional NIRS. The sensor module has two light sources and one detector. Each light source has two wavelengths of near infrared LEDs. We attached the sensor module in the same positions each of the EEG 10-20 system, such as A2, C4, F4, F8, Fp2, O2, P4, T4, and T6. This time we attached the module on mastoid also. The sensor module was attached the head directly for 15 seconds. And we measured the optical signal to confirm the pulsation from each position. Both AC signals and DC signals were detected by the NIRS sensor module. The pulsation was detected at each position of A2, C4, F4, F8, Fp2, O2, P4, T4, T6, as AC signals. And we detected the pulsation at mastoid. There were difference of NIRS signals between the sensor's positions. Because the DC signals were also obtained precisely, we could acquire the hemoglobin parameters by applying the modified Lambert-Beer's Law used in NIRS calculation. The NIRS sensor module detected the optical signal from every part of the head even when the hair existed. Our sensor module showed the utility of the wearable NIRS system calculating the heart rate and hemoglobin parameters associated with bio-physical activities. And the NIRS sensor module also suggested a possibility of the brain imaging by using multi modules.



Disclosures: **H. Eda:** None. **M. Yamazaki:** None. **K. Soeta:** None. **Y. Ohtaki:** None. **M. Sakurai:** None. **T. Yamauchi:** None. **T. Fujita:** None.

Poster

263. G.04. Physiological Methods; G.04.a. Optical methods

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 263.11/AA31

Topic: G.04. Physiological Methods

Title: Can wearable NIRS capture brain activities comparably to EEG?

Authors: *M. YAMAZAKI[1], H. EDA[2];
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Abstract: Introduction Near infrared spectroscopy (NIRS) calculates hemoglobin parameters, such as change in oxygenated hemoglobin (oxyHb) and deoxygenated hemoglobin (deoxyHb), using the near infrared lights around the 800 nm wavelength. The NIRS has been used in brain science, phychology clinical field to evaluate the brain activities. We developed wearable NIRS sensor module and suggested that it can capture physiological information from everywhere in the body (Eda et.al., SfN2014). Purpose of this study is to validate the wearable NIRS as a new method of measuring the brain activities. Methods We conducted EEG and NIRS recording simultaneously. 1)EEG : We attached 19 scalp EEG electrodes on the head according to 10-20 system and recorded EEG data using Neurofax EEG-1200 (NihonKohden) for 3 sessions. The session consisted of both eyes open and close state for 20 seconds each. 2) NIRS : We put two wearable NIRS sensor modules on the frontal head region (between Fpz and Fz) and the occipital head region (between O1 and O2) and measured the hemoglobin parameters (oxyHb and deoxyHb). We compared the signals recorded by EEG and NIRS in both eyes opening state and closing state. Results Eye closing state : α activities were captured predominant in the occipital head region (O1 and O2) immediately after eyes closed. NIRS showed that oxyHb dynamically elevated and decreased deoxyHb 3-5 seconds after the appearance ofα activities in the occipital head region compared to the frontal head region. Eye opening state : EEG showed mixture of low amplitudeα and βactivities without predominant rhythmic activities. NIRS also showed no significant dynamic changes in both oxyHb and deoxyHb. Conclusion This study showed that the wearable NIRS module captured the dynamic oxyHb changes associate with the appearance of α activities. This result is concordant with fMRI studies which hemodynamic response (increased oxyHb and decreased deoxyHb) was shown when the brain activities increased. The wearable NIRS module suggests utility in the assessment of the brain activities as a new measuring tool. Acknowledgements This study was supported by ALPS ELECTRIC CO., LTD, Tokyo, JAPAN[3] and Genial Light. co.,LTD, Hamamatsu, Japan[4]. We received grateful technical supports from Mr.K.Soeta[3], Y.Ootaki[3], M.Sakurai[3], T.Yamauchi[4] and Mr. T.Fujita[4].

Disclosures: **M. Yamazaki:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); ALPS ELECTRIC CO., LTD, Tokyo, JAPAN and Genial Light. co.,LTD, Hamamatsu, Japan. **H. Eda:** None.

Poster

263. G.04. Physiological Methods; G.04.a. Optical methods

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 263.12/AA32

Topic: G.04. Physiological Methods

Title: Spike detection with biophysical models for GCaMP6 and other multivalent calcium indicator proteins

Authors: *D. S. GREENBERG[1], D. J. WALLACE[2], J. T. VOGELSTEIN[3], J. N. D. KERR[2]; [2]Dept. of Behavior and Brain Organization, [1]Res. Ctr. Caesar: A Max Planck Inst., Bonn, Germany; [3]Dept. of Biomed. Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: Two photon-imaging of pressure-injected fluorescent organic molecule calcium indicators can resolve single action potentials from neurons *in vivo*, but recording times are limited to a few hours. Genetically encoded indicators such as GCaMPs promise relatively noninvasive, long-term observation of neural activity. However, GCaMP fluorescence grows non-linearly with increasing numbers of action potentials, have slow kinetics across multiple binding states and show variable protein expression levels and transient shape across neurons. Consequently, previously developed algorithms for determining the action potential discharge pattern underlying the recorded fluorescence transients are substantially less accurate and reliable when they are applied to data acquired using GCaMPs. In order to detect action potentials despite the complex and variable relationship between spiking and fluorescence, we developed a mathematical framework for modeling calcium entry and extrusion, kinetics of transitions across multiple binding states, drifting baseline fluorescence, photon shot noise and variable indicator concentration across neurons. We used a sequential Monte Carlo algorithm with 100K particles to perform spike detection, microscope calibration, and estimation of indicator concentration from fluorescence data alone. In addition we have developed an efficient GPU-based parallel implementation to reduce processing time required for larger datasets. By using a biophysical modeling approach as opposed to "black-box" statistical methods, we were able to carefully control which aspects of our model were shared for all data (such as rate constants) and which could vary across neurons (such as indicator concentration). Importantly, we validated our approach with simultaneous optical and electrical recordings of mouse cortical L2/3 pyramidal neurons expressing GCaMP6s. In comparison to alternative methods, we show a significant increase in correct spike detection, combined with a large reduction in false positive detection rate. Our approach provides an accurate method for converting calcium transients measured from the latest generation of GCaMP indicators into the underlying train of action potentials, and has been designed to also accommodate future alterations to the indicators which result in differences in their binding kinetics.

Disclosures: D.S. Greenberg: None. **D.J. Wallace:** None. **J.T. Vogelstein:** None. **J.N.D. Kerr:** None.

Poster

263. G.04. Physiological Methods; G.04.a. Optical methods

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 263.13/AA33

Topic: G.04. Physiological Methods

Title: An algorithm for identification of structures from fluorescently labeled neuronal populations validated with simultaneous electrophysiological and 2-photon imaging recordings, *in vitro*

Authors: *K.-M. VOIT, D. J. WALLACE, J. N. D. KERR, D. S. GREENBERG;
Behavior and Brain Organization, Res. Ctr. Caesar: A Max Planck Inst., Bonn, Germany

Abstract: While protein-based calcium sensors such as GCaMPs can report neuronal activity longer and less invasively than their synthetic counterparts, they brightly label diverse, intricate and overlapping fluorescent structures, which makes precise isolation of the borders of single-neurons and single structured challenging. These indicators have very low fluorescence in the absence of action potentials. Consequently, small structures (eg. dendrites) from neighboring or nearby neurons which overlap with or lie in close apposition to a neuron of interest can be inadvertently included within that neurons assigned region-of-interest (ROI). Because these indicators also show very large increases in fluorescence following action potential firing, inclusion of even a single pixel from a neighboring structure can result in artifactual fluorescence increases larger than single action potential (AP) responses and with similar rise and decay times. These artifactual transients are frequently large enough to cause false positive errors in AP detection. To combat this problem, we have developed an algorithm to detect, separate and extract fluorescence signals from overlapping fluorescenct structures. Our method is based on non-negative matrix factorization (NMF), with a novel penalization term to enforce both temporal and spatial constraints on the detected signals. The time course of each structure's fluorescence is assumed to consist of low-frequency baseline drift added to sparse activity-associated increases. The number of fluorescence objects is assumed to be limited, both in the complete field of view and at any one pixel where they may overlap. An important aspect for validation of this approach was using simultaneous optical and electrical recordings from mouse L2/3 cortical pyramidal neurons expressing GCaMP6s. Firstly this allowed identification of artifactual signals not associated with the neuron of interest. Secondly, this allowed quantification of the validity of the automated segmentation for use with AP detection algorithms. We demonstrate improved AP-detection from automatically extracted signals compared to ROI-averaged fluorescence kinetics traces. The properties of the current generation of genetically encoded calcium indicators mean that imprecisely assigned ROIs can easily include structures from other labelled neurons which result in artifacts in the calculated kinetic traces. This segmentation approach minimizes errors caused by these indicator properties.

Disclosures: K. Voit: None. **D.J. Wallace:** None. **J.N.D. Kerr:** None. **D.S. Greenberg:** None.

Poster

263. G.04. Physiological Methods; G.04.a. Optical methods

Location: Hall A

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Program#/Poster#: 263.14/AA34

Topic: G.04. Physiological Methods

Support: NWO investment grant

Title: DAQLab: A hierarchical data acquisition and task control software for imaging

Authors: A. NEGREAN, *H. MANSVELDER;
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Abstract: Complex experimentation typically encountered in the neurosciences requires the interaction of multiple instruments and software modules to acquire multidimensional data sets. Data and parameters can then be retrieved for analysis as (data, parameters) = {i = 0..l, j = 0..mi, k = 0..nj, ...} by specifying a value for each of the indices {i, j, k, ...} indexing the data set. In this picture, a change in the experimental design means: a) A change in the order of data indexing indices, b) Addition or removal of data indexing indices, as well as, c) A change of index boundaries {l, mi, nj, ...} and d) A change in the acquisition parameters. Ideally the acquisition software should easily adapt to new experimental conditions and manage multiple devices or software modules that sometimes may function in parallel and exchange data or hardware triggers. In the present work this was accomplished by developing a software framework structured around the concept of hierarchically interacting task controllers (TCs) that are modelled as extended state machines and mediate the interaction between hardware and software modules. In this manner, a change in the experimental design that can be formulated in terms of operations a-d) translates directly to operations on the task tree (TT) graph formed by the TCs: a) Reshuffle of TC nodes, b) Addition and removal of TCs, c) Change in the number of times a parent TC starts its child TCs, d) Change in the acquisition parameters - all adjustable from DAQLab GUI. In addition to this, DAQLab provides a convenient GUI for directing data and specifying hardware trigger relationships. Using this framework we developed a laser scanning and data acquisition software in C/LabWindows consisting of the following TC controlled modules: 1) National Instruments DAQmx hardware control, 2) Laser scanning, 3) XY- and Z-motorized stage, 4) Pockells cell modulator and 5) Data display and storage in HDF5 file format.

Disclosures: **A. Negrean:** None. **H. Mansvelder:** None.

Poster

263. G.04. Physiological Methods; G.04.a. Optical methods

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Topic: G.04. Physiological Methods

Support: ERC Advanced Investigator Grant (DHISP 250128)

Swiss National Research Foundation (131093 and 156393)

Title: Two-photon imaging of light-induced nociceptive processing *in vitro*

Authors: *H. C. JOHANNSSEN[1], H. U. ZEILHOFER[2];
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Abstract: The spinal cord dorsal horn is essential for the processing and relay of peripheral noxious information to several brain regions. In addition to physiological nociceptive encoding, adaptive changes to dorsal horn circuits are known to contribute to chronic pain development, including inflammatory and neuropathic pain conditions. Despite its fundamental significance in nociceptive processing, the spinal cord dorsal horn only recently became accessible for high-resolution optical monitoring of neuronal activity using two-photon imaging in living, anesthetized mice. Similarly, channelrhodopsin-based light activation has just been newly applied to control the activity of peripheral nociceptors. Here, we combine *in vivo* two-photon calcium imaging of primary nociceptors and dorsal horn neuronal circuits with light-activation of primary afferent fibers, which transmit noxious information from the skin to the central nervous system. To selectively activate nociceptive afferent pathways with light, we crossed mice expressing cre-recombinase in peripheral nociceptors (sns::cre mice) with cre-dependent ChRH2-eYFP reporter mice. In double-transgenic sns-ChRH2-eYFP mice, eYFP was detected in peripheral nerve fibers as well as in subpopulations of dorsal root ganglia (DRG) neurons, expressing the markers calcitonin gene-related peptide (CGRP) and Isolectin B4 (IB4), respectively. In sns-ChRH2-eYFP mice, we observed robust nocifensive behaviors triggered by brief (approx. 1s) hindpaw exposure to 473 nm laser light (approx. 15 mW/mm^2). In contrast to negative controls (n = 3), all double-transgenic mice (n = 7) showed paw withdrawal and licking immediately after light stimulation. Using transganglionic tracing with OGB1-dextran, we observed labeling of lumbar DRGs *in vivo* and light-stimulation of the hindpaw evoked calcium signals in OGB1-positive DRG neurons. In addition, we monitored nociceptive processing by second order neurons in the spinal cord of sns-ChRH2-eYFP mice previously injected intraspinally with AAV-GCamP6m. The optogenetic stimulation protocol evoked robust calcium signals in subsets of GCamP6m-expressing dorsal horn neurons *in vivo*. In summary, the approaches presented here allow us to selectively activate and visualize nociceptive signaling at various anatomical levels in living mice.

Disclosures: H.C. Johannssen: None. **H.U. Zeilhofer:** None.

Poster

263. G.04. Physiological Methods; G.04.a. Optical methods

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Topic: G.04. Physiological Methods

Support: NSF IDBR DBI-1353757

NIH DC00566

NIH HD041697

Title: 3D imaging of neurons *in vitro* using a variable focus fiber-coupled microscope

Authors: *B. OZBAY[1], J. T. LOSACCO[2], D. RESTREPO[2], R. CORMACK[3], J. T. GOPINATH[3], V. M. BRIGHT[4], R. WEIR[1], E. A. GIBSON[1];
[1]Bioengineering, [2]Cell & Developmental Biol., Univ. of Colorado Denver, Aurora, CO;
[3]Electrical, Computer and Energy Engin., [4]Mechanical Engin., Univ. of Colorado Boulder, Boulder, CO

Abstract: We have developed a chronically implantable fiber-coupled microscope (FCM) that incorporates an electrowetting variable focus lens to obtain real-time 3D functional images of neurons in a mouse brain. The device is coupled to a laser-scanning microscope through an optical fiber-bundle for lateral scanning with ~2 μm resolution. Axial scanning is accomplished by altering the focus of the electrowetting lens distal to the fiber-bundle and allows for a Z-scan range of ~150 μm. The FCM is manufactured through a 3D-printing process for rapid assembly, and allows for a variety of dimensions depending on the target brain region. The assembly includes a chronically implanted lightweight (< 0.5 g) adapter for the 0.5 mm diameter GRIN objective lens and an easily attachable FCM head for imaging. We have demonstrated the FCM by performing real-time 3D imaging of mitral/tufted cells in the olfactory bulb of a mouse expressing GCaMP6s, a calcium-sensitive fluorescent protein, over multiple sessions. Our FCM shows a robust and customizable implementation of a fast miniature 3D fluorescence imaging system for monitoring brain regions over the long term.

Disclosures: B. Ozbay: None. **J.T. Losacco:** None. **D. Restrepo:** None. **R. Cormack:** None. **J.T. Gopinath:** None. **V.M. Bright:** None. **R. Weir:** None. **E.A. Gibson:** None.

Poster

263. G.04. Physiological Methods; G.04.a. Optical methods

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 263.18/AA37

Topic: G.04. Physiological Methods

Title: Strategic positioning and characterization of the SH-SY5Y human neuroblastoma cell line

Authors: C. A. MANGOLD[1], M. M. SHIPLEY[1], T. J. HUANG[2], *M. L. SZPARA[1]; [1]Biochem. and Mol. Biol., [2]Engin. Sci. and Mechanics, Pennsylvania State Univ., University Park, PA

Abstract: Microfluidic devices are routinely used to study the details of molecular neurobiology, neurovirology, neuronal communication, and neuron growth *in vitro*. Integrating the use of polydimethylsiloxane (PDMS) chambers, substrate gradients, and orienting cues allows for strategic positioning of neurons and guidance of axons during development *in vitro*. Despite these advances, there remains a gap in our ability to precisely position individual cells with minimal stress and to guide synapse formation between adjacent neurons. Combining microfluidic chambers with standing surface acoustic waves (SSAWs) is a novel, gentle, and cost-effective method that enables positioning of single cells and monitoring of cell behavior and function. Cell flow can be directed through design of the microfluidic chamber, while positioning is achieved through variations in acoustic frequency. Additionally, the acoustic focusing platform can be miniaturized, making subsequent downstream analyses such as microscopy or high-throughput drug screening easier. We are adapting SSAW technology to strategically position individual human neurons. These neurons are derived from SH-SY5Y human neuroblastoma cells, a neuro-potent cell line that can be differentiated via an extended process of serum deprivation and introduction of neurotrophic factors. We first tested the ability of SH-SY5Y cells to develop into mature neurons at low density in PDMS microfluidic devices. This process requires culturing cells at low density for 7 days in a closed system with low media volume, which is beyond the typical time frame for neuronal culture in PDMS. Our results indicate we are able to culture SH-SY5Y cells for $\geq$ 7 days to maturity. We confirmed terminal differentiation via immunohistochemical localization of markers for neuronal maturity. Our preliminary data suggests that partially differentiated SH-SY5Y cells survive treatment with SSAW and are able to be positioned at specific acoustic nodes. Ongoing studies are aimed at optimization of PDMS devices, SSAW conditions, and neuronal connectivity. Successful adaptation of this method will provide researchers with a well-controlled platform to study neuron and/or neuroglial signaling, responses to infection, as well as the ability to execute high-throughput drug screening at the level of a single cell or small groups of cells.

Disclosures: C.A. Mangold: None. **M.M. Shipley:** None. **T.J. Huang:** None. **M.L. Szpara:** None.

Poster

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Topic: G.04. Physiological Methods

Support: NEI Grant R01EY022931

Research to Prevent Blindness

Title: *In vivo* characterization of genetic expression of virus-transduced calcium indicators in retinal ganglion cells using a funduscope

Authors: *Y.-C. CHANG[1,2], S. T. WALSTON[1,2], R. H. CHOW[1,3], J. D. WEILAND[1,2,4]; [2]Biomed. Engin., [3]Physiol. & Biophysics, [4]Ophthalmology, [1]USC, Los Angeles, CA

Abstract: Genetically encoded calcium indicators (GECIs) have been widely used for observing neural activity, especially in vision research, because they enable repeated measurement of many cells in parallel at single-cell resolution. For example, some GECIs were developed to test and optimize stimulation strategies for epiretinal prostheses. Technically, the reporters can be delivered to cells via electroporation, biolistics, viral vector transduction, or generation of transgenic animal engineering. Of these methods, viral transduction provides the greatest cellular specificity in the retina. A suitable tool for monitoring GECI genetic expression *in vivo* following transduction is highly desirable. Compared with complex and expensive microscopic imaging, endoscopic fundus imaging is efficient and cost effective. Previous researchers had shown that endoscopes are capable of *in vivo* imaging of the fundus. Thus, we designed a custom endoscope-base fundus system, dividing the optical path into excitation and emission parts to facilitate fluorescence imaging of retina at single-cell resolution. We used a xenon lamp and a removable 469 nm excitation filter to generate illumination of 6-mW power for fluorescence excitation. Excitation light was coupled into the side port of a 3-mm-outer-diameter endoscope, the imaging endface of which was optically coupled by a drop of sterile saline added to the front of the lens of the mouse eye. Fluorescence emission was captured and transmitted via the fundoscope to a digital camera, with a 535-nm emission filter positioned in the optical path. To label the majority of RGCs in adult mouse retina, we designed AAV2-CAG vectors incorporating the GECI GCaMP6f. The virus was administered through intravitreal injection and the fluorescence images of the fundus were recorded every 4 days, starting at 7 days post injection. Representative fluorescence images show that the system clearly resolves individual RGCs and axons. In addition, analysis of RGC fluorescence intensity, normalized to background intensity, demonstrates a consistent rising trend from day 11 to 23 after viral injection, thus indicating a uniform increase of GCaMP6f expression in average. The normalization to background was also validated by parallel studies in which we imaged RGCs of transgenic mice expressing YFP in RGCs. The results prove that the fluorescence-endoscopy fundus system is a powerful and widely accessible tool for evaluating *in vivo* fluorescence reporter expression. Further comparison between *in vivo* and *in vitro* calcium imaging will be accomplished to determine the optimal timing for electrophysiological experiment.

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Poster

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Research to Prevent Blindness

Title: Mapping receptive fields of foveal ganglion cells in the living primate eye

Authors: *L. YIN, Q. YANG, J. ZHANG, D. R. WILLIAMS, W. H. MERIGAN;
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Abstract: The primate fovea dominates vision due to its magnified cortical representation and its exquisite acuity. However, remarkably little is known about the retinal circuitry supporting foveal vision because of the difficulty in recording from foveal ganglion cells (GCs) *in vivo* due to the blurring of stimuli by the optics of the eye and eye motion. To overcome those hurdles, we combine: (a) high-resolution adaptive-optics *in vivo* imaging, (b) real-time visual stimulus stabilization, and (c) optogenetics, to map the receptive fields of hundreds of macaque foveal GCs simultaneously at a spatial resolution of about 4 μm. We expressed the genetically encoded calcium indicator, G-CaMP6s, in foveal GCs by transfection with adeno-associated viral vector. Receptive fields (RFs) were measured using checkerboard stimuli of binary sequences at 100% contrast, refreshed at 0.5 Hz, matching the slow kinetics of GC calcium responses. For each responsive cell, we calculated temporal impulse response for each check by reverse-correlating check contrasts and fluorescence measurements from GC somas. To estimate the RF spatial profile, we calculated a weight for each check by averaging its temporal impulse response across time. The RF center was initially identified as the check having the highest weight. The temporal impulse response for this check was used as a template to decide whether adjacent checks should be included as part of the RF center or surround. Individual cells were typically responsive to only a single check, or two adjacent checks with unequal strength in the center, suggesting a RF only a few microns in diameter. A small fraction of cells were activated by three or more checks, indicating a RF center larger than that of midget cells. We observed a similar proportion of ON-center and OFF-center GCs. By correlating the spatial locations of RFs and somas of GCs, we

show that cell somas are distributed retinotopically, and the radial displacement from RF centers to cell somas matches known anatomy of the fovea. GCs on the temporal and nasal sides of the fovea form two distinct but adjacent clusters of RFs that do not cross the vertical meridian. In the future, we hope that by characterizing RF structures of foveal GCs, we may begin to identify other types of GCs in addition to the dominant midget types, and design specific visual stimuli to probe their visual functions. The ability to study foveal GCs in intact animals will eventually allow us to compare responses in retina to those in higher visual areas.

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Poster

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Support: grant ANR-10-INSB-04-01

Title: Research Scientist

Authors: ***O. ASSAYAG**[1,2], R. CONTI[2], M. GUILLON[2], V. DE SARS[2], V. EMILIANI[2];
[1]Intelligent Imaging Innovations, Göttingen, Germany; [2]Neurophotonics Lab., Paris, France

Abstract: Stimulation of multiple cells through optogenetic tools inevitably suffers from the heterogeneity of the responses, mostly due to different expression levels of the light-gated channels. We have developed an algorithm for holographic multiple cell illumination that allows to independently adjust the light intensity on the targeted cell so that the currents elicited will have the same amplitude notwithstanding different levels of expression. The software is best suited for cell-filling type constructs where the fluorescence intensity is proportional to the density of channels. Fluorescence images are recorded and used to generate the holographic pattern with graded intensity so that more light can be directed onto dim cells with respect to

more fluorescent ones, thus achieving uniform excitation conditions. Experiments were done on cultured CHO cells expressing a p2A construct of YFP and ChR2 (cell-filling). Whole-cell voltage clamp experiments allowed us to verify the proportionality between YFP fluorescence and ChR2HR peak currents. We then characterized the behavior of the peak current as a function of the illumination power. We produced a simple model of the channel activation as a function of light power density which was in good agreement with the data. Based on this model, we derived the equations to implement in our home-made software to obtain equal stimulation on multiple cells with different expression levels. Paired recordings of CHO cells in the whole-cell voltage clamp configuration were performed to verify the software performance. We measured peak currents in conditions of equal patterned light stimulation and in condition of graded stimulation. In n=7 pairs (out of 9 tested) the ratio of peak currents was reliably brought to about 1 (ratio of equal stimulation peak currents ranged from 1.9 to 6.3 (avg =4±2); ratio upon graded stimulation varied from 0.8 to 1.4 (avg 1.1±0.2)). The other 2 pairs tested showed a bad initial correlation between the ratio of the peak currents and the ratio of fluorescence and were excluded from the statistics. This new method will permit uniform excitation of multiple cells independently on their relative expression levels, precisely tuning of the synchronicity of spike generation or photoactivation of caged compound with a specific concentration gradients

Disclosures: **O. Assayag:** None. **R. Conti:** None. **M. Guillon:** None. **V. de Sars:** None. **V. Emiliani:** None.

Poster

263. G.04. Physiological Methods; G.04.a. Optical methods

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Topic: G.04. Physiological Methods

Support: Max Planck Society

Title: Unraveling circuit mechanisms: linking circuits components to behavioral parameters through 3D holographic optogenetics

Authors: ***J. C. DONOVAN**[1,2], M. DAL MASCHIO[1], H. BAIER[1];
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Abstract: Understanding how components within a neural circuit interact to drive behavior is a challenging task. Progress will be made through highly targeted probing of how activity in small numbers of neurons drives network activation, from the perspective of the circuit function, and how these activity patterns lead to behavioral outcomes. To investigate these aspects we have developed a 3-dimensional light shaping system based on computer generated holography, which

can be used to optogenetically activate identified neurons in a spatially precise manner across a circuit of interest, while minimizing contamination in the network activity originating from off-target or broad photostimulation effects . For the study of the neuronal circuits, this activation protocol is combined with simultaneous functional imaging of the network and high-speed behavioral recording, to link the activity patterns induced in the network with the relevant parameters of the triggered behavior. We have taken advantage of this approach to distill a minimal subset of neurons in the zebrafish central nervous system whose activation is sufficient to engage a characteristic motor output and to record simultaneously the circuit activity supporting such a motor program. Assembling together information from induced activation patterns, neural activity, and behavior outcomes provides identification of critical driving elements of the circuit and their functional roles.

Disclosures: J.C. Donovan: None. **M. Dal Maschio:** None. **H. Baier:** None.

Poster

263. G.04. Physiological Methods; G.04.a. Optical methods

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Title: Development of novel fast-response GCaMP6 sensors for monitoring neuronal action potential

Authors: N. HELASSA[1], B. PODOR[2], A. FINE[2], *K. TÖRÖK[1];
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Abstract: Green Fluorescent-Calmodulin Proteins (GCaMPs) have been the reporters of choice for visualizing neuronal network activity *in vivo*. GCaMPs are based on a circularly permutated EGFP molecule (cpEGFP) flanked at the N and C termini by the smooth muscle myosin light chain kinase derived RS20 peptide and calmodulin (CaM), respectively. Upon Ca^{2+} binding, the formation of a tight complex between RS20 and CaM induces a fluorescence enhancement. However, the slow Ca^{2+}-response kinetics of the current GCaMPs does not make them optimal for monitoring action potentials (AP). To accelerate the Ca^{2+} response kinetics of GCaMP6f, we decreased the binding affinity of the Ca^{2+}.CaM.RS20 complex by point mutations in the EF-hands of CaM[1] (mutants EF-1 to EF-4) and in the RS20 target peptide sequence[2] (mutant RS-1). Newly engineered GCaMP6f proteins were characterised *in vitro* in terms of dynamic range,

Ca^{2+} affinity and kinetics at 37°C. Dissociation constants (K_d) for Ca^{2+} obtained from equilibrium Ca^{2+} binding were in the μM range (0.1-3.3 μM) with Hill coefficients from 1.7 to 4.2. The GCaMP6f RS-1 EF-3 mutant had half times ($t_{1/2}$) for Ca^{2+} rise and decay of 1.3 and 2.8 ms, respectively, compared to 10 ms and 63 ms for GCaMP6f. GCaMP6f RS-1 EF-4 had $t_{1/2}$ for Ca^{2+} rise and decay of 3.3 and 3.5 ms, respectively, 3- and 18-fold faster than the corresponding values for GCaMP6f. Fluorescence changes on Ca^{2+} association were highly cooperative and characterized by a rate limiting conformational change. *In vivo* Ca^{2+} responses associated with AP firing patterns were tested in cultured hippocampal slices by two-photon imaging at 28°C. The performance of GCaMP6f RS-1 EF-3 and GCaMP6f RS-1 EF-4 was compared with that of GCaMP6f. Ca^{2+} decay kinetics were determined by monitoring fluorescence changes evoked by 5 AP fired at a 100 Hz. $t_{1/2}$ values for GCaMP6f RS-1 EF-3 and GCaMP6f RS-1 EF-4 were 58 ms and 126 ms, respectively. With an up to 7-fold faster kinetics than GCaMP6f, GCaMP6f RS-1 EF-3 and GCaMP6f RS-1 EF-4 are promising tools for monitoring brain activity *in vivo*.

Disclosures: **N. Helassa:** None. **B. Podor:** None. **A. Fine:** None. **K. Török:** None.

Poster

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Topic: G.04. Physiological Methods

Support: NIH 1-U01-NS090501-01

Title: Probing the retinal circuit with computer generated holography and two photon calcium imaging

Authors: ***G. L. SPAMPINATO**[1,2], E. RONZITTI[3,4], E. PAPAGIAKOUMOU[3,4], H. KHABOU[1,2], D. DALKARA[1,5], S. PICAUD[1,5], O. MARRE[1,5], V. EMILIANI[3,4];
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Abstract: Understanding how neurons integrate information from their inputs requires mapping the functional connections between two successive layers of a neural circuit. A method to systematically measure how the activity of one neuron will influence the next layer is still lacking. Here we have developed such a method by combining two photon computer generated holography and two photon calcium imaging, and tested it in the retina. The retina is an ideal system for studying information processing through different layers. The excitatory signals flow vertically from photoreceptors through the bipolar cells to the retinal ganglion cells, which send spikes down to the optic nerve to the brain. Here we focused on the information transmission between the bipolar and ganglion cell layers. We have designed an optical system combining two

photon computer generated holography stimulation of the bipolar cells and two photon calcium imaging of the ganglion cell layer. Using AAVs, we expressed Channelrhodopsin2 in ON bipolar cells of wild type mice and GCaMP6s in the ganglion cells. Single bipolar cells were stimulated with holographic spots, while imaging the responses of ganglion cells. Our method also allowed us to stimulate several cells simultaneously. Preliminary results showed that single bipolar cell stimulation can evoke unique activation patterns in the ganglion cell population. Our method paves the way to a comprehensive map of the functional connectivity between two layers, by reconstructing the projective field of each bipolar cell and the receptive field of each ganglion cell.

Disclosures: **G.L. Spampinato:** None. **E. Ronzitti:** None. **E. Papagiakoumou:** None. **H. Khabou:** None. **D. Dalkara:** None. **S. Picaud:** None. **O. Marre:** None. **V. Emiliani:** None.

Poster

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Title: Video-rate monitoring of calcium signals with HiLo microscopy

Authors: *E. RONZITTI[1], M. A. LAUTERBACH[1], J. STERNBERG[2,3,4,5], C. WYART[2,3,4,5], V. EMILIANI[1];
[1]Univ. Paris Descartes - CNRS, Paris, France; [2]Inst. du Cerveau et de la Moelle Épinière, Paris, France; [3]INSERM, Paris, France; [4]CNRS, Paris, France; [5]UPMC Univ., Paris, France

Abstract: Three-dimensional reconstruction capability and speed of image acquisition are two essential requirements in neuroscience to study the brain architecture and function *in vitro* and *in vivo*. Many efforts have been made to develop novel strategies and optical approaches that preserve the functional neuronal activity and allow for monitoring signal processing in 3D

networks. Structured illumination methods integrate 3D optical sectioning capabilities with widefield microscopy, generating configurations simple to construct, low-cost, versatile and enabling high acquisition speeds. In particular, the strategy of generating a speckle patterned illumination adopted in the HiLo method offers an optically robust and versatile approach to be implemented on a wide-field fluorescence microscope for three dimensional functional imaging in thick samples. In the present study, we use HiLo microscopy configured in a fast acquisition scheme to record calcium signals from living Zebrafish larva. This approach allows for monitoring calcium activity generated by specific neurons located at different depths, offering an affordable solution for monitoring at high acquisition rates the activity of large ensemble of neurons at subcellular resolution.

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Poster

264. Optical Methods

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Topic: G.04. Physiological Methods

Title: Design and evaluation of an all-optical electrophysiology workstation integrating two-photon imaging, two-photon digital holographic photostimulation, and temporal focusing

Authors: *K. KILBORN[1], O. ASSAYAG[2];
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Abstract: Computer-generated holography (CGH) offers a novel and powerful means to deliver light in a spatially precise manner throughout a 3D volume of tissue for uncaging, optogenetic stimulation, and other photomanipulation applications. A particularly promising use of CGH is to enable “all-optical” electrophysiology where stimulating electrodes are replaced by 3D illumination patterns and recording electrodes are replaced by the expression of genetically-encoded activity reporters in the target neurons of interest. This combination enables significantly greater parallelism in both stimulation and recording, allowing one to investigate circuit-level connectivity, integration of many afferents, and elucidate neural algorithms in a way that is difficult with conventional methods at the circuit scale. Key design factors in such a system are the ability to selectively stimulate individual neurons in a volumetric field of excitation sufficiently large enough to encompass the circuit of interest, the ability to image neurons in the same (or potentially larger) volumetric field of view with sufficient signal-to-noise to detect single-cell responses, and the ability to synchronize stimulation and recording. By using a two-photon source with digital holography, it is possible to stimulate deeper in tissue and with greater axial confinement than with a one-photon source. The addition of temporal focusing

(TF) further improves axial confinement, particularly when the lateral extent of the region of illumination is large (on the order of one or multiple cell bodies). A system is presented which combines CGH and TF with two-photon resonant scanning and arbitrary 3D curve scanning to obtain a 500um x 500um x 500um volume of excitation and view with single-cell resolution. In order to achieve the maximum flexibility in configuring all-optical experiments, it is beneficial for the stimulation and acquisition to be tightly coordinated. The system presented provides such coordination, including facilities to automatically identify stimulation targets from imaging activity, using the image data to directly feed the stimulation patterns in a closed-loop fashion.

Disclosures: **K. Kilborn:** A. Employment/Salary (full or part-time):; 3i. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 3i. **O. Assayag:** A. Employment/Salary (full or part-time):; 3i.

Poster

264. Optical Methods

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Support: Agence Nationale de la Recherche (grant ANR-10-INSB-04-01, France-BioImaging Infrastructure Network)

FRC and the Rotary Club program “Espoir en Tête” 2012

National Science Foundation International Graduate Research Fellowship

Title: Phase-based light shaping improves the spatial specificity of calcium and voltage fluorescence functional imaging

Authors: ***V. ZAMPINI**[1], D. TANESE[1], A. J. FOUST[1], E. PAPAGIAKOUMOU[1], M. CANEPARI[2], V. EMILIANI[1];
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Abstract: Calcium and voltage sensitive reporters have the potential to revolutionize our understanding of the neuronal electrical communications. Calcium and voltage changes can track neuronal activity simultaneously in neuronal compartments difficult to access with standard electrophysiological patch-clamp techniques. However, an efficient epifluorescence detection of rapid fluorescent transients in neighboring labeled structures is limited by contamination of out-of-focus and scattered light. To overcome this limitation, we used phase modulation approaches to distribute light excitation on user-defined regions. This method enables illumination of axons, dendrites and spines of interest to detect calcium and voltage fluorescence changes, without

exciting surrounding structures, thus achieving high spatial specificity while maximizing collected photon flux and signal-to-noise ratio. In CA1 hippocampal pyramidal neurons loaded with a low affinity calcium indicator (OGB488-5N, 1 mM), calcium fluorescent changes correlated with action potentials imaged by shaping light onto neighboring axons and dendrites. Neighboring axons and dendrites of neurons loaded with the voltage dye JPW3028 were also illuminated with shaped light. We observed different kinetics in dendritic back-propagating compared with axonal action potentials, differences not measured in the same structures illuminated with a large "pseudo-widefield" spot of the same excitation density. Shaped illumination reduced baseline fluorescence, increased fractional fluorescence transient amplitudes, and improved spatial discrimination compared to trials acquired with pseudo-widefield illumination of the same regions. In conjunction with the rapidly expanding toolbox of genetically encoded voltage and calcium reporters, light shaping can enable high signal to noise ratio and parallel detection of neural-evoked fluorescence transients from neighboring cells or subcellular compartments in regions with dense expression of genetically targeted fluorescent proteins.

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Poster

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Topic: G.04. Physiological Methods

Support: NIH Grant 1-U01-NS090501-01

ANR-12-BSV5-0011

Title: Three-dimensional simultaneous photoconversion of neuronal ensembles with single-cell resolution

Authors: O. HERNANDEZ[1], E. PAPAGIAKOUMOU[1], C. WYART[2,3,4,5], *V. EMILIANI[1]; [1]Neurophotonics Lab., CNRS-University Paris Descartes, Paris, France; [2]Inst. du Cerveau et de la Moelle Épinière (ICM), Paris, France; [3]INSERM, Paris, France; [4]CNRS, Paris, France; [5]UPMC, Paris, France

Abstract: Genetically encoded light-sensitive channels and reporters enable both neuronal activity optical control and read-out. Full exploitation of these optogenetics tools requires single-cell scale methods to pattern light into neural tissue. Computer Generated Holography can powerfully enhance optogenetics stimulation by efficiently shaping light onto multiple cellular targets. However, a linear proportionality between lateral shape area and axial extent degrades

axial precision for cases demanding extended lateral patterning i.e., to cover entire soma of multiple cells. To address this limitation, we previously combined computer generated holography with temporal focusing to stretch laser pulses outside of the focal plane, which combined with two-photon's nonlinear fluorescence dependence, axially confines fluorescence regardless of lateral extent. However, this configuration restricts nonlinear excitation to a single spatiotemporal focal plane, precluding simultaneous confinement of axially separated light patterns. Here, we report an optical system enabling remote axial displacement of temporally focused holographic patterns, as well as generation of multiple temporally focused holographic targets occupying separate axial planes. The capabilities of the system for axially confined multi-plane illumination are demonstrated by photoconverting, with single-cell resolution, tens of Kaede protein expressing neurons occupying separate axial planes in live zebrafish larvae.

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Poster

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Support: NIH

HHMI

Private Foundations

Title: Optogenetic, calcium-sensing, and chemogenetic mouse models available from the JAX repository

Authors: *M. SASNER, S. F. ROCKWOOD, J. BECKWITH;
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Abstract: The Jackson Laboratory (JAX) Repository distributes mouse lines with optogenetic and calcium-sensing technologies. Opsins are light-activated proteins that alter membrane potential in neurons, so that light stimulation allows control of neuronal activity. Several mouse models express improved/optimized opsins fused to fluorescent proteins, including channelrhodopsin expression directed by specific promoters, as well as Cre-dependent expression of archaerhodopsin, channelrhodopsin or halorhodopsin variants. Variants of GCaMP fluoresce in response to calcium binding, serving as an indication of neuronal activation. These include *Thy1*-promoter driven GCaMP3, GCaMP6f or GCaMP6s transgenic, Tet-dependent GCaMP6s transgenic, and Cre-dependent GCaMP3, GCaMP6f or GCaMP6s expressing mouse

lines. Several strains utilize both Cre-lox and Tet-On/-Off systems. Removal of a floxed-STOP allows Tet-dependent expression of channelrhodopsin (Chronos/EGFP), halorhodopsin (Jaws/EGFP), GCaMP6s, GCaMP6f or a voltage-sensitive FRET chromophore. 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 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) in interacting lineages. 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- or Tet-inducible expression of hM3Dq, hM4Di or rM3Ds. Search Repository holdings using the newly designed JAXMice webpage (*jaxmice.jax.org/query).* To donate mouse strains, use our online submission form *(jax.org/donate-a-mouse*). For a list of newly added models and detailed information, visit our resources for Optogenetics web page at *research.jax.org/grs/optogenetics.html.*

Disclosures: M. Sasner: None. **S.F. Rockwood:** None. **J. Beckwith:** None.

Poster

264. Optical Methods

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Topic: G.04. Physiological Methods

Support: Supported by Howard Hughes Medical Institute

Title: Improved red fluorescent genetically-encoded calcium indicators for *in vitro* imaging

Authors: *H. DANA[1], Y. SUN[1], J. P. HASSEMAN[1], G. TSEGAYE[1], E. R. SCHREITER[1], B.-J. LIN[1], S. D. BRENOWITZ[1], B. MOHAR[1,2], V. JAYARAMAN[1], L. L. LOOGER[1], K. SVOBODA[1], D. S. KIM[1];
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Abstract: Imaging neural activity using genetically-encoded calcium indicators (GECIs) is a widely used method for *in vivo* neurophysiology. GECIs allow tracking of activity in large populations of neurons, measurements from genetically-defined neuronal cell types, and imaging of small neuronal compartments. The most popular GECIs are based on GFP, and their emission spectra are therfore in the green range of the wavelength spectrum (green GECIs). Red GECIs could have advantages for *in vivo* imaging because of reduced absorption and scattering losses in intact tissue. However, so far the molecular properties of red GECIs are inferior to green GECIs

for *in vivo* imaging of activity. Here we present improved red GECIs, jRCaMP1 (based on mRuby) and jRGECO1 (based on mApple), with performance that is comparable to state-of-the-art green GECIs. We characterize the properties of the new red GECIs in cultured neurons and *in vivo*. We further compare the performance of a variety of red and green indicators for population imaging in the visual cortex *in vivo*. Finally, we discuss the limitations of jRCaMP1 and jRGECO1, and test their compatibility with other optical indicators and effectors.

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Poster

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Topic: G.04. Physiological Methods

Support: Howard Hughes Medical Institute

Title: Multi-color genetically-encoded calcium indicators for fast-spiking neurons in *Drosophila*

Authors: ***Y. SUN**, D. HOD, J. P. HASSEMAN, G. TSEGAYE, G. HOLT, E. R. SCHREITER, A. NERN, M. REISER, K. SVOBODA, L. L. LOOGER, V. JAYARAMAN, D. S. KIM; Howard Hughes Med. Inst., HHMI Janelia, Ashburn, VA

Abstract: Genetically-encoded calcium indicators (GECIs) are preferred probes for optical neurophysiology in genetically modifiable model organisms. Current GECIs (e.g. GCaMP6) are optimized for action potential (AP) detection in the low firing rate range (1-50 Hz) and have high calcium affinities (low Kd). The high Hill coefficient of these indicators (2-3 for GCaMP6) narrows their linear range, and their slow decay kinetics limit their ability to track rapid changes in activity that are typical of several types of non-spiking and fast-spiking neurons found in *Drosophila* and other invertebrates. We are developing red and green GECIs for simultaneously monitoring the activity of intermingled populations of distinct classes of such neurons. We mutated GCaMP6 and the high-affinity red fluorescent sensors jRCaMP1 and jRGECO1, screened for useful low-affinity variants in *Drosophila* larval neuromuscular junction (NMJ), and identified GCaMP, RCaMP and RGECO variants with improved calcium detection. In the NMJ these indicators responded monotonically to spike rates from 1Hz to 160Hz, with improved linearity and faster kinetics compared to parent sensors. They enable reliable single trial imaging of neurons across a wide range of firing rates in behaving animals.

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Poster

264. Optical Methods

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Topic: G.04. Physiological Methods

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DARPA Neuro-FAST program

Gatsby Foundation

Title: Multisite two-photon three-dimensional random access *in vitro* calcium imaging

Authors: ***S. J. YANG**[1], W. ALLEN[2], I. V. KAUVAR[1], A. ANDALMAN[3], N. YOUNG[3], C. K. KIM[2], K. DEISSEROTH[3,4,5];
[1]Electrical Engin., Stanford, CA; [2]Neurosci. Program, [3]Bioengineering, [4]Psychiatry and Behavioral Sci., [5]Howard Hughes Med. Inst., Stanford Univ., Stanford, CA

Abstract: A major goal in neurophysiology is to record the activity of large ensembles of neurons simultaneously in awake, behaving animals. This problem is challenging for three dimensional volumes of neurons in scattering tissue. Current approaches to three-dimensional *in vivo* calcium imaging in scattering mammalian tissue utilize multi-photon single-point random access scanning techniques. However, such systems require acousto-optic scanners in order to sample the calcium sensor fast enough and inherently trade off the number of sites recorded against signal acquisition time per site, since each site is visited sequentially. Alternative approaches utilize spatial light modulators in an attempt to overcome this limitation by simultaneously illuminating all sites. However these simultaneous illumination techniques have been limited to ~100 sites in a less-scattering medium, and have not been demonstrated to work *in vivo* or to scale to large neuronal populations. Here we demonstrate multisite random access three-dimensional *in vivo* recording of neural activity in scattering tissue volumes using multifocal spatial-light-modulator-based two-photon illumination and coded detection using an sCMOS camera. Richardson-Lucy deconvolution allows for the reconstruction of cellular activity traces from coded images. We further scale the system by utilizing a regenerative amplifier modified to operate at 920 nm to overcome laser power limitations. The technique operates without fast scanners and is relatively easily implemented. We are able to sample calcium signals at 10 Hz from 106 locations through a cranial window over S1 barrel cortex in head-fixed mice (N=2) virally transduced with GCaMP6m (AAVdj-Camk2a-GCaMP6m)

undergoing whisker stimulation. This technique represents a promising approach to recording the activity of large neuronal populations in three dimensions in the cortex of awake, behaving animals; with further *in vivo* testing and optimization of single-cell spatial resolution, this approach may become a simpler alternative to fast single-point scanning approaches.

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Poster

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R01EY011787

R01MH101218

R41MH100895

DARPA W91NF-14-1-0269

ARO W911NF-12-1-0594 (MURI)

Title: Simultaneous 3-D optogenetics and volumetric imaging on neural circuits *in vitro*

Authors: W. YANG, L. CARILLO-REID, J.-E. K. MILLER, R. YUSTE, *D. S. PETERKA; Columbia Univ., New York, NY

Abstract: Optical recording and manipulation of *in vivo* neural circuits with cellular resolution could be important for understanding cortical function. Despite recent progress, simultaneous optogenetic activation with cellular precision has either been limited to 2-D planes, or a very small number of neurons, and rarely with simultaneous optical imaging. Here we demonstrate a novel paradigm for simultaneous 3D activation and imaging using a dual-laser two-photon microscope. The microscope utilizes a low repetition rate pulse-amplified fiber laser system and a spatial light modulator (SLM) to project 3-D holographic excitation patterns on the cortex of mice *in vivo* for targeted 3-D photoactivation. High speed volumetric imaging is simultaneously performed with a resonance scanner, and the imaging depth controlled by either an electrotunable lens or another SLM. This allows for remote focusing without moving the microscope objective. The system enables simultaneous activation of at least 50 cells simultaneously, using red-shifted opsins, such as C1V1 or ReaChR, while simultaneously

imaging GFP-based sensors such as GCaMP6. This all-optical 3D imaging and manipulation approach achieves simultaneous reading and writing of cortical activity, and should be a powerful tool for the study of neuronal circuits.

Disclosures: W. Yang: None. **L. Carillo-Reid:** None. **J.K. Miller:** None. **R. Yuste:** None. **D.S. Peterka:** None.

Poster

264. Optical Methods

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Topic: G.04. Physiological Methods

Title: Modulation of spinal motoneurons recruitment by mechanosensory feedback during ongoing locomotion

Authors: *S. KNAFO[1], C. DICKEY[2], K. FIDELIN[2], U. BÖHM[2], H. PASCAL-MOUSSELARD[2], C. WYART[2];
[1]Inst. Du Cerveau Et De La Moelle Épinière, Paris, France; [2]Inst. du cerveau et de la Moelle épinière, Paris, France

Abstract: Sensorimotor integration is by definition a closed-loop process, during which mechanosensory feedback modulates ongoing locomotor activity. Traditional “fictive preparations”, in which the spinal cord is isolated and/or the animal paralyzed, provides an “open-loop” access to spinal circuits deprived of muscle contraction and subsequent mechanosensory feedback. Here we developed a bioluminescent approach for monitoring the activity of motor or sensory neurons in the spinal cord during ongoing active locomotion. We estimated the global neural activity of genetically targeted populations of spinal neurons using the bioluminescent reporter GFP-aequorin during auditory-vestibular evoked escapes using a high-speed infrared camera in zebrafish larvae. The intensity of recruitment of spinal motoneurons during active escapes was associated with specific maneuvers: larger for escapes only, than escapes followed by a slow swim and then slow swims. In open-loop fictive locomotion, recruitment of spinal motoneurons was slower and the decay constant of their bioluminescence signal was longer compared to closed-loop active behaviors, suggesting that sensory feedback modulates the recruitment of spinal motoneurons. Using transgenic lines in which GFP-aequorin expression was restricted to different mechanosensory cell types, we showed which spinal sensory neurons were specifically recruited during active locomotion but not during fictive recordings. Using GCaMP-based calcium imaging, we confirmed that sensory cells were differentially recruited during active bending of the tail but not in paralyzed preparations during auditory-vestibular evoked escapes. Hence, we propose an intra-spinal closed-loop neural circuit including intraspinal sensory neurons, which would be mechanically

recruited and, in turn, would modulate the intensity and timing of motoneurons recruitment in the moving spinal cord. These results show a differential recruitment of motor and sensory spinal neurons during active compared to fictive locomotion using genetically targeted reporters emphasizing the importance of spinal sensorimotor integration during active locomotion.

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Poster

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Topic: G.04. Physiological Methods

Title: Single emitter light distribution in homogenous brain tissue for optogenetic applications

Authors: D. ANGELEY, *I. D. GALLAGER, D. ANDERSEN;
Behavioral Neurosci., Circuit Therapeut., Menlo Park, CA

Abstract: The actual distribution of visible light from a single fiber optic probe embedded in the brain has yet to be reported, although its knowledge is central to the accuracy of in-vivo optogenetic targeting, experimental planning and subsequent data analysis. Monte Carlo simulations of optical transport within tissue were performed using a 3-dimensional Henyey-Greenstein volume scatter model. Isotropic detection probes and light delivery fibers were deployed in-vivo by stereotaxic means into the Caudate Putamen (striatum) of rats and into homogeneous regions of ex-vivo fresh porcine brain for experimental validation. To measure light spread in-vivo, the isotropic detection probe was placed in the posterior region of the tissue and the light delivery fiber was inserted anteriorly at a set of predetermined x-y-z locations from distal to proximal. Laser wavelengths of 473, 588, and 635nm were used, as were a range of delivery fiber core diameters and numerical apertures. Measurements validated the modeling of optical transport. Importantly, peak fluence rates are located distal to the delivery fiber output and increase with wavelength. The distribution of light is predominantly spherical at distances from the fiber output greater than ~1mm. The fluence rate distribution is dependent upon the amount of scattering and absorption, and the wavelength used. Equivalent irradiance contours may be determined, and exposed tissue volumes calculated thereby. For example, a 200μm, 0.22NA fiber delivering 10mW of 473nm light into homogenous grey matter with 2% blood volume fraction creates exposure volumes of 15, 5, 0.3mm3 for irradiance thresholds of 0.4, 1, and 10mW/mm2 respectively. The distribution of light is predominantly spherical at distances that are “optically far” from the source (i.e. in the diffusion regime), after which incident directionality is lost. Furthermore, the fiber core diameter and numerical aperture have negligible impact upon the distribution. These findings may significantly influence results observed in

optogenetic behavioral testing and great consideration should be placed on the potential collateral (extracircuital) effects due to viral and light spread within the brain. Experimenters should therefore attempt to minimize the amount of light utilized within *in vivo* experimentation.

Disclosures: **D. Angeley:** A. Employment/Salary (full or part-time):; Circuit Therapeutics. **I.D. Gallager:** A. Employment/Salary (full or part-time):; Circuit Therapeutics. **D. Andersen:** A. Employment/Salary (full or part-time):; Circuit Therapeutics.

Poster

264. Optical Methods

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Program#/Poster#: 264.13/BB7

Topic: G.04. Physiological Methods

Title: Novel organic light emitting diodes for optogenetic experiments

Authors: ***A. SHAH**, A. SRIDHARAN, J. SMITH, J. CHRISTEN, J. MUTHUSWAMY; Neural Microsystems Laboratory,, Arizona State Univ., Tempe, AZ

Abstract: Optogenetics Current LED technologies are not readily scalable to high-throughput neural stimulation arrays due to power and interconnect constraints. In contrast, OLEDs offer distinct advantages such as monolithic fabrication, flexible substrate, power efficient multi-arraying, and orders of magnitude improvement in interconnect density. For the above reasons, OLEDs are a low cost and a high throughput technology. OLEDs reported here were fabricated at ASU's Flexible Display Center. We present *in vivo* results for blue OLEDs, and *in vitro* results for blue and green OLEDs. For *in vivo* testing, the light out of the flat OLED panel was collected into a collimating optical system and focused into the optical fiber. The optical fiber was then used for stimulating neurons in layer 5 of the motor cortex in transgenic mice expressing ChR2(B6.Cg-Tg(Thy1-ChR2/EYFP)9Gfng/J). EMGs evoked in response to electrical/optical stimulation were recorded from the contralateral vastus lateralis muscles. *In vitro* testing of the OLEDs was done in primary cortical neurons in culture transfected with blue light sensitive ChR2 and green light sensitive C1V1tt in separate cultures. The neurons were cultured on a microelectrode array for neuronal recordings. Statistically signicant ($p<0.01$) increase in neural activity was recorded in comparison to the baseline activity. Keywords - neuronal stimulation, activation, microelectrode arrays, light stimulation

Disclosures: **A. Shah:** None. **A. Sridharan:** None. **J. Smith:** None. **J. Christen:** None. **J. Muthuswamy:** None.

Poster

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Program#/Poster#: 264.14/BB8

Topic: G.04. Physiological Methods

Title: Nanomachined tapered optical fibers for optogenetics

Authors: *M. DE VITTORIO[1,3], F. PISANELLO[1], L. SILEO[1], M. PISANELLO[1,3], I. A. OLDENBURG[4], A. DELLA PATRIA[1], J. A. ASSAD[5,2], B. L. SABATINI[4];
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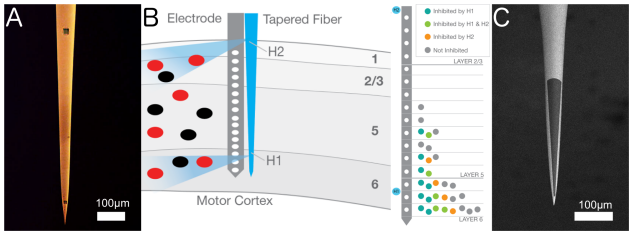
Abstract: Optogenetic control of neural activity is a powerful strategy for causal investigation of neural circuitry. In order to simultaneously control and monitor neural activity in deep brain regions, it is mandatory to develop new light delivery/collection methods and technologies with high spatio-temporal resolution and low-invasiveness. Here we review emerging technologies and new strategies exploited for the experimental in-vivo probing of optogenetic activity for both open-loop and closed loop control of neural circuitry [Grosenick et al, Neuron 86, 106 (2015)]. Special emphasis is given to nanomachined tapered optical fibers, a successfully applied technology for in-vivo light multipoint-emission [Pisanello et al, Neuron 82, 1245 (2014)]. A focused ion beam is used to pattern multiple light windows on the tapered and gold coated section of the fiber to produce independently addressable light sources, slotted emission patterns and light collection windows. Selective and dynamic illumination of different brain regions along the taper have been demonstrated in the mammalian brain *in vivo* by coupling the fiber to a microelectrode array and performing simultaneous extracellular recording and stimulation at multiple sites in the mouse striatum and cerebral cortex with minimal invasiveness.



Disclosures: **M. De Vittorio:** None. **F. Pisanello:** None. **L. Sileo:** None. **M. Pisanello:** None. **I.A. Oldenburg:** None. **A. Della Patria:** None. **J.A. Assad:** None. **B.L. Sabatini:** None.

Poster

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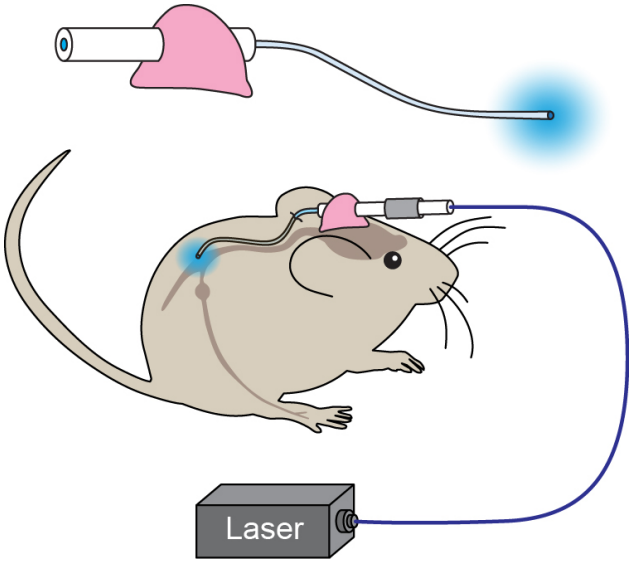
Louise and Alan Edwards Foundation postdoctoral fellowship

Title: Epidural optic fiber implant for spinal optogenetics

Authors: *R. P. BONIN[1], F. WANG[2], M. DESROCHERS-COUTURE[3], M.-E. BOULANGER[4], A. GASECKA[4], D. COTE[4], Y. DE KONINCK[2];
[1]Leslie Dan Fac. of Pharm., Univ. of Toronto, Toronto, ON, Canada; [2]Psychiatry, [3]Psychology, [4]Physics, Laval Univ., Quebec, QC, Canada

Abstract: Optogenetic tools enable the use of light to detect and control neuronal activity with remarkable temporal and cellular precision. Yet, the difficulty in delivering light to the spinal cord of awake, behaving animals has hampered the use of optogenetics in the study of spinal sensory processing. Accordingly, we have developed an epidural optic fiber implant that allows the delivery of light to the spinal cord dorsal horn and dorsal roots of sensory afferents. The epidural optic fiber was implanted in adult mice that expressed the light-activated inhibitory opsin ArchT in Nav1.8-expressing nociceptive afferents (Nav1.8-ArchT) and in wildtype C57Bl/6 mice. Amber light (592 nm) inhibited acute thermal nociception in the Nav1.8-ArchT mice, but did not affect nociception in C57Bl/6 mice. The acute delivery of blue light (488 nm) to the lumbar spinal cord of mice that selectively express channelrhodopsin (ChR2) in Nav1.8+ afferents (Nav1.8-ChR2) produced nocifensive behaviour, while repeated activation of Nav1.8+ afferents in these mice induced long-lasting mechanical hyperalgesia. Finally, inhibition of ArchT-expressing GABAergic interneurons in the lumbar spinal cord induced mechanical but not thermal hyperalgesia. Together, these results demonstrate the utility of our epidural optic fiber for the optogenetic modulation of both sensory afferents and intrinsic spinal cord neurons, permitting studies of activity-dependent nociceptive sensitization and the manipulation of sensory processing pathways within the spinal cord dorsal horn.



Disclosures: **R.P. Bonin:** None. **F. Wang:** None. **M. Desrochers-Couture:** None. **M. Boulanger:** None. **A. Gasecka:** None. **D. Cote:** None. **Y. De Koninck:** None.

Poster

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Title: Tapered fiber optical waveguide for homogeneous *in vivo* light delivery in extended brain structures

Authors: ***F. PISANELLO**[1], I. A. OLDENBURG[2], L. SILEO[1], M. S. EMARA[1,3], A. DELLA PATRIA[1], G. MANDELBAUM[2], M. PISANELLO[1,3], B. SPAGNOLO[1,3], B. L. SABATINI[2], M. DE VITTORIO[1,3];
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Abstract: Although genetic techniques allow targeting well-defined neuronal sub-populations with light-sensitive proteins, most *in vivo* optogenetic experiments are limited by the use of

optical fibers with flat cleaved ends. With such devices light is emitted to a relatively small volume near the fiber facet. Moreover, light delivered into the brain undergoes to both tissue absorption and scattering [Yizar at al, Neuron 71, 9 (2011)], resulting in an exponential decrease of light intensity from the source and uneven illumination. Multipoint-emission tapered fibers [Pisanello et al, Neuron 82, 1245 (2014)] and other approaches [Grosenick et al, Neuron 86, 106 (2015)] can broaden the stimulated area; however, a viable strategy for homogenous illumination of extended brain structures has not yet been developed. Here we present a tapered fiber configuration that redefines the light delivery geometry in the living brain. The tapered fiber, having a very small taper angle (<3°), is designed to gradually and homogenously deliver light along a taper segment that can be tuned from a few hundreds of micrometers up to ~2mm. This is achieved by virtue of gradual loss of the total internal reflection condition along the taper, which acts as a spatial demultiplexer of the optical modes guided into the fiber. Moreover, the sub-micrometer tip (~500nm) allows for smooth insertion into the brain, greatly reducing the overall invasiveness compared to standard light delivery systems. To demonstrate the suitability of this approach for a more uniform illumination of extended brain structures, we have performed tests in the VGAT-ChR2 mouse line, with ChR2 expressed in all inhibitory interneurons. Tapered fibers were used to activate inhibitory neurons along the whole depth of motor cortex, while measuring the evoked effect on cortical neurons. Experiments on several animals show that the light emission geometry of the here-proposed tapered fiber permits sustained inhibition of neural activity at delivered powers 5-times smaller than the threshold recorded for standard flat-cleaved fibers. Moreover, by changing the input angle at the other end of the fiber [Pisanello et al, Neuron 82, 1245 (2014)], tapered fibers also allow the sub-sampling of the region of interest by illuminating only well-defined sub-portion of tissue within the total allowed emission length.

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Poster

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EUROPEAN RESEARCH COUNCIL (ERC-2013-CoG-615699_NEUROKINEMATIKS, D.R.)

Title: A miniature wireless device for movement-triggered deep-brain optical stimulation in rats

Authors: *M. PASQUET, L. PETIT, D. ROBBE;
INMED, MARSEILLE, France

Abstract: Wirelessly controlled optogenetic stimulation systems are now currently available to perform in-vivo neuroscience research on freely behaving animals. Such devices allow performing deep brain stimulation while ensuring a better ethological experimental environment. The production of controlled movements in response to task-specific constraints is one of the fundamental outcomes of brain activity. The understanding of the neuronal basis of motor control would greatly benefit of techniques allowing to deliver selective perturbation of neuronal circuits at specific phases of movement production. In parallel to the stimulation devices evolution, wireless motion quantification devices are now commonly available. They brought unconstrained experimental possibilities and enable many applications in animal research. They are offering the possibility of quantifying, classifying or detecting efficiently all sorts of motion-related elements ranging from simple motion to complex animal behavioral sequences. Despite the interest of both technologies, an all integrated miniaturized headborne solution for performing real-time motion-triggered closed-loop deep-brain optical stimulation in unrestrained laboratory animals such as rats is still lacking. We are developing a miniature wireless device for performing motion-related closed-loop deep-brain optical stimulation on rats. We use a multi-axis movement sensor for motion quantification, a microcontroller performs real-time movement analysis and stimulation triggering, finally the optical deep-brain stimulation function relies on a fibered-LED implant. The motion sensing and computation assembly is coupled to the optical implant by a precisely machined device performing electrical and mechanical connections. The complete system can be worn on the head of a rat and controlled using a Bluetooth link. Both motion analysis and stimulation control are done by a single microcontroller which ensures real-time triggering of the stimulation. Overall, our system has the potential to offer an association between interactive motion sensing functions and deep-brain stimulation in a single device, headborne, and unleashed from many mechanical constraints. We believe that our system will enable all sorts of novel closed-loop motion based experiments on freely moving animals.

Disclosures: **M. Pasquet:** None. **L. Petit:** None. **D. Robbe:** None.

Poster

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EU FP7/2007-2013 n°600925

Title: LED-based neural probes for optogenetics

Authors: ***M. SCHWAERZLE**, F. POTHOF, O. PAUL, P. RUTHER;
Univ. of Freiburg, Freiburg, Germany

Abstract: This paper presents the design, fabrication as well as optical and thermal characterization of miniaturized optrodes with integrated light sources for optogenetic research. Light emitting diode (LED) chips are integrated in cylindrical polyimide (PI) based depth probes [1] or combined with miniaturized Si housings and optical fibers into MEMS-based miniaturized light sources [2,3]. The direct integration of bare LED chips into the neural systems reduces the overall system size and avoids mechanically stiff optical fibers interfacing with external light sources. This facilitates *in vivo* experiments with freely behaving animals. The all-electrical system control enables its integration into wireless headstages. The 40-mm-long depth probe comprises macroelectrodes as well as recording sites with a diameter of 30 µm. Bare LED chips are integrated within this cylindrical probe and electrically connected via a separate PI-based cable. The optical characterization revealed a radiant emittance of 1.03 mW/mm² measured through an aperture of 235 µm (driving current 45 mA; duty cycle 10 %). The thermal behavior is evaluated as a function of duty cycle using an infrared camera. The temperature increase at a duty cycle of 5 % is measured to be below 2.5 K when the depth probe is in contact with a brain phantom. The miniaturized, MEMS-based light sources enable a localized illumination at up to 3×3 independently controlled spots using individual LED chips (size 230×270 μm^2). They are integrated into micromachined Si housings ensuring mechanical stability and their precise alignment with respect to 5-mm-long optical fibers transmitting the light into the neural tissue. A highly flexible PI cable interfaces the LEDs electrically. With housings with volumes smaller than 2.2 mm³ and an optical output intensity of 2.1 mW/mm² at 10 mA and 30 % duty cycle, the 3×3 array is suitable for optogenetic applications [4]. As these devices are fixed on the skull, the temperature increase below 2.2 K is rated uncritical for chronic applications. **References** [1] F. Pothof, et al., *Proc. IEEE EMBS Conf. 2014*, pp. 5244-5247 [2] M. Schwaerzle, et al., *Proc. IEEE EMBS Conf. 2014*, pp. 5252-5255 [3] M. Schwaerzle, et al., *Proc. IEEE Micro Electro Mechanical Systems (MEMS) Conf. 2015*, pp. 162-165 [4] O. Yizhar, et al., *Neuron*, vol. 71, pp. 9-34, 2011

Disclosures: **M. Schwaerzle:** None. **F. Pothof:** None. **O. Paul:** None. **P. Ruther:** None.

Poster

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Topic: G.04. Physiological Methods

Support: BBSRC BB/K016830/1

EPSRC EP/I029141/1

SU2P pilot project

Title: Microscale LED probe for massively parallel optogenetic control at depth

Authors: *S. SAKATA[1], R. SHARF[2], N. MCALINDEN[2], T. TSUNEMATSU[1], E. GU[2], M. D. DAWSON[2], K. MATHIESON[2];
[1]SIPBS, Univ. of Strathclyde, Glasgow, United Kingdom; [2]The Inst. of Photonics, Univ. of Strathclyde, Glasgow, United Kingdom

Abstract: Control of neural activity is a powerful approach to uncover a causal link between neural activity and behavior. Although optogenetics offers innovative approaches to achieve this in a cell-type-specific manner with millisecond precision, it is still challenging to activate cell populations at multiple depths *in vivo*. Here we present two types of microscale light-emitted diode (μLED) array for *in vivo* optogenetic stimulation: sapphire-based and silicon-based μLED arrays. Probes were designed to contain up to 96 μLEDs, which are independently controllable and emit at 450 nm wavelength with an irradiance of up to 2 W/mm^2. To minimize tissue damage, especially the silicon μLED probe has a compact design, with 100 μm width and 30 μm thickness. Thermal properties permit standard operation regimes with minimum temperature increase (<0.5 °C). We confirmed that μLED probes can achieve depth-dependent activation of mouse neocortical neurons *in vivo*. Our devices offer a new optogenetic toolbox to achieve parallel control of neural populations at multiple depths *in vivo*.

Disclosures: **S. Sakata:** None. **R. Sharf:** None. **N. McAlinden:** None. **T. Tsunematsu:** None. **E. Gu:** None. **M.D. Dawson:** None. **K. Mathieson:** None.

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Support: NIH R01 EY018957

NIH P30 EY003176

NIH 5P30EY003176-32

NIH PN2 EY018241

Title: A comprehensive optogenetic toolkit for *in vitro* control of endogenous GABA(A) receptors and synaptic inhibition

Authors: *M.-C. TSAI[1], W.-C. LIN[1], H. ADESNIK[1,2], R. KRAMER[1,2];
[1]Mol. & Cell Biol., Univ. of California, Berkeley, Berkeley, CA; [2]Helen Wills Neurosci. Inst., Berkeley, CA

Abstract: Optogenetic tools are revolutionizing neuroscience. They make it possible to remotely stimulate or inhibit firing of genetically selected neurons and brain regions and thus define their roles in brain circuits and behavior. Since the flow of information through neural circuits depends on synapses, an important next technological step is to bring optogenetic control to the neurotransmitter receptors that underlie synaptic transmission. Our new approach of Optogenetic Pharmacology makes this possible. Here we show that we can confer light-sensitivity on selected isoforms of the GABAa receptors by genetically-engineering the receptors to couple with synthetic Photoswitchable Tethered Ligands (PTLs). We validate in both *ex vivo* preparations and *in vivo* recordings from mouse brain the stable and repeatable photo-control of GABAa receptor-mediated synaptic inhibition. In brain slices, we show that we can photo-antagonize postsynaptic GABAa receptor currents by up to 60%. *In vivo* recordings from visual cortex show that photoswitching GABAa receptors caused a two-fold change of interneuron spike activities in response to visual stimuli. This new technology allows optical control of inhibitory transmission in the living brain for studies of synaptic mechanisms, neural circuit mechanisms, and behavior.

Disclosures: **M. Tsai:** None. **W. Lin:** None. **H. Adesnik:** None. **R. Kramer:** None.

Poster

264. Optical Methods

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: G.04. Physiological Methods

Support: DFG SFB1078, SPP1165, Cluster of Excellence UniCat

IWT

Title: The rhodopsin-guanylyl cyclase of the aquatic fungus Blastocladiella emersonii enables fast optical control of cGMP signaling

Authors: *C. E. GEE[1], U. SCHEIB[2], K. STEHFEST[2], R. FUDIM[2], H. KOERSCHEN[3], P. HEGEMANN[2], T. G. OERTNER[4];
[1]Inst. For Synaptic Physiol., Hamburg, Germany; [2]Humboldt-Universität, Berlin, Germany; [3]Ctr. of Advanced European Studies and Res. (caesar), Bonn, Germany; [4]Ctr. for Mol. Neurobio. Hamburg (ZMNH), Hamburg, Germany

Abstract: Blastocladiomycota fungi form motile zoospores that are guided by sensory photoreceptors to optimal light conditions. Recently, a single rhodopsin coupled to a guanylyl

cyclase domain was discovered in the genome of several members of this family (1). Here we show by heterologous expression that a codon-humanized version of microbial rhodopsin of *Blastocladiella emersonii* is indeed a rhodopsin-guanylyl cyclase (RhGC). Upon light absorption, RhGC (D525) converts in 8 ms after a light flash into a blue-shifted signaling state P380 and recovers within 100 ms. RhGC was well expressed, well tolerated, and rapidly produced cGMP in response to light in *Xenopus* oocytes and mammalian neurons. Rat hippocampal CA1 neurons electroporated with RhGC and the cGMP activated rat cyclic nucleotide gated channel (CNG A2) had normal morphology and electrophysiological properties. The activation spectrum obtained from rat hippocampal CA1 neurons expressing RhGC together with the cGMP activated CNG A2 channel had a maximum at around 530 nm (green light). Cyclic GMP production was light dose-dependent, rapid and reproducible. The light dose required for half-maximal activation in neurons was 1.5 mW mm^{-2}, which is close to the published value for channelrhodopsin of 1.1 mW mm^{-2} (2). Currents activated rapidly (time to onset 120 ± 30 ms) and could be repeatedly and reproducibly activated by short light flashes at 0.2 Hz. In the absence of the CNG A2 channel, almost no endogenous current was activated in CA1 neurons expressing RhGC when illuminated at 530 nm with 19.2 mW mm^{-2} (currents less than -20 pA). Thus, RhGC is poised to become a versatile tool for optogenetic analysis of cGMP-dependent signaling in neurons. 1. Avelar, G. M. *et al.* A rhodopsin-guanylyl cyclase gene fusion functions in visual perception in a fungus. *Current biology : CB* **24**, 1234-1240 (2014). 2. Lin, J. Y., Lin, M. Z., Steinbach, P. & Tsien, R. Y. Characterization of Engineered Channel rhodopsin Variants with Improved Properties and Kinetics. *Biophys J* **96**, 1803-1814 (2009)

Disclosures: C.E. Gee: None. **U. Scheib:** None. **K. Stehfest:** None. **R. Fudim:** None. **H. Koerschen:** None. **P. Hegemann:** None. **T.G. Oertner:** None.

Poster

264. Optical Methods

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Program#/Poster#: 264.22/BB16

Topic: G.04. Physiological Methods

Support: Grant-in-Aid for Scientific Research on Innovative Areas (Comprehensive Brain Science Network) from the Ministry of Education, Science, Sports and Culture of Japan.

Title: Novel transgenic animals for tracing and optogenetics -Flame rats and bistable ChR reporter rats-

Authors: *H. IGARASHI[1], K. KOIZUMI[2], R. KANEKO[3], K. NISHIZAWA[4], Y. YANAGAWA[3], S.-I. MURAMATSU[5], K. KOBAYASHI[4], T. ISHIZUKA[2], H. YAWO[1,2]; [1]Tohoku Univ. Grad. Sch. of Med., Sendai, Japan; [2]Tohoku univ. Grad, Sch. of Life Sci., Sendai,

Japan; [3]Gunma Univ. Grad. Sch. of Med., Maebashi, Japan; [4]Fukushima Med. Univ. Sch. of Med., Fukushima, Japan; [5]Jichi Med. Univ., Tochigi, Japan

Abstract: The Cre/loxP recombination system enables to investigate systemic function of targeted genes, and has been adopted to examine a function of specific gene. For *in vivo* experiments, rats offers potential advantages of larger body size and progressed ability to accomplish more complex behavioral task over mice. Here we evaluated two new transgenic rat strains. One shows strong expression of tdTomato ubiquitously in its whole body (Flame rat) and other is conditional reporter rat line which has a gene of bistable channelrhodopsin (ChR) in the downstream of loxP-flanked STOP cassette. Flame rats; Previously we established a reporter line, NBPR-0734 from three BAC Tg rats, carrying tdTomato gene in the downstream of floxed STOP cassette. By microinjection of Cre-mRNA into fertilized eggs of this reporter rat, two lines that expresses tdTomato ubiquitously were newly established (flame rats). Flame rats were examined its expression of tdTomato in any tissues including brain, lung, liver, spleen and other main organs, and all those were brightly fluorescent positive. Flame rats can be used as an ideal source for tissue/cell transplantation and also beneficial in cell lineage trace studies. They have been successfully deposited in National Bio-Resource Project for the Rat in Japan (NBPR-0789 and -0790). Bistable ChR reporter rats; ChRFR(C167A) is a new bistable ChR (step-function opsin, SFO) which is activated by blue-cyan light and deactivated by yellow-orange light, and is suitable for enhancing the intrinsic activity of neurons at a minimal magnitude of irradiance (Hososhima et al., 2015). ChRFR(C167A)-Venus was evidenced to be expressed conditionally with Cre gene which is virally introduced. This reporter rat line would facilitate the neurophysiological and behavioral studies. All animal procedures were conducted in accordance with the guiding principles of Physiological Society of Japan and NIH. Reference: Hososhima S et al. PLoS One 10:e0119558. doi: 10.1371/journal.pone.0119558.

Disclosures: **H. Igarashi:** None. **K. Koizumi:** None. **R. Kaneko:** None. **K. Nishizawa:** None. **Y. Yanagawa:** None. **S. Muramatsu:** None. **K. Kobayashi:** None. **T. Ishizuka:** None. **H. Yawo:** None.

Poster

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Topic: G.04. Physiological Methods

Support: Marie Curie PIEF-GA-2013-628086

Wellcome Trust 097816/Z/11/A

Title: Using optical activation to redefine gain modulation in detailed biophysical neuron models

Authors: *S. JARVIS[1], K. NIKOLIC[2], S. R. SCHULTZ[3];
[1]Bioengineering, [2]Inst. of Biomed. Engin., [3]Ctr. for Neurotechnology, Imperial Col. London, London, United Kingdom

Abstract: One of the key mechanisms for sensory, motor and cognitive processing within the brain is gain modulation, in which the amplitude of a neuron's response changes while its selectivity remains unaffected. However, as synaptic locations are located throughout the dendritic arbor and dendritic integration is non-linear, it remains unclear how gain is modulated as a function of competing excitatory and inhibitory dendritic input. For this purpose, optogenetics is well-placed to answer this as not only are opsins expressed throughout the dendrites, but also due to the fine temporal and spatial precision that optogenetics offers. Using models of opsins in combination with abstract and biophysically detailed neuron models, we redefine a neuron's gain by co-expressing excitatory (ChR2) and inhibitory (NpHR) opsins throughout the dendritic membrane as well as the soma. By providing external driving input in the form of current injection or as multiple synaptic-like events at multiple locations to mimic inputs for both *in vitro* and *in vivo* scenarios, we find that dendritic morphology strongly indicates a neuron's capacity for gain modulation in abstract models and confirm this in detailed biophysical models of cortical pyramidal cells (PC) and hippocampal stellate cells. In PCs, simultaneous activation in both apical and basal dendrites are necessary for modulating gain. Finally, we evaluate the effects on partial and graded illumination to mimic physical effects of optically illuminating tissue and find that light absorption can decidedly reduce the gain modulation. Consequently, we are able to identify improved experimental illumination strategies that are not only tailored to neuronal morphology but also able to account for this phenomena in modifying the illumination protocol, thereby increasing the robustness of optogenetics for the fine manipulation of neural activity.

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Poster

265. Electrophysiology: Cellular

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Topic: G.04. Physiological Methods

Support: Jerry and Marge Burnett; Human Frontiers Science Program

IET A. F. Harvey Prize

McGovern Institute; MIT Media Lab

George W. Woodruff School of Mechanical Engineering, Georgia Tech

NSF Graduate Research Fellowships Program

NIH 1R01DA029639

NIH 1DP1NS087724

Title: Progress towards high throughput, *in vitro* cell-type identification using coupled electrophysiological and morphological properties

Authors: *G. HOLST[1], W. A. STOY[2], I. KOLB[2], L. LI[3], U. KNOBLICH[3], S. B. KODANDARAMAIAH[4], S. A. SORENSON[3], H. GILL[3], T. JARSKY[3], J. WATERS[3], A. C. SINGER[4], B. YANG[2], G. T. FRANZESI[4], E. S. BOYDEN[4], H. ZENG[3], C. R. FOREST[2]; [1]Mechanical Engin., [2]Georgia Inst. of Technol., Atlanta, GA; [3]Allen Inst. for Brain Sci., Seattle, WA; [4]MIT, Boston, MA

Abstract: There are a number of neuronal cell type classification schemes based on analysis of transcripts, protiens, elepctrophysiology, connectivity, and morphologly although generally these are used independently and lack a one-to-one correspondence between them. We will present progress towards establishing a cell type scheme for layer 5 cells in the mouse visual cortex using coupled *in vivo*, visually-evoked, whole-cell patch clamp electrophysiology and morphological reconstruction on the same cell. We anticipate there to be correlation between the detailed electrophysiological identity and the morphology and connectivity fingerprint that informs the specific functional role of each cell type in the larger network processing of the visual cortex. Similar results in layer 6 cells (Velez-Fort et. al. 2014) have increased our understanding of their exact role and the interactions, and we anticipate there to be similar insights for cells in layer 5. These coupled electrophysiological and morphological experiments are, however, often quite difficult to perform due to low yields and low throughput. Previously, we developed the "autopatcher," an automated *in vivo* patch clamp robot for automatic neuron detection and whole cell recording. Here, we also describe additional robotic tools that enable the first autonomous serial patch clamp recordings. The robot can perform up to 40 fully automated serial recording attempts and compensates for cell health and recording quality in real-time. These tools allow the robot to run unattended for the duration of experiment and include algorithms for automated break-in, amplifier compensation, access resistance monitoring and improvement, and automated electrical and visual stimulation. It also includes electrical and mechanical hardware for pipette storage, filling, wire threading, aligning in the craniotomy, and pneumatic control. The combination of the hardware and algorithms significantly reduces time between trials and eliminates common operator errors resulting in an overall increased throughput and unattended operation.

Disclosures: **G. Holst:** F. Consulting Fees (e.g., advisory boards); Neuromatic Devices, Inc.. **W.A. Stoy:** None. **I. Kolb:** F. Consulting Fees (e.g., advisory boards); Neuromatic Devices, Inc.. **L. Li:** None. **U. Knoblich:** None. **S.B. Kodandaramaiah:** None. **S.A. Sorenson:** None. **H. Gill:** None. **T. Jarsky:** None. **J. Waters:** None. **A.C. Singer:** None. **B. Yang:** None. **G.T. Franzesi:** None. **E.S. Boyden:** None. **H. Zeng:** None. **C.R. Forest:** None.

Poster

265. Electrophysiology: Cellular

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Topic: G.04. Physiological Methods

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NIH grant 1R01DA029639

Title: Oscillatory modulation of action potential firing in hippocampal neurons of awake mice via periodic shunting inhibition

Authors: G. TALEI FRANZESI[1], *A. C. SINGER[1], S. B. KODANDARAMAIAH[1], E. WU[1], B. ARKHURST[1], C. R. FOREST[2], C. BORGERS[3], N. J. KOPELL[4], E. S. BOYDEN[1]; [1]McGovern Inst. and Media Lab, MIT, Cambridge, MA; [2]George W. Woodruff Sch. of Mechanical Engin., Georgia Inst. Of Technol., Atlanta, GA; [3]Tuft Univ., Medford, MA; [4]Boston Univ., Boston, MA

Abstract: Neuronal oscillations are postulated to be fundamental to how the brain encodes, processes, and transmits information, and different oscillations identify distinct network states. Despite striking behavioral and field potential differences between these distinct network states, the cellular mechanisms through which oscillatory dynamics modulate neural firing are difficult to assess in awake, behaving animals. In particular, although inhibition is believed to play a crucial role in mediating the effects of several kinds of oscillations on neural activity, GABAergic inhibition can both shunt neural inputs as well as directly hyperpolarize the neuronal membrane potential, and thus there could be multiple cellular mechanisms by which oscillatory neural activity impacts neuronal firing. To probe these cellular mechanisms we recorded intracellularly in awake, headfixed mice navigating a virtual reality maze, employing a modified autopatcher robot capable of whole cell patch clamp recording in awake mice. As a model region we chose the hippocampal CA1 field, focusing on theta (6-12Hz) and gamma (30-80 Hz) oscillations. We found that intracellular dynamics changed with different network states and oscillatory phases, but, perhaps surprisingly, the membrane voltage did not display rhythmic fluctuations that mimic the local field potential oscillations, consistent with inhibition serving a rhythmic shunting, rather than hyperpolarizing, role. We are currently testing this hypothesis by using somatic current injection via the patch pipette to assess the degree of shunting inhibition

present during various phases of the oscillations. We are also working to create computational models to probe these phenomena. These results may offer new insight into the cellular mechanisms through which oscillations achieve their impact on neural activity in awake, behaving animals.

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Poster

265. Electrophysiology: Cellular

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Topic: G.04. Physiological Methods

Support: Samsung Fellowship

NIH 1R01EY023173

Title: Automated two-photon guided patch-clamp electrophysiology *in vitro*

Authors: *H.-J. SUK[1], I. VAN WELIE[2], C. FOREST[6], E. BOYDEN[3,4,5];
[1]Hlth. Sci. and Technol., [3]Media Arts and Sci., [4]Biol. Engin., [5]Brain and Cognitive Sci., [2]MIT, Cambridge, MA; [6]Bioengineering, Georgia Inst. of Technol., Atlanta, GA

Abstract: Two-photon guided patch-clamp is a powerful tool for studying properties of identified cells *in vivo*. By combining two-photon laser scanning microscopy with whole cell patch-clamp recording, it uniquely enables accurate localization and analysis of individual neurons in the intact brain. However, the complicated and laborious procedures of this technique require much skill and experience, making it difficult to perform in a high-throughput fashion. To address this issue, we have developed a strategy for a robotic system that can automatically complete the tasks involved in two-photon guided patch-clamp recordings, from target cell detection, pipette trajectory calculation and navigation to pipette resistance measurement, pressure modulation and holding voltage control, all of which will be initiated via an easy-to-use user interface. To enable automated imaging and detection of fluorescently labeled cells *in vivo*, we have built a Matlab-based custom module that can be easily integrated into ScanImage, the open-source software commonly used by electrophysiologists for image-guided patch-clamp recordings. The custom module can also automatically locate the tip of a pipette filled with a fluorescent dye and determine the optimal trajectory for the placement of the tip onto the target cell. As the pipette is navigated into the brain tissue by a computer-controlled micromanipulator, the pressure level inside the pipette is sequentially adjusted using the automated pressure box previously developed by Kodandaramaiah et al (2012) to prevent pipette blockage and to

minimize background fluorescence. Meanwhile, our system monitors the pipette resistance and identifies a physical contact between the pipette tip and the cell membrane by detecting a distinct rise in resistance. At this point, a closed-loop pressure and holding voltage controller will be activated for the formation of a gigaohm seal and the whole-cell configuration. By testing our automated patch-clamp strategy on fluorescently labeled parvalbumin-positive interneurons in the mouse cortex, we will optimize the experimental parameters to achieve high robustness of our robotic system. Once fully developed, our system has the potential to become a useful tool for skilled electrophysiologists as well as for those new to the field, as it can eliminate several laborious manual procedures. Furthermore, our system will be highly scalable as the code and algorithms for all of the automated procedures may be extended to multi-cell patch-clamp recordings.

Disclosures: **H. Suk:** None. **I. van Welie:** None. **C. Forest:** None. **E. Boyden:** None.

Poster

265. Electrophysiology: Cellular

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

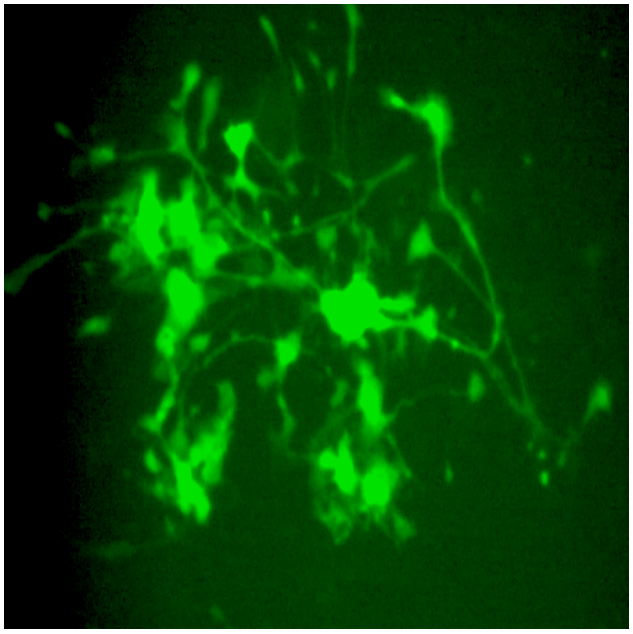
Program#/Poster#: 265.04/BB21

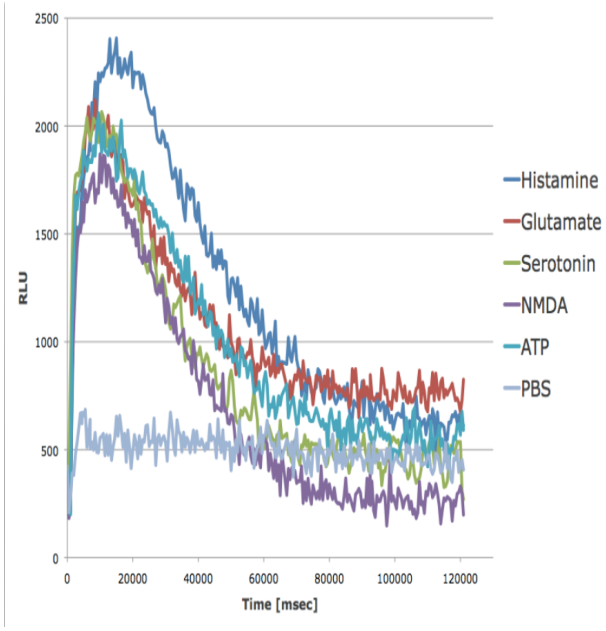
Topic: G.04. Physiological Methods

Title: Human iPSC-derived neurons stably expressing a GCaMP3 calcium sensor demonstrate robust (and temporal) HTS capabilities

Authors: ***G. C. LUERMAN**[1], R. KETTENHOFEN[2], A. EHLICH[2], T. PALM[2], H. BOHLEN[2]; [1]Axiogenesis Inc, Plymouth Meeting, PA; [2]Axiogenesis AG, Cologne, Germany

Abstract: Human induced pluripotent stem cell-derived neurons provide a powerful preclinical cellular tool for predicting/modeling pharmacological behavior, especially as it relates to ion channel modulation, neurotoxicity, and network activity. The combination of high-throughput fluorescence detection technology (Hamamatsu FDSS 7000EX) with extracellular and intracellular calcium and/or voltage sensors permits the acquisition of fast calcium and voltage (transient) events. We compared the effects of multiple ion channel and neurotransmitter modulators, as well as neurotoxic compounds on commercially available iPS-derived dopaminergic and peripheral neurons either stably expressing a GCaMP3 calcium sensor or loaded with a calcium-binding dye (fluo-4). Results from acute ion channel block were comparable between the two methods of detection. However, temporal effects from chronic applications of chemotherapeutics or neurotoxic drugs (e.g. Vinblastine) were only able to be detected with the GCaMP3 sensor. In addition to ease of use, reproducibility, and scalability, these results provide strong rationale for the use of integrated calcium sensor technology in high throughput neuronal safety/toxicity or drug development studies.





Disclosures: **G.C. Luerman:** None. **R. Kettenhofen:** None. **A. Ehlich:** None. **T. Palm:** None. **H. Bohlen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CEO, Axiogenesis AG.

Poster

265. Electrophysiology: Cellular

Location: Hall A

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Program#/Poster#: 265.05/BB22

Topic: G.04. Physiological Methods

Support: Bernstein Center Computational Neuroscience Munich A2

Title: Testing the role of SK channels in frequency tuning with a novel dynamic clamp system for sharp electrodes

Authors: *H. POLDER[1], J. PRESERN[2], J. LOOSER[1], J. BENDA[2];
[1]npi electronic GMBH, Tamm, Germany; [2]Inst. for Neurobio., Eberhard Karls Univ., Tuebingen, Germany

Abstract: The dynamic clamp is a powerful closed-loop technique for intracellular recordings that allows one to introduce artificial membrane conductances into neurons in a graded manner, without pharmacology or genetics. So far, the method was limited to low-resistance (patch) electrodes, which are not suitable for all the cell types. Injecting current trough high-resistance sharp electrodes requires usage of a current-clamp amplifier in discontinuous mode, with high-frequency switching between current injection and voltage measurement. Because of the Nyquist theorem the switching cycle of the amplifier needs to be more than twice of the frequency of the dynamic clamp software loop. This can be a major limitation because such high switching frequencies might be difficult to achieve. We slightly modified the SEC-05LX amplifier (npi electronic GmbH, Tamm, Germany) such that its switching cycle is triggered by the dynamic clamp loop, thus halving the required switching frequency. Controlled by the RELACS software (www.relacs.net) this allows for sampling rates of up to 50 kHz. Potassium currents not only shape the afterhyperpolarization following action potentials but also modify their signal processing properties. For example, pyramidal cells (PC) of the three segments of the electrosensory lateral line (ELL) lobe of weakly electric fish receive identical input, but the presence of SK-type potassium currents in the PCs of one of the segments shifts their sensitivity to higher frequencies. We used this dynamic clamp system to introduce SK type potassium currents into superficial PCs of the centromedial segment lacking this current. This shifted the frequency tuning of the cells towards higher frequencies as in PCs of the lateral segment that express SK currents.

Disclosures: **H. Polder:** A. Employment/Salary (full or part-time):; npi electronic GmbH. **J. Presern:** None. **J. Looser:** A. Employment/Salary (full or part-time):; npi electronic GmbH. **J. Benda:** None.

Poster

265. Electrophysiology: Cellular

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 265.06/BB23

Topic: G.04. Physiological Methods

Title: HumSilencer: A smart and simple Axon Digidata 1550 series feature for eliminating line-frequency noises

Authors: *K.-C. TANG, B. BLIZARD, K. SRIDHAR, W. DANILO, B. MAERTZ, M. YOUNGQUIST;
Mol. Devices, LLC., Sunnyvale, CA

Abstract: Electrical hum is the most common source of background noise in electrophysiology experiments. It is caused by the alternating current (AC) of the electrical mains delivered via power outlets. This background noise can obscure the biological signals of interest, making analysis difficult, if not impossible. Typical methods for mitigating this type of electrical noise can be time consuming and are only partially effective. In this study, we examined the noise elimination performance of the newly developed Axon™ Digidata® 1550 series Low-Noise Data Acquisition System plus HumSilencer™ Adaptive Noise Cancellation. We found that HumSilencer removed 50/60 Hz interference within 50 ms after it was enabled. If the 50/60 Hz noise pattern changed, the HumSilencer rapidly adapted and subtracted the new noise pattern within one second. In addition, it eliminated noise amplitudes at the digitizer's analog input of up to 20 V, peak-to-peak. We also examined if the HumSilencer algorithm distorts acquired biological signals. Our results showed that it did not distort evoked end-plate potential and action potential analyzed parameters, such as, peak amplitudes, time of peaks, half-widths, rise times, rise slopes, decay times, decay slopes, and several others. More importantly, the HumSilencer was able to distinguish between 50/60 Hz noise that was caused by background interference and 50/60 Hz frequencies within the biological signal and only removed the former. Together, this evidence shows that HumSilencer provides a smart and simple way for background interference elimination and ushers in a new level of ease and confidence in electrophysiology data acquisition. HumSilencer is not a filter and does not have a filtering effect on acquired biological signals; nor does the HumSilencer system cause signal distortion, such as frequency change, amplitude attenuation, phase shift, or DC voltage change.

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Poster

265. Electrophysiology: Cellular

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Topic: G.04. Physiological Methods

Support: AHA Grant #13SDG16990083

Title: 3d data mapping and real-time experiment control and visualization in brain slices

Authors: ***M. E. MILLER**[1], M. A. NAVARRO[2], J. V. K. HIBBARD[2], T. W. NIVIN[2], A. A. PETERS[2], L. S. MILESCU[2];
[1]Life Sci., [2]Biol. Sci., Univ. of Missouri, Columbia, MO

Abstract: We have designed software that streamlines electrophysiology and imaging experiments in brain slices and enhances data collection and analysis. The experiment is interfaced with a 3D scene viewer, where the rig, the brain slice, and the recorded data are represented to scale. Within this viewer, the user can visualize a live image of the sample and 3D renderings of the recording electrodes, with real-time position feedback. Furthermore, the user can control the instruments and visualize their status in real-time, and can reload previously executed experiments and run simulations. Multiple types of experimental data can be integrated into a spatial and temporal map of the brain slice. These data can consist of low-magnification maps of the entire brain slice, for spatial context, and any other types of high-resolution structural and functional images, together with time-resolved electrical and optical signals. The entire data collection can be visualized within the 3D scene viewer. These ideas can be applied to any other types of experiments where high-resolution data are recorded within a larger sample, at different spatial and temporal coordinates.

Disclosures: **M.E. Miller:** None. **M.A. Navarro:** None. **J.V.K. Hibbard:** None. **T.W. Nivin:** None. **A.A. Peters:** None. **L.S. Milescu:** None.

Poster

265. Electrophysiology: Cellular

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Program#/Poster#: 265.08/BB25

Topic: G.04. Physiological Methods

Title: Volume effect of localized injection on PO2, single unit activity, and fMRI

Authors: ***D. P. AKSENOV**[1], K. RUTILA[2], L. LI[1], M. MILLER[1], R. LINSENMEIER[2], A. WYRWICZ[1];
[1]NorthShore Univ. HealthSystem, Evanston, IL; [2]Northwestern Univ., Evanston, IL

Abstract: The local injection of neurotransmitter agonists and antagonists to modulate functions of neurons is a widely-used technique that avoids many of the confounding effects of systemic drug injections, which affect the whole brain and can change input to the recorded area of interest. Localized drug delivery in the brain is typically accomplished via either iontophoresis, reverse microdialysis or microinjection under pressure. Compared to iontophoresis, localized microinjection allows the delivery of larger volumes which can affect a much larger area of interest, but is often accompanied by mechanical displacement of the tissue, known as “volume

effect" (VE). VE can cause changes in oxygen tension (PO2), single unit recordings, and fMRI signal. This study describes the changes in these signals associated with VE, as well as an approach for minimizing these effects. fMRI data, single unit recordings, and oxygen tension measurements were performed to characterize the signal changes resulting from the local injection of artificial cerebrospinal fluid (ACSF) in the whisker barrel cortex of the rabbit. fMRI experiments were performed on a 9.4T Bruker BioSpec imaging spectrometer. Imaging data were acquired from four consecutive slices using single-shot gradient-echo EPI pulse sequence (TR=2s and TE=11ms). The slices included the whisker cortex and whisker thalamus. Single unit activity was recorded from the whisker barrel cortex using gold-silver wires, attached to a chronically implanted microdrive. To record PO2 changes an oxygen sensitive electrode was polarized to −0.7 V with respect to a silver-chloride reference electrode, and the current was measured with a Keithley model 614 electrometer. Our results indicate that combining local microinjection with fMRI, single unit recording, and PO2 is a feasible approach, but the technical issues particular to each technique require careful consideration. The localized injection can produce significant intensity changes in MR images in addition to effects on electrophysiological recordings, such as changes in waveforms and magnitude of the spikes. The fMRI changes are localized to the vicinity of the injection needle, and diminish over time due to diffusion of the injected volume. VE also affects the PO2 signal in a volume-dependent manner, and the changes were found to be long-lasting. Experiments involving local injections therefore should carefully consider the impact of VE in their design. Our results indicate that a slow speed of injection, limited volume and sufficient post-injection waiting period can significantly mitigate the effects of VE.

Disclosures: D.P. Aksenov: None. **K. Rutila:** None. **L. Li:** None. **M. Miller:** None. **R. Linsenmeier:** None. **A. Wyrwicz:** None.

Poster

265. Electrophysiology: Cellular

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 265.09/BB26

Topic: G.04. Physiological Methods

Title: Nonapplication of neuroscience data to medical treatment

Authors: *G. S. WASSERMAN;
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Abstract: My life's work involved intracellularly recording sensory responses in single photoreceptor cells in *Limulus,* the horseshoe crab. Twice this work led to possible treatments for behavioral disorders. The first involved the invention of cochlear implants. Around 1980, Dr. Richard T. Miyamoto of Indiana U. Medical School came to Purdue and told us that implants

restored hearing to the deaf but they could not understand speech. I asked him how the implants coded sound and learned that they did it the way that hi-fi audio systems do -- by stimulating auditory nerves of the deaf with faithful electrical *copies* of physical sounds. I then showed him my recordings of the way sensory receptors temporally *dispersed* them. He then sent cochlear implant patients to my laboratory where I acoustically inserted temporal dispersion and response compression into the signal path. The result was *immediate* and *useful* comprehension of spoken language. Within a year, the incorporation of such modifications into cochlear implants enabled *legions* of sufferers to converse easily. This achievement was recognized by the award of a US Patent (Sensory Prostheses: US 4611596 A) and other honors. Years later, my last trio of graduate students (Amanda Bolbecker, Jia Li, and Corrinne Lim) reversed the line of investigation and began to examine the efferent neural signals that the brain sends *to* neural receptors. They found profound temporal effects of such efference: Substance P *speeds* receptor responses while Octopamine *slows* them. It was obvious that such efference allows the brain to adjust the temporal properties of its inputs so that they are appropriate for whatever external situation engages an organism's attention. About that time, a new tool was invented by Erich Sutter. It is the multifocal electroretinogram (mfERG) and it makes it possible to record retinal activity from a contact lens electrode placed on the eye. My understanding is that *hundreds* of mfERG system have been produced and, with funding from the NIH, are in the hands of many scientists in various institutions. I have spent more than a decade trying to get just *one* of these investigators to determine if changes in the behavioral attention of a person fitted with an mfERG system changes the pattern of activity in that person's neurovisual responses to well controlled stimuli. If that were true, it would lead to *new* ways of understanding and treating attention disorders and thereby aiding *millions* of sufferers. Not *one* investigator has *reported* undertaking *any* such investigation. Clearly *something* has changed quite radically in the culture of science in recent times.

Disclosures: G.S. Wasserman: None.

Poster

265. Electrophysiology: Cellular

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 265.10/BB27

Topic: G.04. Physiological Methods

Title: Voltage-gated calcium channels regulate neural precursor cell migration in the directed current electric field

Authors: *A. STEIGER[1], H. ZHAO[2], Z. BINDA[2], H. YE[2];
[2]Biol., [1]Loyola Univ. Chicago, Chicago, IL

Abstract: Effective stem cell transplantation is dependent upon the cells' ability to restore function after migration, differentiation, and integration with the host's damaged tissue. Taking a bioengineering approach, recent studies have attempted to use an electric field to guide neural precursor cell (NPC) migration (termed electrotaxis) and differentiation. However, the mechanism of NPC electrotaxis is largely unknown. Previous studies have documented that direct current (DC) electric field can guide the undifferentiated NPCs towards cathode migration, but not the differentiated cells (Babona-Pilipos et al., 2011). We propose that the difference between undifferentiated and differentiated cell migration is largely mediated by newly-expressed ion channels on the differentiated cells, particularly the voltage-gated calcium channels (VGCCs), which allows for calcium influx when the membrane is depolarized in the DC electric field. Western Blot analysis suggested the presence of L-type VGCCs in the differentiated cell population, but not in the undifferentiated NPCs. Immunocytochemical staining indicated the presence of L-type VGCCs on the differentiated neurons, but not the undifferentiated cells. Pharmacological blockage of the L-type VGCCs in the differentiated cells promoted neuronal migration toward the cathode. Taken together, these data elucidate the crucial role of VGCCs in EF-guided migration, and provide a potential ionic target for the control of stem cell migration under EF guidance.

Disclosures: **A. Steiger:** None. **H. Zhao:** None. **Z. Binda:** None. **H. Ye:** None.

Poster

265. Electrophysiology: Cellular

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 265.11/BB28

Topic: G.04. Physiological Methods

Title: An automated patch-clamp platform (CytoPatch™) for neuronal ligand- and voltage-gated ion channels pharmacology and recordings in cell lines and dissociated neurons

Authors: T. KNOTT[1], *S. FRECH[1], O. SCHEEL[1], J. EISFELD[1], B. AMUZESCU[1], S. WIERSCHKE[1], J. KUDOLO[1], K.-H. LIN[1], J. WOLFART[2];
[1]Cytocentrics Biosci. GmbH, Rostock, Germany; [2]Oscar Langendorff Inst. of Physiol., Univ. of Rostock, Rostock, Germany

Abstract: In recent years, automated patch clamp has been established as a standard tool for drug discovery and screening in recombinant cell lines. However, when using primary or freshly dissociated cells, the seal quality and low signal-to-noise ratio is of high importance. The CytoPatch™ is a high performance cellular electrophysiology platform using an innovative Cytocentering™ technology based on quartz pipette tips embedded in a silicon microfluidic chip. The chip includes orifices and channels that capture cells in suspension and convey them to the pipette tip, allowing higher quality and stability gigaseals, using physiological external and

internal solutions. The low-volume microfluidic circuitry (100 nL perfusion chamber volume) ensures successful captures with only 150 cells/seal in 0.1 μL, reduced compound consumption (perfusion flow rates < 10 μL/min), and permits very fast wash-in and wash-out speeds. The system features fast signal sampling frequencies (10/20/40/80 kHz) and works in voltage- and current-clamp mode, with automated capacitive transient cancellation and series resistance compensation. Perforated whole-cell configurations are achievable, as well as several types of patch configurations. To demonstrate the performance of the system and suitability for neuroscience-related experiments, we present several examples of recordings in ligand-gated and sensory transduction neuronal-specific ion channels: activation by glutamate in 10-ms applications of GluA2 AMPA receptors transiently transfected in HEK293 cells (rise time < 3.2 ms), mechanosensitive stimulation (140 mbar/50 ms) of Piezo1 and 2 channels in cultured Neuro2A cells and inhibition by ruthenium red (90 μM), pH 6.0-evoked inward current transients by activation of ASIC1a/1b channels in HEK293 cells. We also present voltage- and current-clamp recordings in primary sensory neurons freshly dissociated from rat dorsal root ganglia, as well as recordings at different temperatures in dissociated rat anterior hypothalamic neurons. These results demonstrate the feasibility of complex *in vitro* electrophysiology automated patch-clamp assays with applications for advanced neurophysiology, pathophysiology and pharmacology studies.

Disclosures: **T. Knott:** Other; Cytocentrics Bioscience GmbH. **S. Frech:** Other; Cytocentrics Bioscience GmbH. **O. Scheel:** Other; Cytocentrics Bioscience GmbH. **J. Eisfeld:** Other; Cytocentrics Bioscience GmbH. **B. Amuzescu:** Other; Cytocentrics Bioscience GmbH. **S. Wierschke:** Other; Cytocentrics Bioscience GmbH. **J. Kudolo:** Other; Cytocentrics Bioscience GmbH. **K. Lin:** Other; Cytocentrics Bioscience GmbH. **J. Wolfart:** None.

Poster

265. Electrophysiology: Cellular

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 265.12/BB29

Topic: G.04. Physiological Methods

Title: Glycine-induced currents are insensitive to the glycine receptor α1 subunit-specific blocker, cyanotriphenylborate, in older circling mice

Authors: *J. PRADHAN, SR[1], S. AHN[2];
[1]Kathmandu Ctr. For Genomics and Res. Laborat, Kathmandu, Nepal; [2]Physiol., Dankook Univ., Cheonan, Korea, Republic of

Abstract: The pharmacologic characteristics of glycine receptors (GlyRs) in the lateral superior olive (LSO) of circling mice, animal model for inherited deafness, were investigated using a GlyR α1 subunit-specific receptor blocker (cyanotriphenylborate [CTB]). There was a

statistically significant age-dependent increase in the antagonistic effect of CTB in heterozygous (+/cir) mice. In postnatal (P)0-P3 heterozygous (+/cir) mice, glycine currents evoked by glycine puffs were reduced to 20.4 ± 2.6, 37.1 ± 3.1, and 63.9 ± 2.5% at 0.1, 1, and 10 μM CTB (n = 13) compared to controls, while the glycine currents were reduced to 22.3 ± 3.5, 52.9 ± 4.1, and 78.3 ± 3.5% at 0.1, 1, and 10 μM CTB (n = 7) in P8-P12 heterozygous (+/cir) mice. In contrast, the antagonistic effect of CTB was not strong and even less than that of younger animals in older homozygous (cir/cir) mice. In P0-P3 homozygous (cir/cir) mice, the extent of inhibition was 20.2 ± 3.7, 37.8 ± 4.3, and 66.8 ± 4.2% at 0.1, 1, and 10 μM CTB (n = 6) compared to controls, while the extent of inhibition was 18.7 ± 2.4, 28.1 ± 3.9, and 39.1 ± 8.2% (n = 6) in P8-P12 homozygous (cir/cir) mice. The age dependent decrease in the antagonistic effect of CTB indicates the abnormal development of the α1 subunit-containing GlyRs in homozygous (cir/cir) mice.

Disclosures: J. Pradhan: None. **S. Ahn:** None.

Poster

265. Electrophysiology: Cellular

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Topic: G.04. Physiological Methods

Support: EU FP7-PEOPLE-IEF "INCA-NANEP", contract n. 328214

Flanders Research Foundation (FWO), contract no. G088812N

Belgian Science Policy Office (BELSPO)

Title: Fluorescent nanodiamonds: a novel tool for detecting neuronal activity

Authors: *A. M. MONACO[1], J. MOTYLEWSKI[1], S. K. R. SINGAM[2], E. GOOVAERTS[2], M. GIUGLIANO[1,3,4];
[1]Dept. of Biomed. Sci., [2]Dept. of Physics, Univ. of Antwerp, Wilrijk - Antwerpen, Belgium; [3]Brain Mind Institute, Swiss Federal Inst. of Technol. Lausanne, Lausanne, Switzerland; [4]Dept. Computer Sci., Univ. of Sheffield, Sheffield, United Kingdom

Abstract: Understanding the complex phenomena underlying specific brain functions has always been a challenge for neuroscientists. Recently, the new discoveries in the field of Nanotechnology provided researchers with novel tools that allow studying the brain on its own scale. One of the most fruitful results of this new interdisciplinary approach is the use of carbon-based nanomaterials as nanostructured substrates for cellular growth and regeneration, as labels for cellular imaging and as nanoscale electrodes. However, these applications find limitations in their spatial and temporal resolution, as well as in their invasive character, and this calls for the

development of non-invasive techniques, which allow localizing brain structures on the order of micrometres and measuring neural function on a temporal scale of few milliseconds. To overcome these limitations, we are exploiting the use of Nitrogen Vacancy Nanodiamonds (NV-NDs) to directly evaluate the local electromagnetic fields generated by neuronal activity by means of spin sensitive photon detection. These Nanodiamonds, in fact, are one of the most powerful sensors of magnetic field, thanks to the high sensitivity of the electron spin states of their Nitrogen Vacancy centres to light and to magnetic field. Here we present preliminary results on incubating NV-NDs with primary cortical neurons. Firstly, we evaluated viability of neurons when incubated with NV-NDs, finding that NV-NDs do not affect neuronal viability and morphology compared to conventional glass coverslips. We then studied, by means of DIC and fluorescence microscopy, the localization of these NV-NDs with respect to living neurons, in presence and in absence of a magnetic field, in order to vary the fluorescence of the NV centres. These encouraging preparatory results are fundamental for the forthcoming electrophysiological testing of contingent effects of NV-NDs on neuronal electrical activity and for optical quantum imaging, which will allow a contactless mapping of neuronal activity by monitoring weak electromagnetic field in neurons.

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Poster

266. Methods: Electrophysiology

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Topic: G.04. Physiological Methods

Support: Swiss National Foundation Ambizione Grant PZ00P3_132245

ERC Advanced Grant NeuroCMOS under contract number AdG 267351

Title: Connectivity analysis of whole-network recordings obtained by a high-spatiotemporal-resolution microelectrode array

Authors: ***J. MUELLER**, D. J. BAKKUM, A. HIERLEMANN;
ETH Zurich, Basel, Switzerland

Abstract: To investigate the plasticity of ensembles of multiple neuronal cells over time requires means to quantify the strength of many synaptic connections. The traditional method to assess synaptic strength relies on paired patch-clamp recordings, which is difficult to do for many pairs at the same time. Instead, functional connectivity in a neuronal network has been inferred by analyzing the relative timing of extracellular action-potentials. Recently, various algorithms to infer such functional connectivity have been proposed. However, most of these algorithms can

only approximate connectivity because conventional microelectrode arrays drastically under-sample network activity. Here we utilize a recently developed high-density microelectrode array (MEA), based on complementary-metal-oxide semiconductor (CMOS) technology, to record from potentially every neuron in a small cultured network. The microelectrode array features 26,400 platinum microelectrodes (5×9 μm^2), arranged in a grid-like configuration of 3.8×2.1 mm^2 area at a center-to-center pitch of 17.5 μm. An array of switches and wires residing below the electrodes is used to connect a subset of these electrodes to 1024 low-noise readout channels (2.4 μV_{rms} noise in the action-potential signal band, 300 Hz - 10kHz) and 32 dual-mode voltage and current stimulation units, all residing at the periphery of the electrode array. The electrode-to-readout channel routing can be reconfigured within milliseconds to adapt the electrode selection to the neuronal culture under study and to scan neural activity on all electrodes. Signals are sampled at 20 kHz and 10-bit resolution through on-chip analog-to-digital converters. Low-density cultures of dissociated E18 rat cortical neurons were grown on top of the MEA. Experiments were started after 2 weeks of culturing. First, the entire array was electrically scanned to find neurons that exhibit spontaneous action potentials, and to identify all putative single cells in the culture. Once all cells have been identified, 1024 electrodes that captured electrical signals from neurons were selected in a second step to record activity over several hours. The large number of tightly packed microelectrodes, together with the low-noise recording amplifiers, enabled us to identify up to about a thousand single cells and to record their activity over days and weeks. By confining the growth of neurons to the electrode area, potentially every spike from every neuron and the connectivity throughout the whole network can be investigated.

Disclosures: **J. Mueller:** None. **D.J. Bakkum:** None. **A. Hierlemann:** None.

Poster

266. Methods: Electrophysiology

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Topic: G.04. Physiological Methods

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Title: Neurophysiological investigation of spontaneous BOLD fluctuations in the orbitofrontal cortex and anterior temporal lobe

Authors: *M. J. SUTTERER[1], C. K. KOVACH[2], P. E. GANDER[2], M. A. HOWARD, III[2], M. W. VOSS[3];
[1]Dept. of Neurol., [2]Dept. of Neurosurg., [3]Dept. of Psychology, Univ. of Iowa, Iowa City, IA

Abstract: Resting-state functional connectivity (RSFC) in fMRI has been demonstrated to be a powerful tool for noninvasive brain mapping, showing particular utility in imaging clinical populations. Despite this, limitations of standard BOLD imaging apply, including issues of signal dropout in temporal and orbitofrontal cortex. Concerns about signal dropout in these areas have limited large scale efforts to characterize the connectivity properties of the human brain, with the properties of the orbitofrontal and anterior temporal lobes largely ignored. More focused studies of intrinsic brain networks have identified both the orbitofrontal cortex and the anterior temporal lobes as important nodes in the default mode network (DMN), and altered connectivity in these nodes of the DMN has been characterized as a feature of several neurological and psychiatric disorders. To better understand the neurophysiological properties of spontaneous BOLD fluctuations in these regions, we investigated the concordance of resting-state BOLD connectivity in the orbitofrontal and anterior temporal lobe with oscillations in high-gamma power in a series of patients who were implanted for intracranial electrocortiography (ECoG). Each patient was implanted with 100-256 surface grid and penetrating depth electrodes, including multiple contacts covering the orbitofrontal cortex, the anterior temporal lobe, amygdala, and hippocampus. All patients completed 12 minutes of resting-state fMRI imaging prior to electrode placement, and at least 10 minutes of resting-state ECoG recording. For each patient we generated spherical seed regions of interest corresponding to each electrode contact (registered to MNI space) and extracted for each contact the low-frequency ($0.008 < f < 0.08$) BOLD time-series, and the low-frequency ($0.01 < f < 0.1$) oscillation in high gamma (70-150 Hz) power. We organized the resultant BOLD and ECoG correlation matrices using the Infomap clustering algorithm, and examined the correlation between the ECoG and BOLD connectivity matrices. We observed significant correspondence ($p < 0.05$ under permutation test) between the correlation patterns of spontaneous BOLD fluctuations and low-frequency oscillations in high-gamma power in contacts in the orbitofrontal and anterior temporal lobe similar to that observed in other regions of the brain ($0.3 < r < 0.7$). This correspondence persists even after accounting for the linear distance between contacts. These data support the neurophysiological basis of fMRI-based resting state connectivity in the orbitofrontal cortex and anterior temporal lobe.

Disclosures: M.J. Sutterer: None. **C.K. Kovach:** None. **P.E. Gander:** None. **M.A. Howard:** None. **M.W. Voss:** None.

Poster

266. Methods: Electrophysiology

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Topic: G.04. Physiological Methods

Support: NIH/NIBIB R01 EB016101

Title: Fast-scan cyclic voltammetric measurements of stimulation-induced dopamine release with chronically implanted carbon fibers in awake non-human primate

Authors: *H. N. SCHWERDT[1,2], H. SHIMAZU[2], K.-I. AMEMORI[2], S. HONG[2], J. C. SY[1], K. C. SPENCER[1,3], P. L. TIERNEY[2], Y. YANG[1], H. YERRAMREDDY[2], C. DAGDEVIREN[1], K. RAMADI[1], R. S. LANGER[1], M. J. CIMA[1,3], A. M. GRAYBIEL[2];
[1]Koch Inst. for Integrative Cancer Res., [2]McGovern Inst. for Brain Res., [3]Dept. of Materials Sci. and Engin., MIT, Cambridge, MA

Abstract: Measurement of rapid changes in dopamine concentration via fast-scan cyclic voltammetry (FSCV) has transformed current understanding of neurotransmitter dynamics and their relation to neuronal electrical signaling events. These phenomena are intimately related to motor and neuropsychiatric disorders. Continuous and rapid sampling of neurochemicals may be used in the future as a diagnostic tool and also to improve therapeutic measures for Parkinson's disease and many other disorders. As a first step toward this goal, we used FSCV to measure striatal dopamine in an awake rhesus macaque from carbon fiber microelectrodes (CFMs) implanted in the caudate nucleus for over a month. Polyacrylonitrile-derived carbon fibers 7 μm in diameter and cut to exposed lengths of 100 - 250 μm were used as the electrochemical sensing interface. A triangular voltage waveform was applied at −0.4 V - 1.3 V at 400 V/s every 100 ms while recording current produced at the CFMs. Phasic dopamine release was evoked by targeted medial forebrain bundle (MFB) stimulation, as commonly used to assess sensitivity of CFMs to dopamine in rodents. MFB stimulation was generated at 60, 120, and 200 Hz, for 11 - 48 biphasic 1 ms pulses, and with amplitudes of 50 - 400 μA. Local striatal stimulation also produced dopamine signals as recorded by CFM tips 500 μm away. MFB stimulation was shown to reproducibly evoke dopamine release in both the caudate nucleus and putamen over a depth of ca. 0.9 mm through the MFB as measured across separate acute recording sessions spanning several days along with recordings 1 month post-implantation. The chemical selectivity of the measurements was evaluated via principle component regression as well as by chemical modulation (0.05 - 0.1 mg/kg intramuscular injections of the D2 receptor antagonist, raclopride). Only signals with signal-to-noise ratios > 7 and correlation coefficients (r) > 0.75 were considered to reflect changes in dopamine concentration. Raclopride induced up to 230% and 130% amplification of the peak stimulation-induced dopamine oxidation current from both the acutely implanted and month-long implanted CFMs, respectively. The frequency of endogenous (non-stimulation evoked) dopamine transients recorded by the CFMs was also compared before and after raclopride in both the acute and chronic settings. Collectively, these experiments demonstrate the robust measurement of rapid changes in dopamine from the striatum over prolonged periods in the non-human primate.

Disclosures: **H.N. Schwerdt:** None. **H. Shimazu:** None. **K. Amemori:** None. **S. Hong:** None. **J.C. Sy:** None. **K.C. Spencer:** None. **P.L. Tierney:** None. **Y. Yang:** None. **H. Yerramreddy:** None. **C. Dagdeviren:** None. **K. Ramadi:** None. **R.S. Langer:** None. **M.J. Cima:** None. **A.M. Graybiel:** None.

Poster

266. Methods: Electrophysiology

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Topic: G.04. Physiological Methods

Support: NSF IGERT 0903622

NSF STC EBICS CBET 090939511

Andrew T. Yang Award

Title: Self-rolled-up 3D microtube arrays for enhanced control and guidance of hippocampal neurons in synthetic circuits

Authors: *O. V. CANGELLARIS[1], P. FROETER[2], X. LI[2], M. U. GILLETTE[3];
[1]Bioengineering, Univ. of Illinois, Urbana, IL; [2]Electrical and Computer Engineerinig, [3]Bioengineering, Cell and Developmental Biol., Univ. of Illinois, Urbana-Champaign, IL

Abstract: Directing neurons to form pre-determined circuits is a fundamental goal of neuroengineering with the intention of treating neurological disorders and neurodegenerative diseases. Until recently, only aggregate populations of neurons were able to be studied and characterized in culture. Through the use of a novel arrayed platform of strain-induced self-rolled-up 3D architectures, we are able to measure and provide new insights into mechanisms driving organization of neuronal architecture at the level of a single cell [1, 2]. The array elements, transparent microtubes (2.7-4.4 m diameter), are formed using an ultrathin silicon nitride (SiNx) film that attaches to transparent substrates under conditions that control the size, site, and pattern of the array. Furthermore, functionalization of this substrate with electrodes allows for local application of electric fields (EFs) to accomplish precise manipulation of the directionality and growth patterns of neurons. In order to establish a baseline for the alignment imposed by the topography of the non-powered (inert) microtubes, we cultured hippocampal neurons from post-natal day 1/2 Long Evans BluGill rats at low-density (~250 cells/mm^2) and developed a method for quantifying the degree of alignment. Compared to control substrates, the neurites were significantly more aligned toward the zero degree reference on the inert microtubes. We then studied neuron growth on powered microtubes to determine how the presence of EFs enhances alignment beyond that imposed by the topography. These interactions will inform future experiments for optimization of the stimulation parameters and platform

geometry in order to tune our control over neuron growth. Application of this technology will enhance our ability to construct intentional neural circuits through array design and manipulation of individual neurons, and can be adapted to address challenges in neural repair and neuroregeneration, such as reinnervation. [1] P. Froeter *et al., Nanotechnology* (2013), [2] P. Froeter *et al., ACS Nano* (2014)

Disclosures: O.V. Cangellaris: None. **P. Froeter:** None. **X. Li:** None. **M.U. Gillette:** None.

Poster

266. Methods: Electrophysiology

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Topic: G.04. Physiological Methods

Support: CIHR

Brain & Behavior Research Foundation

Canadian Foundation for Innovation

Heart and Stroke Foundation

Title: Target-specific modulation of descending prefrontal cortical inputs to the dorsal raphe nucleus by endocannabinoids

Authors: *S. D. GEDDES[1,2], S. ASSADZADA[1], D. LEMELIN[1,2], A. SOKOLOVSKI[1,3], R. BERGERON[3,1], S. HAJ-DAHMANE[4], J.-C. BEIQUE[1,2,5];
[1]Univ. of Ottawa, Ottawa, ON, Canada; [2]Canadian Partnership for Stroke Recovery, Ottawa, ON, Canada; [3]Ottawa Hosp. Res. Inst., Ottawa, ON, Canada; [4]Res. Inst. on Addictions, University at Buffalo, NY; [5]Ctr. for Neural Dynamics, Ottawa, ON, Canada

Abstract: The serotonin (5-HT) system has long been implicated in mood regulation. The coding features of 5-HT neurons *per se* are however complex and multifaceted and it has historically been difficult to capture them in a simple and unifying framework. At least part of this complexity likely arises from the sole nature of the synaptic network in which 5-HT neurons are embedded. Indeed, the dorsal raphe nucleus (DRN), where the majority of 5-HT neurons reside, receives strong innervation from a vast array of subcortical and cortical regions. It has further been historically difficult to study in isolation these descending inputs in order to identify with precision how they modulate the excitability of the DRN subnetwork. For instance, the medial prefrontal cortex (mPFC) sends long-range axons to the DRN but the basic processing features of this input to the DRN is still elusive. The details of this top-down control from the mPFC to the DRN are of particular interest, in part because of its role in stress processing and in mediating antidepressant-like effects. Here, using a combination of immunohistochemistry,

optogenetics and electrophysiological whole-cell recordings we dissect out the functional properties of the mPFC-DRN projections. We found that the mPFC inputs to the DRN: 1) are glutamatergic; 2) mono-synaptically activate both 5-HT neurons and local GABA neurons located primarily in the lateral wings of the DRN; 3) are permissive to strong feedforward inhibition; 4) are modulated by endocannabinoids. We further identify a target-specificity in the CB_1R-mediated neuromodulation of mPFC inputs to the DRN that results in a powerful gating of PFC information flow in the DRN by favoring the direct excitatory drive to 5-HT neurons at the expense of the feedforward inhibition. The precise elucidation of how information flow is dynamically regulated by neuromodulators in this mood-related network may lead to the development of informed pharmacological strategies for the treatment of affective disorders.

Disclosures: S.D. Geddes: None. **S. Assadzada:** None. **D. Lemelin:** None. **A. Sokolovski:** None. **R. Bergeron:** None. **S. Haj-Dahmane:** None. **J. Beique:** None.

Poster

266. Methods: Electrophysiology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 266.06/BB36

Topic: G.04. Physiological Methods

Title: Neck collar: A novel non-invasive system for restricting head and body movements in rats for behavioral task performance and simultaneous neuron activity recording

Authors: *Y. TATEYAMA, K. OYAMA, C. LO, T. IIJIMA, K.-I. TSUTSUI;
Div. Sys Neurosci, Tohoku Univ, Grad Sch. Life Sci., Sendai, Japan

Abstract: In behavioral neuroscience, it is necessary to restrain movement of the head and/or the body of the animals in order to reduce movements irrelevant to the behavioral task. In monkeys, fixation of the head has been a major technique in behavioral neurophysiology. On the other hand, in rodents, most behavioral experiments have been conducted under freely-moving condition. While there is an increasing importance of rodents as a model subject in modern systems neuroscience following recent advancements in molecular biological techniques, there is still a gap between data obtained from head-fixed monkeys and freely-moving rodents. In order to fill this gap, it is necessary to conduct behavioral physiological experiments in rodents under head-fixed condition. However, head-fixation requires surgical procedures to implant the head-fixation device onto the animal's skull, which essentially induce risks of intracranial infection, or necrosis and softening of the bone. Therefore, a head-fixation method that does not require the surgical procedure would be useful. In this study, we developed a novel "neck collar system" for restricting head and body movements during the performance of the behavioral task and simultaneous neuron activity recording. We trained rats to perform a delayed response task, in which rats were required to lick a spout toward the remembered visual cue or away from it after

a delay period with neck collar system. We also conducted single-unit recordings with implanted multi-wire electrode in the medial prefrontal cortex from two rats which were trained in the delayed pro-licking task. We successfully recorded the single-unit activity from the neck-restrained rats with low noise. These results suggest that this system can be used in a wide range of behavioral experiments, including electrophysiological recordings.

Disclosures: **Y. Tateyama:** None. **K. Oyama:** None. **C. Lo:** None. **T. Iijima:** None. **K. Tsutsui:** None.

Poster

266. Methods: Electrophysiology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 266.07/BB37

Topic: G.04. Physiological Methods

Support: Hope Center for Neurological Disorders 2014 Pilot Award

Title: Novel *in vitro* assay of embryonic stem cell-derived neural network properties

Authors: *J. R. GAMBLE, S. SAKIYAMA-ELBERT, D. L. BARBOUR;
Biomed. Engin., Washington Univ. In St Louis, Saint Louis, MO

Abstract: Recent stem cell transplantation therapies for spinal cord injury (SCI) have elicited encouraging though limited improvement in motor and sensory function using spinal cord neural progenitors and embryonic stem cells (ES). This recovery appears to be promoted through various mechanisms, including the creation of new spinal circuits 1) externally, between grafted neuronal populations and the endogenous host circuit, and 2) internally, within the transplant. Current assays of transplant efficacy in animal models of SCI include gross measures of electrophysiological input/output, as well as molecular quantifications of graft neurite outgrowth and synapse formation. Missing is any assessment of the functional connectivity within the graft network, owing to the insurmountable challenges making these measurements *in vivo*. This shortcoming poses a significant barrier to designing effective SCI therapies because functional interactions involving transplanted ES-derived neuronal populations will greatly affect the transmission of activity through the transplant. We have developed a novel high-throughput *in vitro* assay combining ES technology and microelectrode array (MEA) platforms to study functional connectivity of candidate neuronal populations directly for the first time. To induce transplant candidate populations enriched for Chx10-expressing V2a interneurons, which are excitatory and ipsilaterally projecting spinal cord neurons *in vivo*, mouse RW4 ESs were subjected to an appropriate 2-/4+ protocol *in vitro* prior to being dissociated and cultured on 60-channel MEAs. Recorded spike waveforms were sorted offline using thresholding, principal components analysis and k-means clustering. After two weeks in culture, neural network activity

revealed two regimes of activity: synchronized network bursting and spontaneous random firing. Different patterns of putative neuron-neuron connectivity, including monosynaptic and disynaptic connections, were inferred from spike-sorted activity using spike-time cross correlation histograms. The ability to monitor various types of synaptic connections simultaneously across a candidate transplant population has multiple implications: 1) the strength and category of connections can be monitored during manipulations such as electrical stimulation 2) the neurotransmitter(s) underlying these connections can be identified with the use of specific neurotransmitter antagonists and 3) network formation and remodeling can be monitored over extended time periods. These outcomes are essential for rationally improving transplantation therapies for SCI.

Disclosures: J.R. Gamble: None. **S. Sakiyama-Elbert:** None. **D.L. Barbour:** None.

Poster

266. Methods: Electrophysiology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 266.08/BB38

Topic: G.04. Physiological Methods

Support: NSF BRAIN EAGER DBI-1450767

NIH GM008804

Title: Simultaneous detection of dopamine release and neural activity

Authors: *M. L. HEIEN[1], K. L. PARENT[2], D. F. HILL[3], J.-P. WIEGAND[4], M. A. MILLER[4], C. W. ATCHERLEY[8], S. L. COWEN[5,6,7];
[2]Dept. of Chem. and Biochem., [3]Dept. of Physiol., [4]Dept. of Neurosci., [5]Dept. of Psychology, [6]Evelyn F. McKnight Brain Inst., [7]ARL Div. of Neural System, Memory & Aging, [1]Univ. of Arizona, Tucson, AZ; [8]Dept. of Res., Mayo Clin., Scottsdale, AZ

Abstract: Complex behaviors rely on coordinated firing of neurons in local networks which are dynamically regulated by neuromodulators such as dopamine. However, tools for simultaneous monitoring of neural assemblies and dopamine dynamics have not previously been developed due to difficulties in integrating electrophysiological and electrochemical hardware. We have engineered a measurement platform capable of tandem measurements of dopamine and neural activity. This platform consists of high-density electrode arrays for measurement of single-neuron activity and local-field potentials in conjunction with a separate carbon electrode for measurement of real-time dopamine release using fast-scan cyclic voltammetry. Electrochemical instrumentation was improved to allow a common reference to be utilized for both electrophysiological and electrochemical measurements resulting in a higher signal-to-noise ratio for all measurements. To prevent damage to sensitive instrumentation, current between the

implanted electrodes and the neural recording amplifier circuitry is interrupted during the application of the voltammetric waveform. *In vitro* testing was carried out in a porcine gelatin to mimic brain conductivity and 500 μV sine waves with various frequencies (10 - 3000 Hz) near the electrode array) to simulate neural oscillations. It was found that all frequencies tested were rapidly recovered following resumption of neural recordings in relation to the time between waveforms (200 ms).

Disclosures: **M.L. Heien:** None. **K.L. Parent:** None. **D.F. Hill:** None. **J. Wiegand:** None. **M.A. Miller:** None. **C.W. Atcherley:** None. **S.L. Cowen:** None.

Poster

266. Methods: Electrophysiology

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 266.09/BB39

Topic: G.04. Physiological Methods

Support: NIH 1R01NS078095-01A1

Title: Isoflurane dose dependent functional connectivity in the rodent somatosensory cortex

Authors: ***M. NEZAFATI**[1], J. C. W. BILLINGS[2], W. PAN[1], S. D. KEILHOLZ[1]; [1]Biomed. Engin., Georgia Inst. of Technology/Emory Univ., Atlanta, GA; [2]Emory Univ., Atlanta, GA

Abstract: Resting state functional connectivity measurements are sensitive to network alternations caused by neurological and psychiatric disorders. Multimodal studies in animal models help us to understand the basis of these connectivity measurements so the alternations in humans can be interpreted and potentially used for diagnosis or treatment. Local field potential measurements provide a picture of brain function undistorted by hemodynamic filtering. Although utilizing anesthetic agents is inevitable to study the animal models, their effect on the functional connectivity of the brain networks is still unknown. We examined correlated LFP power as a function of sedation depth to characterize functional connectivity in a dose-dependent measure. Male Sprague Dawley rats weighing 200-210 g were acclimated to the operational environment for 10 days prior to the recording day using a custom restraining set up and time frame designed to reduce animal stress during data acquisition. To avoid pain, Lidocaine paste was applied to the sites of mechanical restraint and those exposed during surgery. The animal was anesthetized using 2% Isoflurane for electrode implantation. Interhemispheric connectivity measurement was performed using vertical implantation of Ag/AgCl microelectrodes at the bilateral sites of primary somatosensory of the forelimb. The anesthetic concentration was reduced by the factor of 0.3% every 45 minutes till it reached 0.5%. The data was collected every 10 minutes at the rate of 1000Hz for 420 seconds. The animals' physiological parameters were

monitored during the experiment no sign of struggle or stress was observed even at very low doses. The acquired data shows significant correlation among the bilateral regions of somatosensory cortex that increased in proportion to the concentration of anesthetic agent. These findings are in agreement with BOLD -LFP based measurements made by Pan et al [1] and shows that the change in correlation has a neural origin and is approximately linear even at very low doses. [1] Pan, W.-J. et al. (2011). Brain Connectivity, 1(2), 119-131.

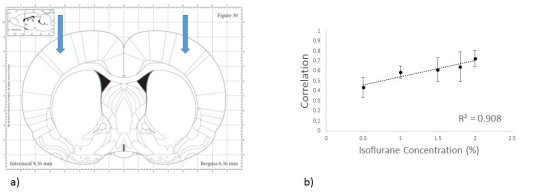


Figure1) a- Illustrates the coronal view of somatosensory cortex and the location of implanted electrodes; b- shows the effect of anesthetic concentration between hemispheres

Disclosures: **M. Nezafati:** None. **J.C.W. Billings:** None. **W. Pan:** None. **S.D. Keilholz:** None.

Poster

266. Methods: Electrophysiology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 266.10/BB40

Topic: G.04. Physiological Methods

Support: Intramural Research Program of the National Institute of Mental Health

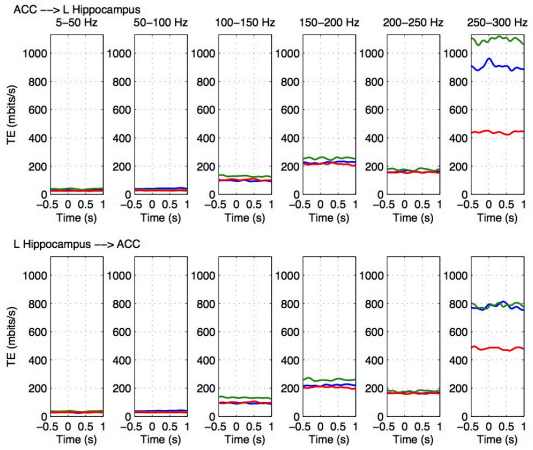
Fetzer Memorial Trust - Fetzer Franklin

Title: Understanding frequency dependencies in transfer entropy estimates

Authors: *S. E. ROBINSON[1], A. J. MANDELL[2];
[1]MEG Core Group, NIH/NIMH, Bethesda, MD; [2]Psychiatry, Univ. of California San Diego Sch. of Med., La Jolla, CA

Abstract: We have applied a unique temporo-dynamic symbolic transfer entropy (tdSTE) analysis to a MEG recording of normal subject performing a working memory (n-back) task, in order to determine if directional information transfer is distributed uniformly across all frequencies. Data were acquired from a 275-channel instrument (CTF Systems, Inc.) at a sample rate of 1200 Hz in a bandpass of DC-300 Hz. A comb-notch filter removed 60 Hz and its harmonics. Data consisted of 18 blocks of 25-seconds duration, alternating among 0, 1, and 2-back tasks. Transfer entropy (TE) was estimated independently for each of the tasks for 5-50 Hz,

50-100 Hz, 100-150 Hz, 150-200 Hz, 200-250 Hz, 250-300 Hz, and 50-300 Hz. TE magnitudes were lowest for the 5-50 Hz bandpass, peaked at 150-200 Hz (375 mbits/sec), and then rose sharply at 250-300 Hz (see figure, for example). Rhythmic activity and oscillations reduce the TE estimates, explaining why the 5-50 Hz band had the smallest TE values. At the highest frequencies one would expect TE to be smallest due to the declining signal-to-noise ratio. However, the observed sharp rise in TE for the 250-300 Hz (as large as 1,100 mbits/sec) suggests that multi-unit activity rather than local field potentials dominate this frequency range. This was tested against empty MSR data to determine if this was due to instrumental noise. Differences in information transfer for each of the n-back tasks could be observed for all frequency ranges, including the 50-300 Hz bandpass. In conclusion, we caution that TE estimates should not be interpreted as measures of absolute connection strength.



Disclosures: **S.E. Robinson:** None. **A.J. Mandell:** None.

Poster

266. Methods: Electrophysiology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 266.11/BB41

Topic: G.04. Physiological Methods

Support: Carnot Institute

CONACYT Scholarship

Title: A millisecond short-latency reprogrammable silicon chip solution for the pre-processing of electrophysiological data: Towards a real-time closed-loop approach

Authors: L. S. MONDRAGON[1], N. OUARTI[2], *E. BURGUIERE[3];
[1]Brain and spine Inst. - UPMC – INSERM U1127 – Pitie-Salpetriere Hosp., PARIS, France;

[2]Inst. des Systèmes Intelligents et de Robotique, Univ. Pierre et Marie Curie-Paris 6, CNRS UMR 7222, PARIS, France; [3]BEBG Team, 3eme etage, Brain and spine Inst. - UPMC – INSERM U1127 –, Paris, France

Abstract: Closed-loop electrophysiology systems require to detect and respond to neural events within short and strict timing constraints of millisecond range. Currently, this approach has been mainly performed in processor-based systems with the major inconvenient that algorithms are executed under general purpose operating systems that are sensitive to dispatch latency variability. Thus, to improve the closed-loop system approach, there is a necessity to develop a platform that improves the throughput from neural data streams to signal processing within well-controlled short time limit. These new systems would open a wide possibility for accurate closed-loop experimentation and brain-computer interfaces. To address this question we used a field-programmable gate array (FPGA) to propose a modular design for automatic fast pre-processing, including artifact removal and action potential detection. Designs were carefully optimized by implementing low-memory algorithms to reduce time response below a millisecond range. Here, we present a design that has been tested using intra- and extra-cellular *in vivo* recordings, and synthetic data. This design has been validated with a set of performance indicators that confirm the feasibility of hardware implementation of this system. The benefit of this approach makes it suitable for time-critical tasks such as online closed-loop optogenetic interventions, as the artifact removal module avoid false positives in action potential detection within sub millisecond processing time. By taking advantage of FPGA parallelism and the short latency of this approach we discuss the possibility of adding other data processing modules. As an example, we will discuss the pertinence of an automatic pattern recognition standalone module to discover biomarkers related to behavioral events in a mouse model of obsessive compulsive disorders.

Disclosures: **L.S. Mondragon:** None. **N. Ouarti:** None. **E. Burguiere:** None.

Poster

266. Methods: Electrophysiology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 266.12/BB42

Topic: G.04. Physiological Methods

Support: NIH Grant P41-RR14075

Title: Whole-brain, sub-second data collection with little cost using simultaneous multi-slice/multiband acquisition in task-evoked fMRI

Authors: *S. A. MCMAINS[1], R. M. HUTCHISON[1], R. W. MAIR[1,2];
[1]Ctr. for Brain Sci., Harvard Univ., Cambridge, MA; [2]Dept of Radiology, Harvard Med. Sch., Athinoula A Martinos Ctr. for Biomed. Imaging, Charlestown, MA

Abstract: Slice-accelerated EPI using multiband (MB) RF pulses that allow for simultaneous multi-slice (SMS) acquisition of BOLD contrast images can significantly enhance the temporal and spatial resolution of fMRI by acquiring up to 8 non-contiguous slices at the same time, thus enabling whole-brain sub-second TRs. Here we studied visual cortex response at a variety of MB accelerations and TR reductions to investigate whether there were any costs associated with parameters that allowed for whole-brain, sub-second data collection at 2mm resolution. 6 subjects were scanned (3.0T Siemens Tim Trio) with a 32-ch head coil while they performed a fixation task and blocks of flashing checkerboards were presented to alternating visual fields. BOLD scans were acquired at 3mm and 2mm isotropic resolutions, a max TR of 3s, and MB accelerations of 0 (conventional BOLD sequence), 1 (MB sequence, no acceleration), 4 and 8 (Siemens WIP 770A). Beta and t-statistics were extracted from regions localized with a separate scan in visual cortex. With a TR of 3s, there were 91 timepoints, while for TR= 1.25/0.75/0.7s, there were 184/307/328 timepoints. There were no significant differences in betas for any parameters, or in t-statistics for levels of MB when holding the TR constant. Shortening the TR increased t-statistics significantly. This advantage was reduced when temporal autocorrelations in the noise were modeled. An event-related study was also conducted for 2mm voxels to compare 3s TR (MB1) versus 750ms TR (MB8). Betas were larger for the MB8 scans, likely due to improved characterization of the hemodynamic response, even though stimulus onset was jittered to the TR. The results suggest that whole brain coverage with high spatial and temporal resolution can be achieved using SMS with little to no cost in terms of BOLD signal sensitivity, as measured by betas and t-statistics, even though time-series SNR decreased significantly at high MB factors.

Disclosures: S.A. McMains: None. **R.M. Hutchison:** None. **R.W. Mair:** None.

Poster

266. Methods: Electrophysiology

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 266.13/BB43

Topic: G.04. Physiological Methods

Title: Point source networks: relationship between dynamics of cortical/sub-cortical neuron firing and regional cortical imaging in mouse cortex

Authors: *D. XIAO[1,2], M. P. VANNI[1], A. CHAN[1], A. C. CHEN[2], T. H. MURPHY[1];
[1]Kinsmen Lab, Dept. of Psychiatry, Vancouver, BC, Canada; [2]Beijing Inst. for Brain Disorders, Capital Med. Univ., Beijing, China

Abstract: A more complete understanding of brain function will require measurement techniques which monitor large-scale neuronal activity across multiple brain areas and relate this to single neuron properties. We present a step towards achieving this aim: by simultaneous *in vivo* recording of cortical/subcortical single unit activity while wide-field cortical imaging during spontaneous activity. We use glass pipettes to record single unit activity in target sites, and use wide-field cortical imaging (GCaMP) to determine large-scale neuronal activity. By employing spike-triggered averaging we established temporal relations between single neuron firing and regional cortical calcium imaging. We apply the method in cortex and establish expected regional maps that are associated with spiking activity at single cortical points. Extension of the electrophysiological assessment to sub-cortical structures permits the relationships between these regions and maps of cortical activity to be determined. We find that unique wide-field regional cortical calcium imaging patterns are highly correlated with spiking activity from single neurons. Spike evoked maps should be useful for assessing the functional relationship between single neurons and wide-field activity in *in vivo* models of neurological disease.

Disclosures: D. Xiao: None. **M.P. Vanni:** None. **A. Chan:** None. **A.C. Chen:** None. **T.H. Murphy:** None.

Poster

267. Electrode Arrays I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 267.01/BB44

Topic: G.04. Physiological Methods

Support: CIHR

Polaris

Title: The use of thomas electrodes and traditional tetrodes in an *in vitro* reloadable hyperdrive

Authors: *M. KESLER, H. STEENLAND, K. LIN, B. L. MCNAUGHTON;
Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: Electrode technology for extracellular multi- and single- unit recording typically involves either rigid single wire electrodes which can penetrate both deep and superficial structures or nichrome tetrodes which have an excellent ability to isolate neurons for long periods of time but tend to lack structural rigidity. Thomas quartz-glass electrodes possess both structural rigidity and potential tetrode configurations to better isolate neurons from both superficial and deep cortical layers. While these electrodes have been on the market for some time, their use has limited due to their cost and inherent difficulties in their preparation. Moreover, there are no reports for the use of these electrodes in high-density hyperdrives. Here, we standardized a protocol for the use of Thomas single electrodes and tetrodes in high-density

Kloosterman-style hyperdrives. We find that quartz glass electrode material costs could be cut in half by simply tapering electrodes with fused/molten sodium hydroxide and beveling them with a grinder. We also show that sodium hydroxide is an excellent etchant to remove the insulation from the electrode so that electrical contact can be made. Furthermore, we present a flexible connection and new design for an electrode interface board to permit easy connection and disconnection from recording electronics. Flex connectors also permitted replacement of defective electrodes from a hyperdrive and subsequent replacement in an awake unrestrained rat, extending the life and quality of recordings. We also present data which show that single Thomas electrodes can penetrate rat dura matter and in some cases bone window for high quality recording of both superficial (i.e. cortex) layers and deep brain structures (e.g. ventral tegmental area and reuniens nucleus), with stability over multiple days. Furthermore, Thomas electrodes were also found to be ideal for reliably detecting high-amplitude positive spikes (HAPS) (as previously described by Gold et al., JNP, 2009 and Guld, Med. Elect. Bio. Engng. 1964) and so they offer insight into the identity of these rare cells.

Disclosures: **M. Kesler:** None. **H. Steenland:** None. **K. Lin:** None. **B.L. McNaughton:** None.

Poster

267. Electrode Arrays I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 267.02/BB45

Topic: G.04. Physiological Methods

Support: Hungarian Scientific Research Found (OTKA) K113147

EU-FP7-ERC-2013-Starting grant (No. 337075)

the ‘Momentum’ program of the Hungarian Academy of Sciences (LP2013-62/2013)

the ‘Excellence’ program of the Hungarian Academy of Sciences (KEP-1.2/2014)

Title: Determination of spatio- temporal input current patterns of single hippocampal neurons based on extracellular potential measurements

Authors: *Z. SOMOGYVARI[1,2], Z. BENKO[1], J. Z. JALICS[3], L. ROUX[4], A. BERENYI[4,5]; [1]Dept. of Theory, Wigner Res. Ctr. For Physics, Budapest, Hungary; [2]Dept. of Neurol. and Epileptology, Natl. Inst. of Clin. Neurosciences, Budapest, Hungary; [3]Dept. of Mathematics and Statistics, Youngstown State Univ., Youngstown, OH; [4]Sch. of Med., The Neurosci. Inst. New York Univ., New York, NY; [5]MTA-SZTE ‘Momentum’ Oscillatory Neural Networks Res. Group, Univ. of Szeged, Dept. of Physiol., Szeged, Hungary

Abstract: One of the main obstacle to decipher the information processing and the neural communication in the brain is the lack of any experimental technique which is able to measure

the spatio-temporal distribution of synaptic currents on individual neurons in freely behaving animals. Thus, we developed a new micro electric imaging technique, which is able to determine the currents flowing on single hippocampal, neocortical or thalamic neurons during action potentials based on the extracellular electric potentials recorded by micro electrode array. We have shown the differences of cell-type specific input current patterns preceding and causing the action potentials during different oscillatory states of hippocampus. The layers and subfields of the hippocampus have been identified based on the recorded electrical signals, by using our electroanatomy concept and latter verified by histology. The types of the extracellular recorded and clustered cells were determined based on their electrophysiological characteristics and their spatial tuning. Analyzing the temporal dynamics of the cell type specific micro-field potentials we have found, that the onset of the synaptic currents preceding the action potential was the shortest in the CA1 region: 8.9 and 11.4 ms for pyramidal and interneurons respectively). We have found longer onset times in the dentate gyrus (12.4 ms for granular cells and 16.7-31.2 ms for interneurons). Finally, the longest onset times were found in the CA3 pyramidal neurons (40 ms), while the onset times of the interneurons were shorter in this region (7.6-21 ms). As the dynamics of the total synaptic current is depending on the natural statistics of the synaptic activations, measuring the temporal aspects of net synaptic currents could lead to better understanding of the neural code, by refining our knowledge about the input-output transformation implemented by the neurons.

Disclosures: Z. Somogyvari: None. **Z. Benko:** None. **J.Z. Jalics:** None. **L. Roux:** None. **A. Berenyi:** None.

Poster

267. Electrode Arrays I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 267.03/BB46

Topic: G.04. Physiological Methods

Title: The open ephys gui: Plugin-based software for high-channel-count electrophysiology

Authors: *A. CUEVAS LOPEZ[1], Y. A. PATEL[2], J. VOIGTS[3], J. H. SIEGLE[4];
[1]Dept. de Ingeniería Electrónica, Univ. Politècnica De València, Valencia, Spain; [2]Georgia Inst. of Technol., Atlanta, GA; [3]MIT, Cambridge, MA; [4]Allen Inst. for Brain Sci., Seattle, WA

Abstract: Software for acquiring extracellular electrophysiology data should ideally be robust, scalable, and easy to extend. To meet these requirements, we have developed the Open Ephys GUI, an open-source graphical user interface for processing electrophysiology data based on a plugin architecture. Essential functions for electrophysiology experiments, from filtering to spike detection to visualization, are encapsulated within discrete modules, which can be compiled separately from the base application. The behavior of each module can be customized

independently, without the need to understand the entire code base. This makes it much easier for end users to tailor the software to the specific needs of their experiments. Our software was developed in C++ (based on the JUCE library), but plugins can be written in Python or Julia, a language with Matlab-like syntax. Although plugin-based applications are the standard in the audio recording industry, they have yet to catch on for extracellular electrophysiology. The advantages of such an architecture are most apparent when real-time signal processing is needed for the purpose of delivering closed-loop feedback. Closed-loop experiments have the potential to greatly enhance our understanding of neural function, but are not widely used due to the difficulty of implementing them with currently available tools. We hope that as more neuroscientists contribute general-purpose, reusable modules to the Open Ephys GUI, such experiments will become more commonplace as the barrier to entry is lowered.

Disclosures: **A. Cuevas Lopez:** None. **Y.A. Patel:** None. **J. Voigts:** None. **J.H. Siegle:** None.

Poster

267. Electrode Arrays I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 267.04/BB47

Topic: G.04. Physiological Methods

Support: Pirogov Russian National Research Medical University Grant 340-OK-14

Title: Analysis of channel cross-talk in microwire bundles: microwires go far enough from each other to record activity of different neurons

Authors: *L. N. VASILEVA, I. V. BONDAR;
Inst. of Higher Nervous Activity, Moscow, Russian Federation

Abstract: Single-unit extracellular recording continues to be a widely-used method to study brain activity over relatively short periods of time. Chronic recordings with implantable devices can reveal long-term changes in brain activity critical for complex behaviors. We make use of Ni-Cr microwire bundles to maintain high signal-to-noise ratio over time (Kruger et al., 2010) and record activity of the same single-units for up to one year (McMahon et al., 2014). In contrast to arrays with regularly spaced recording sites, each recording site on the microwire bundles has a unique trajectory while being inserted into the brain and the experimenter has no information regarding individual microwire positions. Despite this, statistical methods can assess relative distances between microelectrodes by assessing the similarity of observed activity between different channels. In this study we aimed to define channels recording the same neuronal activity by multidimensional scaling and cross-correlation analysis. Recordings were performed in hippocampus of 9 chronically-implanted rats and in one anesthetized rat. First, we assessed relative distance between microwires by multidimensional scaling. We calculated

correlation coefficients between local field potentials (0-500 Hz) of 100 ms length on different channels as a similarity measure. Then we applied the multidimensional scaling method to establish relative distances between microwires. Microwires turned out to gather into consistent clusters and the indicated positions remained highly stable regardless of which data segment was analyzed. Our second goal was to evaluate the probability of recording the activity of the same single unit on different microwires within the bundle. For that purpose cross-correlation analysis was used. Raw signal was band-pass filtered (500-10000 Hz) and action potentials were detected by crossing an amplitude threshold. We used principal component analysis for spike sorting (implemented on Spike2 software, Cambridge Electronic Design, UK). Finally, we assessed the cross-correlation between the activity of single-units on different channels. A prominent central peak in cross-correlation histograms suggested the same single unit was present on two of the channels. When compared to the remaining channels, only 6 % exhibited similarly correlated single-unit activity.

Disclosures: L.N. Vasileva: None. **I.V. Bondar:** None.

Poster

267. Electrode Arrays I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 267.05/BB48

Topic: G.04. Physiological Methods

Support: RFBR Grant 13-04-12094

RFBR Grant 13-04-12078

Title: Long-term isolation of same single unit activity: stability criteria and estimation of longevity

Authors: *I. V. BONDAR[1], L. N. VASILEVA[1], A. M. BADAKVA[2];
[1]Inst. of Higher Nervous Activity and Neurophysiol. RAS, Moscow, Russian Federation; [2]RF SRC-Institute of Biomed. Problems RAS, Moscow, Russian Federation

Abstract: Once started neuronal activity persists for the whole cell lifespan. Neuronal discharge magnitude can vary depending on the brain state: arousal, sensory information flow, administration of chemical substances etc. To establish main principles of brain function we need to track activity of single and multiple neurons over extended period of time. Currently, technical capabilities allow to place registration device in close proximity of the neuron and leave it there for long-term single-unit recording. More recently, possibility to observe activity of the same neuron for a long period of time met serious criticism, but recent publications provide a new point of view on the problem of stable neuronal recordings (McMahon et al., 2014). It is important to elaborate well-defined criteria for evaluation of stability of recorded activity

(Jackson & Fetz, 2007; Dickey et al., 2009; Fraser & Schwartz, 2012). Taking in account background discharge of neurons we made attempt to develop stability criteria for evaluation of neuronal activity. Two rhesus macaques were implanted with guide tubes over M1 under general anesthesia. Each microwire bundle contained 16 microwires (diameter 12 or 18 μm) insulated with polyimide. Microwire bundles were inserted into M1 through the guide tubes. Neurophysiological recordings were performed every 3-4 days. Spikes were detected by crossing an amplitude threshold in raw data with Spike 2 software (CED, UK). Correlation coefficients were calculated between average spike forms belonging to the same and different neurons. Stable recorded neurons showed high level correlation coefficients between average spike forms (over 0.98). Based only on the spike forms classification of stable recorded units yields ambiguous result. Additional comparison form of interspike interval histogram decreased misclassification probability considerably. Using proposed criterion we were able to identify 82 neurons that were recoded for more than one day. In a remarkable case activity of one neuron was observed for 94 days.

Disclosures: I.V. Bondar: None. **L.N. Vasileva:** None. **A.M. Badakva:** None.

Poster

267. Electrode Arrays I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 267.06/BB49

Topic: G.04. Physiological Methods

Support: NINDS Grant U01NS090557

Title: Next generation of large-scale, semi-chronic micromanipulator systems for measuring neuronal activity in distributed neural circuits 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 large-scale, semi-chronic recording instruments that permit the implantation of hundreds of independently movable microelectrodes in behaving non-human primates. These devices can be flexibly configured to enable the long-term measurement of neuronal activity from distributed circuits spanning the depth and breadth of the brain. The instruments are now in widespread use in laboratories in North America, Europe and Asia. Findings from these studies have revealed several weaknesses in our original designs. These include inconsistencies in the reliability of the

actuator mechanism, problems associated with fluid leakage into the devices, and limitations in the manufacturing of the devices. Here, we report results from new designs that eliminate or alleviate each of these potential problems. Electrode position is controlled by rotation of a fixed lead screw (125 μm/turn resolution) that advances or retracts a cylindrical shuttle to which the electrode is bonded. We have greatly improved the reliability of this design by constructing the shuttle to have a cross-sectional teardrop shape and by fabricating the actuator block with matching hole dimensions using 3D printing technology. We have also implemented a procedure to inject a calibrated amount of sterile silicone grease into the electrode guide holes on the bottom of the actuator block. Together these design changes have significantly improved the performance and reliability of the system. Future improvements will include 1) reduction of the inter-electrode spacing to permit higher density recording, 2) the incorporation of injection cannulae for intra-cerebral drug injection, 3) longer lead screws to permit access to the ventral surface of the brain, and 4) the incorporation of zero-insertion-force connectors to increase reliability and ease of connecting to the headstage amplifiers.

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

267. Electrode Arrays I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 267.07/BB50

Topic: G.04. Physiological Methods

Support: The National Research Foundation of Korea (NRF) Grant 2014R1A2A2A09 052449

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The Smart IT Convergence System Research Center funded by the Ministry of Education, Science and Technology as Global Frontier Project (CISS-2012M3A6A6054204)

Title: Ultrasound-induced long term potentiation of the spontaneous neural activity recorded from hippocampal neurons cultured on multi-electrode arrays

Authors: ***S. HWANG**[1], H. JEONG[1], S. KIM[1], Y. LEE[1], T.-S. KIM[3], S. JUN[2,4]; [2]Dept. of Electronics Engin., [1]Ewha Womans Univ., Seoul, Korea, Republic of; [3]Kyung Hee Univ., Gyeonggi-do, Korea, Republic of; [4]3Department of Brain and Cognitive Sci., , Ewha Womans Univ., Seoul, Korea, Republic of

Abstract: Ultrasound is an emerging non-invasive neuromodulation approach due to its advantages of the noninvasiveness, high spatial selectivity and high penetrating power. In the past few years, several studies have been performed to examine the effect of ultrasonic

neuromodulation in an animal model. However, the underlying mechanisms have not been clarified yet. In the previous study, we reported that the low-intensity, low-frequency ultrasound (LILFU) increases the neuronal activity using cultured hippocampal neurons on microelectrode arrays with simultaneous calcium imaging. In the present study, we showed that ultrasound induced the long term potentiation (LTP) of spontaneous neural activity. Primary hippocampal neurons were dissociated from embryonic 17-day gestation Sprague Dawley rat hippocampi and seeded on microelectrode arrays at the density of 1500 cells/mm2. After DIV 14, LILFU was applied at the intervals of 20 min to the cultured neural networks and the individual spontaneous action potentials (APs) were recorded before, during and after ultrasound stimulation for 4 times and analyzed. Repeated ultrasound stimulation lead to the increase of the frequency of APs and the increased activity remained even after 20 min resting time in the absence of ultrasound stimulation. Additionally, in order to figure out the synaptic change after ultrasound application, immunocytochemistry was performed. This study indicates that ultrasound can induced the synaptic changes in order to treat various neurological diseases.

Disclosures: **S. Hwang:** None. **H. Jeong:** None. **S. Kim:** None. **Y. Lee:** None. **T. Kim:** None. **S. Jun:** None.

Poster

267. Electrode Arrays I

Location: Hall A

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Topic: G.04. Physiological Methods

Support: Fogarty International Center of the NIH R21TW009384

NSF IIP Div Of Industrial Innovation & Partnersh, Eng award# 1248654

NSF IIP Div Of Industrial Innovation & Partnersh, Eng award# 1430833

Title: Semi-automated high-frequency oscillation detection from tripolar concentric ring electrode electroencephalography

Authors: ***W. G. BESIO**[1], M. ABTAHI[2], I. E. MARTÍNEZ-JUÁREZ[3], J. N. GAITANIS[4]; [1]Electrical Computer and Biomed. Engin. Dept., Univ. of Rhode Island/Cremedical Corp., Kingston, RI; [2]Electrical Computer and Biomed. Engin. Dept., Univ. of Rhode Island, Kingston, RI; [3]Epilepsy Clin. and Clin. Epileptology Fellowship, Mexico's Natl. Inst. of Neurol. and Neurosurg. MVS, and Natl. Autonomous Univ. of Mexico, Mexico City, Mexico; [4]Pediatric Neurol., Tufts Med. Ctr., Boston, MA

Abstract: Epilepsy is one of the most frequent neurological diseases with an overall incidence between 0.5 and 1% [1]. Electroencephalography (EEG) records the electrical activity from large

groups of neurons in the brain. The EEG has excellent temporal resolution however, it typically has poor signal quality due to movement artifacts and muscle contractions. To overcome these problems with EEG, tripolar concentric ring electrodes (TCREs) have been developed by Besio [2]. Two bipolar differences of the potentials from the three closely spaced elements perform the tripolar Laplacian derivation first described in [2] as a weighted sum. It was shown that, compared with conventional EEG signals, tripolar EEG (tEEG) has nearly 4-fold (374%) the signal to noise ratio and less than a tenth (8.27%) the mutual information [2-3]. Further, there are reports that high-frequency oscillations (HFOs) can be attributed to epileptic brain tissue [1, 4] but are not consistently detected in scalp EEG. Methods: Our recording protocol was approved by the IRB committees and did not interfere with the clinical EEG recording and evaluation. The tEEG recordings, from eight patients having seizures, were performed concurrently with the clinical EEG with TCREs place just behind the EEG sensors. We used a modified version of the algorithm reported by [4] for detection of HFOs. We performed the Fourier transform to calculate the power spectrum over consecutive one second epochs using a Hamming window and summed the power for each frequency within the window. A threshold was set at the mean plus one standard deviation of the spectrum to determine where/when HFO activity was present. Results: We found that in all eight patients we were able to detect HFOs in the tEEG but none in the EEG. The semi-automatic detection worked well for all patients directing us where to look for HFOs. We were able to detect HFOs up to two-hours prior to the clinical seizures and up to 425 Hz all from the scalp surface. Conclusion: Using the threshold of the power spectrum lead to HFO detection in the tEEG for these eight patients. [1] Jacobs J, et al. "High-frequency oscillations (HFOs) in clinical epilepsy" Prog in Neurobiol 2012; 98(3): 302-315. [2] Besio WG., et al. "Tri-polar concentric ring electrode development for Laplacian electroencephalography" IEEE Trans BME 2006; 53(5): 926-933. [3] Koka, K, and WG. Besio. "Improvement of spatial selectivity and decrease of mutual information of tri-polar concentric ring electrodes." J. of Neurosci Meth 2007;165(2): 216-222. [4] Gardner Ab., et al. "Human and Automated Detection of High Frequency Oscillations in Clinical Intracranial EEG Recording" J. of Clin Neurophys 2007; 118: 1134-1143.

Disclosures: **W.G. Besio:** A. Employment/Salary (full or part-time):; CREmedical Corp. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); stock. **M. Abtahi:** None. **I.E. Martínez-Juárez:** None. **J.N. Gaitanis:** None.

Poster

267. Electrode Arrays I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 267.09/BB52

Topic: G.04. Physiological Methods

Support: NIH Grant NS0885762 (KLS)

Title: Miniature wireless and batteryless device for longitudinal recording and stimulating of bioelectric events in small animals

Authors: *K. L. SEBURN[1], R. BERCICH[2], Z. WANG[3], D. PEDERSON[3], H. MEI[2], P. P. IRAZOQUI[2];
[1]The Jackson Lab., Bar Harbor, ME; [2]Weldon Sch. of Biomed. Engin., [3]Electrical and Computer Engin., Purdue Univ., West Lafayette, IN

Abstract: Longitudinal collection of bioelectric data in small animals such as mice and rats is typically performed using tethered headstages or battery-powered implants which, despite offering some sophisticated features, still pose practical and experimental limitations to the testing environment and device lifespan. To overcome these obstacles a fully wireless (telemetry and power) miniature, chronically implantable recording and stimulating device has been developed that employs resonantly coupled filter energy transfer (RCFET) to provide system power. This device and its auxiliary hardware represent a comprehensive and non-proprietary solution for chronic collection and stimulation of bioelectric events. Once implanted the device is able to non-invasively and continuously record electrophysiological information and stimulate excitable tissue in conscious, untethered, freely behaving animals. The device is equipped with two differential recording channels and one stimulating channel. All device logic is mediated by an ARM® Cortex™ M0 microcontroller. Bidirectional communication between the device and a base station allows for adjustment of functional specifications such as sampling rate up to 25kS/s, ADC resolution, and stimulus parameters. Data is telemetered to the base station and then to a custom software application for real-time display and storage. The power needed to drive the microelectronics is continuously coupled between an external coil and a thin (0.13mm), flexible harvesting coil. With a total volume of less than 0.2cc and mass of 0.7g, it is smaller and lighter than any commercially available system with the same or fewer capabilities. To date, we have successfully recorded electromyograms (EMG) of voluntary evoked muscle activity from conscious, freely moving wild-type rats and mice for several consecutive days. We also recorded spontaneous fibrillations/fasciculations from a conscious mouse carrying a laminin α2 mutation that causes muscular dystrophy (MDC1A). This type of aberrant, spontaneous muscle activity is characteristic of this model, and it is an example of the type of data that is now accessible, but that has previously been technically difficult or impossible to obtain longitudinally from conscious animals. Additional data collection using other mouse models of neuromuscular disease is currently underway. Importantly, although the current emphasis is on EMG recording to facilitate longitudinal study of our neuromuscular disease models, the system is versatile such that with only minor modifications to the electrode configuration, it may find utility for other neurophysiological recordings.

Disclosures: K.L. Seburn: None. **R. Bercich:** None. **Z. Wang:** None. **D. Pederson:** None. **H. Mei:** None. **P.P. Irazoqui:** None.

Poster

267. Electrode Arrays I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 267.10/BB53

Topic: G.04. Physiological Methods

Support: NSF 1152658

Title: The path of least resistance: minimizing vascular damage from electrode array insertions

Authors: *W. J. JOHNSTON[1,2], N. GAUDREAULT[2,3], D. DENMAN[2], B. LONG[2], H. PENG[2], T. J. BLANCHE[2,3];
[1]Univ. of Chicago, Chicago, IL; [2]Allen Inst. for Brain Sci., Seattle, WA; [3]Univ. of California, Berkeley, Berkeley, CA

Abstract: Extracellular electrophysiological recordings are an essential tool for studying neural activity. However, the signal quality, number of isolatable single units, and stability over time can be extremely variable from one implantation to the next. One possible explanation for this variability is the extent of damage to the vasculature caused by the electrode, and the concomitant local hypoxia and inflammatory response. Targeting electrode insertions to avoid the microvasculature may thus improve recording reliability. Preserving the integrity of the blood-brain-barrier may also improve the outcome of chronic implants. Here, we study the disruption of blood vessels caused by planar silicon probes. We developed a detailed 3D neurovascular model in Vaa3d (http://vaa3d.org) based on high resolution *in vivo* 2-photon imaging of mouse cortical neurovasculature using fluorescently labeled dextrans, up to a depth of 600µm. Our preliminary results, based on manually estimating the fraction of cut vessels, indicated that damage at the insertion site and the surrounding neuropil increases supra-linearly with probe size. The smallest probes (25µm wide) induced uniform damage over all depths, whereas larger probes (>100µm) produced a conical zone of damage with greater vascular disruption in deeper layers. We used the model to expand on this result by simulating the insertion of electrodes of various sizes, tiling all possible locations and orientations within an image volume. These simulations allow us to estimate the damage from a “blind” insertion of arbitrary electrode size with respect to number, length, diameter and predominant orientation of vascular segments in the ~0.5mm3 volume of imaged tissue. Such maps can also be used for targeting actual probe insertions. We confirmed our model’s predictions by comparing reconstructions obtained before and after a real electrode insertion. Finally, we used our model to explore several potentially crucial predictors of damage, such as intersection of large feeder vessels that are important for regional circulation, and show that, with respect to these more specific metrics, larger electrodes may be successfully targeted to achieve an impact similar to that caused by a much smaller electrode inserted blindly. Results from this study demonstrate the value of developing smaller, high-density electrode arrays, and suggest that actively avoiding intracortical microvasculature may improve neural recording outcomes.

Disclosures: **W.J. Johnston:** None. **N. Gaudreault:** None. **D. Denman:** None. **B. Long:** None. **H. Peng:** None. **T.J. Blanche:** None.

Poster

267. Electrode Arrays I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 267.11/BB54

Topic: G.04. Physiological Methods

Support: DARPA Grant W911NF-14-1-0172

DARPA Grant W911NF-14-1-0156

Title: A High-resolution flexible electrode manufacturing with a platform 3-d printing system

Authors: ***D. R. MERRILL**, T. J. PARK, C. F. SMITH, D. A. MCDONNALL, K. S. GUILLORY;
Ripple, Salt Lake City, UT

Abstract: We describe a flexible electrode manufacturing process based on robotic deposition of alternating layers of nonconductive polymers and polymers doped with electrically conductive particles forming the electrodes and interconnections. This process facilitates production of composite structures with feature sizes below 100 μm, and supports synthesis of the electrode array and encapsulation of integrated electronics at low cost. We have performed flexural testing on electrodes, demonstrating electrical and mechanical reliability for over 100 million flexions. As an initial application, we have developed a wireless recording system for short-term electrocorticographic (ECoG) monitoring. Reliable monitoring is necessary to guide accurate resection of cortical seizure foci in epilepsy patients who do not respond to drug therapies, but who are candidates for surgical treatment. State-of-the-art monitoring relies on percutaneous leads which may be a source of infection, and relatively stiff electrode arrays which may cause hemorrhaging and unacceptable foreign body response. The system described here comprises a wireless transcutaneous system using infrared data transmission which mitigates infection risk, and a manufacturing system for production of highly flexible electrode arrays which mitigates the foreign body response of traditional electrodes, together allowing the appropriate substantially larger epilepsy patient base to receive surgical treatment. Although the system design targets an underserved epilepsy population, the solutions offered by these technologies are also well-suited to providing a long-term brain-machine interface.

Disclosures: **D.R. Merrill:** A. Employment/Salary (full or part-time):; Ripple LLC. **T.J. Park:** A. Employment/Salary (full or part-time):; Ripple LLC. **C.F. Smith:** A. Employment/Salary (full or part-time):; Ripple LLC. **D.A. McDonnall:** A. Employment/Salary (full or part-time):; Ripple LLC. **K.S. Guillory:** A. Employment/Salary (full or part-time):; Ripple LLC.

Poster

267. Electrode Arrays I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 267.12/BB55

Topic: G.04. Physiological Methods

Title: Highly-flexible package and silk fibroin assisted needle penetration for 5-μm-diameter neuroprobe device

Authors: *D. TEO[1], H. SAWAHATA[1], S. YAMAGIWA[1], A. MORIYA[1], H. OI[2], Y. ANDO[2], R. NUMANO[3], M. ISHIDA[1,2], K. KOIDA[2], T. KAWANO[1];
[1]Dept. of Electrical and Electric Information Engin., [2]Electronics-Inspired Interdisciplinary Res. Institute(EIIRIS), [3]Dept. of Envrn. and Life Sci., Toyohashi Univ. of Technol., Toyohashi-Shi, Japan

Abstract: To achieve low invasive neuronal activity recordings, we have proposed a very fine (<10 μm in diameter) and high-density multi-electrode array fabricated by the selective vapor−liquid−solid (VLS) growth of silicon 'whisker' wires (Fujishiro et al., 2014). However, it is difficult to insert such out-of-plane needle-electrode into certain parts of the brain such as the subdural spaces and the inner sulcus due to the rigid, large silicon substrate (>5 mm × 8 mm). Here we newly developed a neural electrode device with the polyimide-based flexible printed circuit (FPC, 12.5 μm in thickness). The silicon whisker needle-electrode (3 μm in diameter, 160 μm in length) was fabricated on a low resistance 1mm×1mm silicon block which was assembled to a FPC package using electrically conductive epoxy. Electrical impedance was reduced (~100kΩ at 1 kHz) with the platinum black plating. Neuronal activity was recorded from the mouse's primary somasensory cortex (S1) to evaluate the recording capability of the FPC packaged needle-electrode. As a result, electrode was able to penetrate through the cerebral cortex by surface tension between the silicon block and cortical surfaces, without applying any external force. After the penetration, single unit activity and local field potential (LFP) signals were stably recorded for a long period (>80 minutes). Additionally, for future application such as device placement into subdural and inner sulcus spaces, the needle electrode was entirely covered with silk fibroin, which is soluble in water (cerebrospinal fluid). In the acute experiments, approximately 30 minutes after this device was placed on a wet surface of mouse cortex, the silk fibroin layer was dissolved and the needle-electrode successfully recorded the cortical activity (LFP) from the cortical tissue. These results suggest the feasibility of long-term recording of neuronal activity from subdural and inner sulcus spaces of large animals, including monkey. The silk fibroin remain solidify for minutes, allowing electrode to be effectively protected before being slid into the targeted cortical area. Furthermore, the high flexibility of the FPC is able to mitigate the vibration caused by throbbing or organism movement, therefore the electrode was able to be stably fixed to the brain tissue. If the single unit recording from the

cortical area in the narrow space can be achieved, this proposed device is expected to become a powerful tool to contribute significantly to neurophysiology.

Disclosures: D. Teo: None. **H. Sawahata:** None. **S. Yamagiwa:** None. **A. Moriya:** None. **H. Oi:** None. **Y. Ando:** None. **R. Numano:** None. **M. Ishida:** None. **K. Koida:** None. **T. Kawano:** None.

Poster

267. Electrode Arrays I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 267.13/BB56

Topic: G.04. Physiological Methods

Title: A neuroelectronic device based on nanocoax arrays

Authors: J. R. NAUGHTON[1], J. M. VARELA[2], J. N. LUNDBERG[2], T. J. CONNOLLY[3], M. J. BURNS[1], T. C. CHILES[3], J. P. CHRISTIANSON[2], *M. J. NAUGHTON[1];
[1]Dept. of Physics, [2]Dept. of Psychology, [3]Dept. of Biol., Boston Col., Chestnut Hill, MA

Abstract: We report on the development of a nanocoax-based neuroelectronic array. A nanocoax consists of concentric conductor core, a dielectric annulus and an outer conductor shield. Computer simulations with this architecture indicate that a nanocoax array can pixelate local field potentials (LFPs) at a spatial pitch far smaller than bare wire sensors of equal size. Moreover, the shielded nature of the nanocoax provides improved signal-to-noise ratio at any pitch. We have developed fabrication techniques in which nanocoax arrays, consisting of individual coaxes as small as 300 nm diameter, can be deployed as multielectrode arrays for neural LFP recordings. First, we made extracellular LFP recordings from leech Hirudo medicinalis ganglion sacs. Biphasic waveforms with amplitude and duration akin to published leech action potentials were evident. Next, we cultured HEK293-Channelrhodopsin2 cells (HEK-ChR2) on 5×6 arrays of 2 um coaxes. Brief application of blue light (0 to 30 mW/mm^2) evoked negative LFPs with a linear optical dose-response relationship. Importantly, optically-evoked LFPs were only observed on nanocoax sensors found to be in direct contact with HEK-ChR2 as determined after recording with fluorescent microscopy. These results encourage future development of nanocoax electrode arrays for optogenetic neural recordings with high spatial and electronic resolution.

Disclosures: J.R. Naughton: None. **J.M. Varela:** None. **J.N. Lundberg:** None. **T.J. Connolly:** None. **M.J. Burns:** None. **T.C. Chiles:** None. **J.P. Christianson:** None. **M.J. Naughton:** None.

Poster

267. Electrode Arrays I

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Topic: G.04. Physiological Methods

Support: RENVISION project Grant agreement n°600847

Title: Toward pan-retinal characterization of ganglion cells responses to finely controlled spatio-temporal light stimuli on 4096 CMOS-MEAs

Authors: *A. MACCIONE[1], S. DI MARCO[2], G. HILGEN[3], S. PIRMORADIAN[4], M. HENNIG[4], E. SERNAGOR[3], L. BERDONDINI[1];
[1]Inst. Italiano Di Tecnologia, Genova, Italy; [2]Univ. degli Studi dell'Aquila, Aquila, Italy; [3]Newcastle Univ., Newcastle, United Kingdom; [4]Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: Advances in the field of CMOS multielectrode array sensors [1] for extracellular recordings with sub-millisecond resolution of neuronal electrical activity allows scaling up the number of the simultaneously recorded single-units from few tens up to several thousands. This offers a novel opportunity to perform fine-grain yet large-scale analysis of brain circuits to investigate how they develop and to gather novel knowledge about information processing at the level of large neuronal populations. In particular we focused on recording from the retinal ganglion cell (RGC) layer in retinal wholemounts by taking advantage of CMOS-MEAs with 4096 electrodes (7.12 mm^2 active area, 42 μm electrode pitch) to investigate the effects of inner and outer lateral inhibition in mediating the responses of RGCs to light stimuli. In order to provide finely controlled spatio-temporal light stimuli, a visual stimulator based on a DLP custom system was integrated in the recording platform to focus and project light patterns on local or large retina areas at a 4 x 4 μm2 spatial resolution and with sub-millisecond temporal precision. Here, we present preliminary results on functional RGC classification at pan-retinal scale (hundreds to thousand well responding neurons). We first characterized the spatial frequency sensitivity of RGCs based on Fourier analysis computed on responses to moving bars at different spatial frequencies. We show how the receptive field properties and the spatial tuning are influenced by varying the extent of the stimulus projected onto the retina. We then quantified RGCs receptive fields using full field stimulation and linear models by reverse correlation. Although broadly distributed, response kinetics had a clear dorsoventral gradient: RGCs in more ventral locations responded more slowly, and receptive fields of Off cells were smaller in ventral than in dorsal locations. This potentially obscures the classification of cell types based on kinetic measures. In contrast, full field responses yielded more distinct groups of neurons with different response polarities and transient versus sustained activity. [1] L. Berdondini, A. Bosca, T. Nieus, and A. Maccione, "Active Pixel Sensor Multielectrode Array for High Spatiotemporal Resolution," in *Nanotechnology and Neuroscience: Nano-electronic, Photonic and Mechanical*

Neuronal Interfacing SE - 7, M. De Vittorio, L. Martiradonna, and J. Assad, Eds. Springer New York, 2014, pp. 207-238.

Disclosures: **A. Maccione:** None. **S. Di Marco:** None. **G. Hilgen:** None. **S. Pirmoradian:** None. **M. Hennig:** None. **E. Sernagor:** None. **L. Berdondini:** None.

Poster

267. Electrode Arrays I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 267.15/BB58

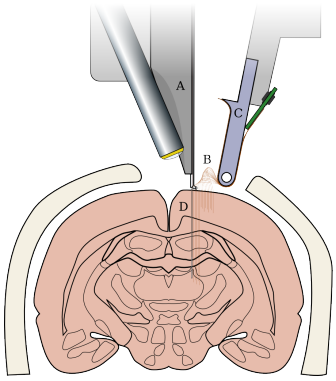
Topic: G.04. Physiological Methods

Support: UCSF EYE RAP 2013

Title: Flexible electrodes and insertion machine for stable, minimally-invasive neural recording

Authors: ***T. L. HANSON**[1], M. M. MAHARBIZ[2], P. N. SABES[1];
[1]Physiol., UCSF, San Francisco, CA; [2]Electrical Engin., Univ. of California, Berkeley, Berkeley, CA

Abstract: Current approaches to interfacing with the nervous system rely mainly on stiff electrode materials, which work remarkably well, but suffer degradation from chronic immune response due to mechanical impedance mismatch and blood-brain barrier disruption. Current technology also poses limits on recording depth, spacing, and location. In this project, we are working to ameliorate these issues through a system of very fine and flexible electrodes, a robotic system for manipulating and implanting these electrodes, and a means for integrating electrodes with neural processing chips. We have fabricated preliminary flexible 5μm x 16μm polymer electrodes, and have demonstrated their manual and automated insertion into an agarose tissue proxy, and both ex-vivo and in-vivo brains using etched and laser micro-welded 25μm and 13μm diameter tungsten needles. We have also fabricated and tested several inserter robots; the most recent design uses replaceable needle and electrode cartridges, the latter to which electrodes are mounted. Electrodes are lithographically fabricated in groups of 64, and include a peel-away backing, which holds the fine wires and keeps them from tangling until they are inserted, and provides a more robust means of handling and mounting the structures. To integrate these electrodes with neural processing chips, we have tested electroplated bond sites with aluminum wirebonding. We will describe the development and operation of the system, as well as preliminary functional tests in rats.



Not-to-scale schematic of the ``**sewing machine**'' method for inserting *fine and flexible* electrodes into the brain.
Inserter head, **A**, moves in three dimensions to individually pick flexible electrodes **B** from a replaceable, sterilizable cartridge **C**.
Electrodes are then inserted with a fine needle **D** into the brain.
The needle is retracted, leaving the electrode in place, and the inserter head moves to pick a new electrode.
Note electrodes and needle are enlarged to make them visible. In practice, it is not possible to manipulate either manually -- *a specialized machine is required*.

Disclosures: **T.L. Hanson:** None. **M.M. Maharbiz:** None. **P.N. Sabes:** None.

Poster

267. Electrode Arrays I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 267.16/BB59

Topic: G.04. Physiological Methods

Support: NIH MH079511 to HTB

NIH DA035443 to KMW

Title: Simultaneous *in vivo* single-unit recording and fast-scan cyclic voltammetry in the behaving rat

Authors: *A. G. HOWE, K. M. WASSUM, H. T. BLAIR;
UCLA, Dept. of Psychology, Los Angeles, CA

Abstract: In-vivo fast-scan cyclic voltammetry (FSCV) allows rapid sampling of electroactive neurochemical concentrations (e.g., dopamine) from targeted brain regions in behaving animals. Relationships between neuronal firing and dopamine function can be investigated by performing FSCV simultaneously with single-unit recording. Previous approaches to this have relied on fast switching between the two techniques, so that unit recordings were interleaved with FSCV voltage sweeps (Takmakov et al., 2011; PMC3160449); a disadvantage of this approach is that unit recordings are occluded during FSCV scans, resulting in loss of ~10% of the single unit spikes. Moreover, recording directly from the FSCV probe limits sampling of single units to the immediate vicinity of the FSCV probe, precluding analysis of neural population activity. Here we introduce a new approach with zero data loss and the ability to record hundreds of single units throughout the brain. Following conventional FSCV methodology, a custom-made voltammetric potentiostat was used to apply a triangular waveform (-0.4 to 1.3 V, 8.5 ms waveform width) to a carbon-fiber microelectrode implanted in the nucleus accumbens of a

behaving rat through a head-mounted voltammetric amplifier; 16 tetrodes simultaneously recorded units from nucleus accumbens, ventral tegmental area, and hippocampus. A highly regular voltage artifact generated by the FSCV scan was removed from unit recording signals via a 4-step offline analysis process, to yield full recovery of all single-unit waveforms originally occluded by the FSCV scanning artifact. These units were then classified using conventional spike-sorting techniques. Future work is targeted towards online artifact removal to achieve real-time single unit spike sorting simultaneously with FSCV dopamine recordings. This approach allows a circuit-level analysis of the relationship between ventral striatal dopamine concentration changes with striatal, ventral tegmental, and hippocampal neuronal activity.

Disclosures: **A.G. Howe:** None. **K.M. Wassum:** None. **H.T. Blair:** None.

Poster

267. Electrode Arrays I

Location: Hall A

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Topic: G.04. Physiological Methods

Support: EPSRC Grant EP/K015141/1

EPSRC Grant EP/I005102/2

Simons Foundation 325512

Wellcome Trust 95668

Wellcome Trust 95669

Title: Long-term recordings with immobile silicon probes in the mouse cortex

Authors: ***M. OKUN**, M. CARANDINI, K. D. HARRIS;
UCL, London, United Kingdom

Abstract: One of the main experimental approaches in systems neuroscience involves extracellular chronic recording of population activity in awake behaving mammals, especially rodents. Chronic recordings have traditionally been performed with self-fabricated tetrodes, but in recent years the emphasis has shifted to silicon probes, and this trend will be further reinforced by the forthcoming new generation of high-density, high-count probes. There is a widespread belief that to use silicon probes in long-term chronic recording, one must attach them to microdrives. Microdrives allow one to advance the recording sites inside the brain. The common concern is that an immobile silicon probe rigidly affixed to the skull would introduce too much damage to the surrounding neural tissue, compromising the ability to observe high quality spiking activity. Here we report that, contrary to this opinion, it is possible to obtain high quality

recordings in both head-fixed and freely moving animals for at least several months following the implantation of immobile chronic probes into the mouse cortex. We implanted mice with a head plate and 16- or 32-channel Neuronexus CM-type probes, with tetrode and octrode organization of contacts. The probes were positioned in the deep layers of the primary visual cortex (V1). To assess the stability of well-isolated single units recorded with the probes we utilized the highly reproducible responses in mouse V1. We recorded responses to gratings across four consecutive days in mice head fixed in the same position relative to the screen showing the stimuli. Spike sorting was performed on all sessions together, using standard procedures, oblivious to any sensory response properties. Qualitatively, neurons' responses were similar to acute recordings performed in the lab and conserved across days. Quantitative analysis showed that PSTHs computed from half of the trials on one day predict 71% ± 25% (median and MAD) of the explainable variability in the PSTH from half of the trials on the next day (i.e., 100% is taken to be the level of PSTH variability explained by the other half of trials in the same recording session). Thus, although electrode drift cannot be completely eliminated, our results suggest that immobile silicon probes represent a straightforward and reliable technique to obtain stable, long-term population recordings in mice.

Disclosures: **M. Okun:** None. **M. Carandini:** None. **K.D. Harris:** None.

Poster

267. Electrode Arrays I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 267.18/BB61

Topic: G.04. Physiological Methods

Title: Predicting current density induced by transcranial direct current stimulation (tDCS) in the human motor cortex using an optimized electrode configuration and high-resolution head models

Authors: ***M. E. RASMUSSEN**[1,2], P. LUU[1], E. ANDERSON[1], K. K. MORGAN[3,1], A. R. GUNN[1], D. M. TUCKER[3,1];
[1]Electrical Geodesics, Inc., Eugene, OR; [2]Human Physiol., [3]Psychology, Univ. of Oregon, Eugene, OR

Abstract: Transcranial direct current stimulation (tDCS) is emerging as a promising research and clinical tool to modulate excitability of the human cortex. Both noninvasive and inexpensive, tDCS utilizes weak electrical current (0.5-2mA) conventionally administered through patch electrodes positioned on the scalp over a target cortical region of interest. While anodal stimulation typically facilitates cortical excitability through neuronal membrane depolarization and cathodal stimulation typically inhibits through hyperpolarization, there exists a widespread inconsistency in efficacy and duration of neuromodulation effects. This unpredictability proves problematic for dosage considerations particularly in clinical applications. For example, the use

of tDCS for the suppression of epilepsy necessitates both focal and predictable current induction in order to provide maximal therapeutic benefits. In this study we utilized high-resolution head models, derived from MRI images, to characterize each individual's head tissues to predict the current density at the hand region of the primary motor cortex using 1) a standard M1-supraorbital patch electrode configuration and 2) a dense-array, target optimized electrode configuration and then compared the current density at the target region associated with each configuration. The results show that the M1-supraorbital patch electrode configuration often produced maximal current density at non-target locations and sometimes with the opposite current direction than intended. In contrast, the dense-array, target optimized electrode configuration produced maximal current density in at the target location and always with the intended current direction. The results suggest that variability of tDCS after-effects observed in previous research may be directly attributable to the variability in current density and direction associated with conventional M1-supraorbital patch electrode configuration, and that to reduce the variability dense-array, target optimized electrode configuration should be employed.

Disclosures: **M.E. Rasmussen:** A. Employment/Salary (full or part-time):; Electrical Geodesics, Inc. **P. Luu:** A. Employment/Salary (full or part-time):; Electrical Geodesics, Inc. **E. Anderson:** A. Employment/Salary (full or part-time):; Electrical Geodesics, Inc. **K.K. Morgan:** A. Employment/Salary (full or part-time):; Electrical Geodesics, Inc. **A.R. Gunn:** A. Employment/Salary (full or part-time):; Electrical Geodesics, Inc. **D.M. Tucker:** A. Employment/Salary (full or part-time):; Electrical Geodesics, Inc..

Poster

267. Electrode Arrays I

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Program#/Poster#: 267.19/BB62

Topic: G.04. Physiological Methods

Support: Intramural Research Program of the National Institute of Mental Health, National Institutes of Health, and Department of Health and Human Services

Title: Effect of shank spacing and probe sharpening on insertion force and dimpling depth in Matrix Array 3D probe implantation into the primate cortex

Authors: ***A. E. SNELLINGS**[1], M. FUKUSHIMA[2], J. JEON[1], R. J. VETTER[1], M. MISHKIN[2], R. C. SAUNDERS[2];
[1]NeuroNexus Technologies, Ann Arbor, MI; [2]Lab. of Neuropsychology, NIMH/NIH, Bethesda, MD

Abstract: The Matrix ArrayTM is a 3-dimensional silicon-based microelectrode array that can be used to obtain high-density recordings from large populations of neurons in a volume of

tissue. The Matrix ArrayTM is comprised of multiple two-dimensional 32-channel probes that are stacked in a platform with a fixed amount of spacing, typically producing a 64-, 128- or 256-channel array. It is implanted with a variable-speed computer controlled insertion motor that allows for precise, low speed insertions. In this study we examine the so-called "pincushion" effect for 3D probe implants in a primate model. We measure the insertion force required to insert a Matrix ArrayTM, as well as the depth of dimpling prior to pial penetration, across several implantation speeds (0.1 mm/s, 0.3 mm/s, 0.5 mm/s, 1.5 mm/s, 4.5 mm/s, 6.0 mm/s, 8.0 mm/s). We tested three different inter-platform spacings (0.3 mm, 0.6 mm, 1.0 mm), two different inter-shank spacings (0.2 mm, 0.4 mm), and standard probe tips (e.g. chisel shaped) vs. sharpened tips (e.g. chisel shaped, tapered to a point) to fully characterize this design space. Initial results indicate that both spacing and probe sharpness are factors that influence the dependent variables. In the first comparison, which tested two-dimensional probes with shank spacing of 200 microns ("M4x8-2mm-100-200-704") across the seven insertion speeds, the standard-tipped array required 2.90 g ± 0.32 g of force to insert and produced a 2.3 mm ± 0.14 mm dimple prior to insertion while the sharpened array required only 1.91 g ± 0.12 g of insertion force and produced a 1.85 mm ± 0.13 mm dimple before insertion. Similarly, a three-dimensional 128-channel Matrix ArrayTM comprised of four two-dimensional probes with standard tips and 200 um shank spacing ("M4x8-2mm-100-200-704") with 300 μm platform spacing required 7.32 g ± 0.30 g of insertion force and produced a 3.6 mm ± 0.07 mm dimple (across insertion speeds 0.5 mm/s, 1.5 mm/s, 4.5 mm/s, 6.0 mm/s, 8.0 mm/s) while an array comprised of the same 32-channel probes but with sharpened tips and 600 μm platform spacing required only 3.27 ± 0.29 g of insertion force and produced a 1.80 ± 0.11 mm dimple. Testing in this study is on-going, and the final results will span the parameter space described above.

Disclosures: **A.E. Snellings:** A. Employment/Salary (full or part-time):; NeuroNexus Technologies. **M. Fukushima:** None. **J. Jeon:** A. Employment/Salary (full or part-time):; NeuroNexus Technologies. **R.J. Vetter:** A. Employment/Salary (full or part-time):; NeuroNexus Technologies. **M. Mishkin:** None. **R.C. Saunders:** None.

Poster

267. Electrode Arrays I

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Program#/Poster#: 267.20/BB63

Topic: G.04. Physiological Methods

Support: NIH Grant 5R21NS084492

Title: Tunable microelectrode arrays for monitoring neurons in deep brain structures

Authors: ***S. PALANISWAMY**[1], A. SRIDHARAN[1], J. MUTHUSWAMY[1], M. BAKER[2], M. OKANDAN[2];

[1]Biomed. engineering, Arizona State Univ., Tempe, AZ; [2]Biomed. engineering, Sandia Natl. Labs, Albuquerque, NM

Abstract: We report here a scalable technology using micro scale actuators and movable microelectrodes for the unprecedented capability of seeking and recording/stimulating targeted single neurons in deep brain structures up to 10 mm deep (with 6 μm displacement resolution) in behaving or head-restrained animals. Using a combination of a unique micro bonding technique and a modified flip-chip process, we have now extended the repertoire of our previously reported movable microelectrode arrays to enable us to mate conventional stainless steel and Pt/Ir microelectrode arrays of desired lengths to steerable polysilicon shafts. We have tested scalable prototypes in rigorous bench top tests to assess electrical and mechanical stability under long-term implantation. The method allows a wide variety of electrode configurations to be realized such as a rectangular or circular array configuration or other arbitrary geometries optimal for specific regions of the brain with inter-electrode distance as low as 25 um. The autonomous moveable MEA is coupled with robotic tracking algorithms that operate in one of 3 phases - seek (to seek neurons of interest), optimize (fine positioning after identifying neuron of interest to maximize signal quality) or maintain (maintain the quality of neural recording over long periods of time). We will report results from *in vivo* experiments.

Disclosures: S. Palaniswamy: None. **A. Sridharan:** None. **J. Muthuswamy:** None. **M. Baker:** None. **M. Okandan:** None.

Poster

267. Electrode Arrays I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 267.21/BB64

Topic: G.04. Physiological Methods

Title: Characterization of somatosensory and motor cortices in awake common marmosets using carbon nanotube coated ECoG electrodes

Authors: *A. KOSUGI[1,2], M. TAKEMI[1,2,3], E. CASTAGNOLA[4], A. ANSALDO[4], D. RICCI[4], K. SATO[1], T. NAKAMURA[1], B. TIA[4], K. SEKI[5], L. FADIGA[4], A. IRIKI[2], J. USHIBA[2,6]; [1]Grad. Sch. of Sci. and Technol., Keio Univ., Kanagawa, Japan; [2]Lab. for Symbolic Cognitive Develop., RIKEN Brain Sci. Inst., Saitama, Japan; [3]Danish Res. Ctr. for Magnetic Resonance, Copenhagen Univ. Hosp. Hvidovre, Hvidovre, Denmark; [4]Inst. Italiano di Tecnologia, Ctr. for Translational Neurophysiol., Univ. of Ferrara, Ferrara, Italy; [5]Dept. of Neurophysiol., Natl. Inst. of Neurosci., Tokyo, Japan; [6]Depertment of Biosci. and Informatics, Keio Univ. Facalty of Sci. and Technol., Kanagawa, Japan

Abstract: Assessing neural dynamics in the sensorimotor cortex gives a valuable source of information to study motor control and its functional recovery from neurological deficits. Among various recording procedures, Electrocorticogram (ECoG) is considered to be appropriate to capture characteristic neural activities in sensorimotor cortex because ECoG provides more reliable measurements than penetrating electrodes into the cortex and ECoG carry rich information sufficient for decoding forelimb movements. In the current study, we aimed at investigating ECoG signals in common marmosets, which has been attracting much attention in the field of neuroscience as a primate model in both basic and clinical studies. However, for ECoG recordings in common marmosets, localization of the motor cortex is essential because there is no cortical landmark on their sensorimotor cortex. Here, the purpose of this study was to characterize neural activities of the motor and somatosensory cortices in marmosets, by using custom-made ECoG electrode arrays that can be used for both epidural cortical stimulation (ECS) and recording of cortical neural activity. In order for detailed motor cortex stimulation mapping, recording Electromyographic (EMG) activity was necessary to evaluate motor evoked potential (MEP) by ECS as well. In this study, at first, we developed methods for chronic implantation of ECoG electrode arrays and EMG wire electrodes, and then identified the motor and somatosensory maps in the cortex. We implemented EMG wire electrodes in 7 forelimb muscles and ECoG electrode arrays epidural in common marmosets (N=2). After the implantation, ECS was applied through ECoG electrode arrays and MEP of each forelimb muscle was recorded. Somatosensory evoked potentials induced by electrical stimulation of peripheral nerve were also recorded by the same electrode arrays. With ECS and neural recordings in the same electrodes, we obtained combined motor and somatosensory maps of the forelimb area in the cortex in the same individuals. These maps were consistent with the anatomical findings in previous studies. The current results suggest that our ECoG electrode arrays can reliably assess both MEP and somatosensory somatotopy with sufficient spatial resolution. Implantation of EMG and ECoG electrodes enabled both cortical stimulation and recording of cortical neural activity in high reproducibility, and also recording in awake. Moreover, the present method may enable activity-dependent cortical stimulation for inducing functional reorganization in the cortex.

Disclosures: **A. Kosugi:** None. **M. Takemi:** None. **E. Castagnola:** None. **A. Ansaldo:** None. **D. Ricci:** None. **K. Sato:** None. **T. Nakamura:** None. **B. Tia:** None. **K. Seki:** None. **L. Fadiga:** None. **A. Iriki:** None. **J. Ushiba:** None.

Poster

267. Electrode Arrays I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: G.04. Physiological Methods

Support: Marie-Curie FP7 NAMASEN ITN - 264872

Title: 4096 CMOS multielectrode arrays reveal lognormal firing patterns in hippocampal neuronal networks and allow to characterize early distribution changes induced by amyloid-beta toxicity

Authors: *H. AMIN, A. MACCIONE, L. BERDONDINI;
Fondazione Inst. Italiano Di Tecnologia (IIT), Genova, Italy

Abstract: Lognormal-like distributions of neuronal firing patterns in large populations have been recently reported for *in vivo* brain circuits and their role to the structural and functional properties of brain networks has been investigated (Mizuseki and Buzsáki 2013). By taking advantage from recently developed high density multielectrode arrays (MEAs) based on Complementary Metal Oxide Semiconductor (CMOS) technology, that enable simultaneous *in vitro* recordings from 4096 electrodes (Berdondini et al. 2005), we have recently demonstrated that lognormal-like firing patterns can be unveiled also from *in vitro* grown hippocampal neuronal networks and can be estimated already after 10 minutes of recording (Amin et al. 2015). On the other hand, a growing body of evidence suggests that soluble oligomers of amyloid-beta (Aβ1-42) are cytotoxic and their aggregates can inhibit many critical neuronal activities such as long-term potentiation (LTP) (Lambert et al. 1998), thus representing an hallmark of Alzheimer's disease (AD). Here, by analyzing changes (shifts) in the lognormal-like distributions of extracellular firing patterns recorded by 4096 CMOS-MEAs and quantified in a logarithmic histogram by means of Gaussian fit parameters, we have investigated the functional effects induced by oligomeric Aβ toxicity on the network activity of hippocampal neuronal cultures grown on-chip for 21 days *in vitro* (DIVs). Results show, in a nutshell, that firing patterns of untreated control cultures exhibit skewed distributions with non-significant inter-culture differences and tend, over development, to shift from the left to the right (i.e., toward higher firing rates). Always with very low variability, cultures treated with Aβ manifest over 26 hours a salient shift from the right to the left (i.e., toward low spiking frequencies) and loose a lognormal-like distribution after 19 hours. Overall, these results show that large-scale multielectrode array recordings combined with the analysis of the firing rates distribution within a network undergoing functional changes, provides a way to investigate the induced functional re-organization occurring at the cellular scale within the network. This suggests that this methodology might be a valuable approach to investigate neuronal networks beyond their characterization based on simple network-wide activity parameters (e.g. Mean Firing Rate and Mean Bursting Rate), and can find applications for studying neurodegeneration, for developing analytical tools for neuropharmacology, and for the development of computational neuronal network models.

Disclosures: H. Amin: None. **A. Maccione:** None. **L. Berdondini:** None.

Poster

267. Electrode Arrays I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 267.23/BB66

Topic: G.04. Physiological Methods

Support: R01EB016407

Title: The Real-Time eXperiment Interface: a closed-loop data acquisition system with sub-millisecond latencies for electrophysiology

Authors: *A. GEORGE[1], Y. PATEL[2], F. ORTEGA[1], J. WHITE[3], D. CHRISTINI[1], A. DORVAL[4], R. BUTERA[5];
[1]Weill Cornell Med. Col., New York, NY; [2]Georgia Tech, Atlanta, GA; [3]Boston Univ., Boston, MA; [4]Univ. of Utah, Salt Lake City, UT; [5]Georgia Tech., Atlanta, GA

Abstract: To understand causal interactions between neural activity and function, both function and neural activity need to be monitored with timing precision at the sub-millisecond scale. This requires enabling of closed-loop, hard real-time technologies that are capable of controlling stimulation (optogenetic, electrical, thermal, etc) dependent upon some functional measure (behavior, motor action, etc) within a deterministic period. While monitoring neural activity at precise time scales is commonplace, there is an important unmet need in a cost-effective sub-millisecond closed-loop real-time framework capable of interacting with neural activity and function at the appropriate timescales. To enable investigation of neural activity and function at these time scales, we have developed the Real-Time eXperiment Interface (RTXI) - a versatile interface based off of real-time Linux that enables deterministic closed-loop monitoring, stimulation, and control of single-cell, network, animal, and human electrophysiology experiments. RTXI is a free and open source platform currently employed by over 65 labs around the world. At the base of RTXI are built-in modules such as a high-speed oscilloscope, signal generator, and common filters. The value of RTXI arises from it's plugin-based architecture, enabling community-developed modules that enable numerous options for customizing closed-loop control. Such an architecture enables resource sharing and maximizes reproducibility of experimental setups and results. Most recently, RTXI's user interface and underlying architecture have been rewritten to provide up to 32 16-bit I/O channels, with worst-case jitter less than 5 microseconds. Addtional information is available on our website (http://www.rtxi.org), and our code repository is open on GitHub (https://github.com/RTXI).

Disclosures: **A. George:** None. **Y. Patel:** None. **F. Ortega:** None. **J. White:** None. **D. Christini:** None. **A. Dorval:** None. **R. Butera:** None.

Poster

267. Electrode Arrays I

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Topic: G.04. Physiological Methods

Support: This study was funded by NIH-NCCAM T32 AT002688.

Title: Event-related potential correlates of mindfulness meditation aptitude

Authors: *R. M. ATCHLEY, D. KLEE, T. MEMMOTT, E. GOODRICH, B. OKEN; Neurol., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Background: Mindfulness meditation interventions produce some clinical benefit, but the neurophysiological mechanisms underlying these benefits and how they are acquired are poorly understood. This study assesses a physiological marker of meditation aptitude using EEG. Objectives: The primary objective was to measure changes in various event-related potentials across three groups: naïve, novice, and experienced meditators. Heart rate, eye blinks, respiration, galvanic skin response, salivary cortisol, and blood pressure were also assessed. Methods: Forty-three healthy adults (M age = 49 years) participated in the study. Participants completed a variety of cognitive tasks while EEG was recorded. These tasks included a target tone detection task and a breath counting task that also served as a meditation condition for those with meditation experience. Participants were instructed to respond to target tones with a button press in the first task (Tones), and then ignore the tones as they sounded in the background while breath counting. Statistical analyses focused on event-related potential responses to target tones. Results: Mixed 2x3 ANOVAs revealed a task effect for P3 amplitudes ($p < .001$). As expected, the P3 was attenuated during the breath counting task in comparison to the Tones task. When comparing meditators to non-meditators, there was a task by group interaction for P3 ($p = .035$) with a greater decrement in P3 amplitude in the meditators compared to non-meditators. Furthermore, both experienced meditators ($p = .003$) and novice meditators ($p = .004$) exhibited less task-related change in N2 in comparison to controls. Conclusions: Meditators had stronger P3 responses to target tones when they were instructed to attend to them, and a greater attenuation of P3 responses to the same tones when they were instructed to ignore them during the breath counting session. The results of this study provide initial steps in the identification of ERP markers of mindfulness meditation aptitude. This information has potential to improve mindfulness meditation interventions by allowing objective assessment of meditation quality.

Disclosures: R.M. Atchley: None. **D. Klee:** None. **T. Memmott:** None. **E. Goodrich:** None. **B. Oken:** None.

Poster

267. Electrode Arrays I

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Topic: G.04. Physiological Methods

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engineering-dentistry interdisciplinary research grant jointly funded by college of engineering and college of dentistry, Seoul National University

Title: A novel micro-fabricated tetrode

Authors: *S. SHIN[1], J.-H. KIM[2], J. JEONG[1], J. PARK[1], T. GWON[1], S.-H. LEE[2], S. KIM[1]; [1]Dept. of Electrical and Computer Engin., Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Dept. of Biol. Sci., KAIST, Daejeon, Korea, Republic of

Abstract: For decades, microwire-based tetrodes have been widely and usefully used for *in vivo* -neural signal recording experiments. The advantage of microwire-based tetrodes among various neural electrode arrays is that its structure allows electrode sites to have easy access to nearby intact neurons. However, the fabrication process of the microwire-based tetrodes requires a mastery of manual skills and the resulting device tends to be unregulated. In this presentation, we suggest a polymer-based tetrode fabricated by micro-fabrication processes, so that, the dimensions in the device are well controlled and the devices are mass producible.
The suggested neural probes are made with liquid crystal polymer (LCP) and they have four contacts at the tip. The fabrication involves bonding multiple layer LCP films by thermal lamination and exposing electrode sites by laser micromachining. Among the LCP films, two LCP layers are used for covers and the others contain metal patterns to form the tip contacts. After thermally laminating the LCP films, the four tip contacts are made by cut-exposing the thickness of the electroplated metals. The four tip contacts have enough contact areas and electrochemical impedance to ensure good quality of neural signal recordings. ($1000\mu m^2$, 88.63 ± 49.34 kohm at 1kHz) To demonstrate recording capability of the fabricated polymer-based tetrodes, *in vivo* recording experiments were done. Spontaneous activity was successfully recorded from the primary visual cortex of the rodents using the LCP-based tetrode.
In summary, we present a polymer-based tetrode fabricated by micro-fabrication processes. The suggested LCP-based tetrodes can be exquisitely fabricated by micro-fabrication processes and laser micromachining. And its use has been proven by through *in vivo* neural signal recording tests.

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Poster

267. Electrode Arrays I

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: G.04. Physiological Methods

Support: NIH grant MH079511 to HTB

Title: OvalDrive36: A chronically implantable microdrive system for brain-wide single-unit recording, optogenetic stimulation, and cyclic voltammetry in behaving rats

Authors: *H. T. BLAIR, IV[1], A. G. HOWE[1], R. M. DE GUZMAN[2];
[1]Dept Psychology, UCLA, Los Angeles, CA; [2]Univ. at Albany, Albany, NY

Abstract: OvalDrive36 (open-ephys.atlassian.net/wiki/display/OEW/OvalDrive36) is a chronically implantable microdrive system for rats that allows up to 36 individually movable probes to accurately target any desired combination of neural structures throughout the brain. The oval shape of the drive places almost the entire anteroposterior, mediolateral, and dorsoventral extent of the rat brain within range of the probes, and also permits simultaneous targeting of brain regions that are widely separated from each other by distances of up to 2 cm. The advancement path of each probe tip can be precisely specified by importing brain atlas diagrams into a computer-aided design (CAD) software template, which is then used to construct a targeting cone that is fabricated on a 3D printer. The CAD design process makes it easy to specify complex probe trajectories, including angled paths that avoid obstacles such as bone and blood vessels. The targeting cone is fitted onto a drive core that features a shuttle-in-groove design, which assures micrometer stability of each probe tip along the entire length of its 15 mm advancement path. Each of the 36 shuttle grooves can be fitted with different types of probe shuttles: 1) microwire shuttles that hold tetrodes or bipolar stimulating electrodes for neurophysiology, 2) optical fiber shuttles equipped with light shields and ferrule receptacles for plugging in light sources, 3) carbon nanofiber shuttles for fast-scan cyclic voltammetry (FSCV) studies, or 4) injector cannula shuttles for intracranial drug infusions. The drive core contains a large central payload space which can fully enclose a wireless telemetry transmitter, and also contains pedestals upon which up to four electrode interface connector boards can be mounted. These combined features make OvalDrive36 well suited for experiments employing convergent methods of electrophysiology, optogenetics, voltammetry, and neuropharmacology.

Disclosures: **H.T. Blair:** None. **A.G. Howe:** None. **R.M. De Guzman:** None.

Poster

267. Electrode Arrays I

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Topic: G.04. Physiological Methods

Support: Grant-in-Aid for Scientific Research (A)

Grant-in-Aid for Young Scientists (A)

PRESTO program

Title: Self–curling and –sticking flexible thin–film ECoG array device

Authors: *S. YAMAGIWA[1], H. SAWAHATA[1], M. ISHIDA[1,2], T. KAWANO[1];
[1]Toyohashi Univ. of Technol., Toyohashi-Shi, Aichi Pref., Japan; [2]Electronics-Inspired Interdisciplinary Res. Inst. (EIIRIS), Toyohashi Univ. of Technol., Toyohashi-Shi, Aichi Pref., Japan

Abstract: An array of ECoG electrodes, which enable electrical interface between electronics and human brain, potentially offers low-invasive ECoG−based brain-machine interface and epilepsy detection. However, thick and stiff substrate−based conventional ECoG devices are still problematic because it is difficult to sufficiently cover hemispherical shaped brain and sulci. A way to cover the brain with ECoG electrodes is the use of a sub-10-μm thick substrate with a great flexibility. Handling of such flexible thin-film device can be improved by using a stiff substrate of silk fibroin, which is a dissolvable material in brain [D. H. Kim et al, Nature materials, 9, 2010]. However, the silk fibroin is a foreign-body material in human brain. To overcome the issue of handling of flexible thin-film devices, here we propose an easy-to-use "self-actuating" flexible thin-film ECoG device. The device is based on a biocompatible parylene-N/-C sandwiched substrate with the self-curled property, but it is flatten out and stuck to brain surface once the substrate contacts with solution over the brain. To realize the curled parylene-film, herein we use the differences of linear expansion coefficient between the parylene-C (35 ppm) and the parylene-N (69 ppm). After calculations of mechanical properties of parylene-C/-N substrate and preliminary experiments, the parylene-C/-N film ECoG array device was fabricated. First of the device fabrication, a sacrificial layer of titanium was sputtered on a silicon substrate. After the first layer of parylene-C and thin parylene-N were deposited, platinum-electrodes and interconnections were formed by sputtering and etching. After the device metallization, an insulator of parylene-N was deposited. To enhance the sticking properties of the device, hydrophobic surface of the parylene-N was changed to hydrophilic property by oxygen plasma treatment. The parylene-N/-C was etched by oxygen plasma by using titanium hard-mask. Finally, the titanium layers were etched by ammonium solution to release the film device. The width, length, and thickness of the fabricated parylene-C/-N film ECoG

substrate were 1 cm, 5 cm and 5 μm respectively. Each ECoG electrode with the diameter of 1 mm exhibited the electrical impedance of 3 kΩ at 1 kHz in room temperature saline. *In vivo* experiments using a rat's brain demonstrated that the fabricated ECoG device stuck to the brain surface and detected evoked potentials from the visual cortex (V1) during the visual stimulation. These results indicated that the proposed self-actuating flexible thin-film ECoG device can be used in ECoG recordings.

Disclosures: **S. Yamagiwa:** None. **H. Sawahata:** None. **M. Ishida:** None. **T. Kawano:** None.

Poster

267. Electrode Arrays I

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Title: phy: a fast, next-generation spike sorting and data analysis framework for high-channel-count electrophysiology

Authors: ***M. L. D. HUNTER**, D. F. M. GOODMAN, S. N. KADIR, N. A. STEINMETZ, K. D. HARRIS, C. ROSSANT;
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Abstract: The brain operates through the parallel activity of large sets of neurons. To understand brain function, it is thus essential to record the simultaneous activity of these populations. A leading method for large-scale neuronal recordings is extracellular electrophysiology with multi-site recording probes. Advances in microfabrication technology have recently enabled the production of electrodes with close to a thousand sites, allowing for recording of thousands of neurons simultaneously. In order to draw scientific conclusions from such data, the problem of "spike sorting" - grouping recorded action potentials into clusters putatively corresponding to individual neurons - must be solved. Traditional spike sorting methods scale poorly, if at all, to this new generation of electrodes. Not only are the error rates of traditional methods high, but they also typically require weeks or months of computation time, on servers with several hundred gigabytes of RAM, followed by multiple days of manual curation per experiment. To make spike sorting efficient in terms of both human and computer time therefore requires careful consideration of algorithms, data access patterns, caching, and underlying file storage structures

when writing code to perform scientific analyses. We developed “phy”: a new, fast open-source framework for extracellular electrophysiology data analysis. phy is based upon a previous set of tools (Klusta-Suite), but rewritten from the ground up in Python. It includes a customisable pipeline for spike sorting with inbuilt functions for efficient spike detection and automated clustering, and a fast GUI based on Vispy views for manual curation and arbitrary analyses. An improved ‘wizard’ guides a human operator through the manual curation process, greatly reducing the operator burden required by presenting choices in under 500ms. Many of the internal data structures of KlustaSuite have been changed, to reduce disk and memory usage and computation time by orders of magnitude for large electrodes. All the functions for accessing data are abstracted away, so it is easy to write and benchmark new algorithms, read different file formats, or access and modify any data with documented Python functions. We demonstrate the ease and speed of clustering an 128-channel dataset from new IMEC-manufactured electrodes, and run various sample common analysis functions as an example of the framework’s extensibility.

Disclosures: **M.L.D. Hunter:** None. **D.F.M. Goodman:** None. **S.N. Kadir:** None. **N.A. Steinmetz:** None. **K.D. Harris:** None. **C. Rossant:** None.

Poster

267. Electrode Arrays I

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 267.29/BB72

Topic: G.04. Physiological Methods

Title: A 512-channels, whole array readout, CMOS implantable probe for acute recordings from the brain

Authors: *L. BERDONDINI, G. ANGOTZI, M. MALERBA;
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Abstract: Over the last 40 years, much progress has been made in the development of implantable neural recording probes based on MEMs and individual electrode wiring technologies. Current devices permit to record action potentials simultaneously from hundreds of neurons, but it is still unclear which technology will permit to step toward several thousands of single-neurons in the near future. An emerging approach is based on the adoption of CMOS technology to realize dense and large-scale multielectrode array (CMOS-MEA) probes [Berdondini et al., 2014]. CMOS-MEAs were extensively demonstrated in-vitro [Hierlemann et al.,2011] over the last decade and more recently also in-vivo [Lopez et al.,2014]. This allows to realize microelectronic micro-machined probes that permit to implement arrays of active electrode-pixels with local amplification and filtering circuits, as well as addressing and multiplexing circuits on the same substrate. The design of CMOS probes enabling whole array

recordings at sub-millisecond resolution from densely integrated microelectrodes on single shafts would potentially allow to literally image spiking activity and field potentials in multiple brain circuits at the same time, as demonstrated so far in-vitro on brain slices [4], cell cultures and retina whole mounts [Maccione et. al, 2014]. However, such CMOS-MEA probes must meet very demanding constraints in terms of sizes and power consumption imposed by their implantation into brain tissue. Making the shafts extremely small with smooth surfaces and rounded corners can minimize brain tissue damages at the time of insertion. Also, smart power-management circuit solutions and resource sharing approaches can effectively contribute in reducing power consumption much below the 40mW limit for a mouse brain. Here, we present a complete probe system based on a fully multiplexed CMOS neural probe that was designed for in-vivo acute recordings with a scalable circuit architecture. In particular, a first prototype of a single-shaft probe with 512 electrodes was realized in a standard CMOS 0.18µm technology and post-processed to structure the shaft with a wedge-like geometry of 30µm in thickness at the tip and 80µm at the base. Preliminary results on electrical, mechanical and implantation tests demonstrate the feasibility of realizing large-scale recording implantable probes.

Disclosures: **L. Berdondini:** None. **G. Angotzi:** None. **M. Malerba:** None.

Poster

267. Electrode Arrays I

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Support: R01DA034178-03 (NIH/NIDA)

CBET-1263785 (NSF)

Alfred P. Sloan Research Fellowship

McKnight Technical Innovations in Neuroscience Award

Title: Large-scale multi-regional neural recordings reveal altered frontostriatal network dynamics following cocaine administration

Authors: *W. C. SMITH[1], L. CLAAR[2], M. ROSENBERG[2], V. CHANG[1], N. CHONG[1], S. SHAH[1], S. MASMANIDIS[2];
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Abstract: It is increasingly understood that drugs of abuse alter brain circuit function at the network scale. This places limitations on approaches that study the neurobiological basis of addiction in one brain region at a time. However, it remains unclear how network dynamics are altered between different regions at the resolution of single cells and spikes. We hypothesize that

cocaine administration reduces frontostriatal functional connectivity, in part due to the well-known effect of frontal cortex hypoactivity. To address this hypothesis we utilize novel 512 channel silicon microprobes to simultaneously record in two interconnected areas, the medial prefrontal cortex (mPFC) and nucleus accumbens core (NAcC), which are strongly implicated in mediating the addictive properties of cocaine. To study network activity and behavioral changes that accompany cocaine administration, we have established a conditioned odor preference test for head-restrained mice. When animals are tested with brief presentations of two odors after cocaine conditioning, they exhibit a conditioned response by dilating their pupils and running on a spherical treadmill preferentially after the cocaine-paired cue, but not a saline-paired cue. The microprobes enable recordings of around 100 single units per area, providing novel opportunities for studying how firing and local field potentials within and between the mPFC and NAcC are correlated during different behavioral states. We find that intra-cortical and intra-striatal interneuron firing patterns at rest become decorrelated after chronic drug administration, while putative projection neurons from both regions also decouple within their populations to a lesser extent. Encoding of the odor stimuli changes after drug exposure, whereby a significant proportion of neurons shifts from non-discriminating to odor discriminating. Prefrontal hypoactivity after drug treatment is observed in the form of lower baseline firing and altered burst firing patterns. Both pyramidal neurons and putative fast spiking interneurons burst less frequently compared to saline-injected controls. Interestingly, pyramidal cells shift to having more spikes per burst compared to controls, while cortical interneurons have fewer spikes per burst, suggesting a change in information processing intracortically. Future work will focus on solidifying these findings and attempting to rescue drug-conditioned behaviors by altering frontostriatal network synchrony.

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Poster

268. Neuron Stimulation Methods

Location: Hall A

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Program#/Poster#: 268.01/BB74

Topic: G.04. Physiological Methods

Title: Novel electrical stimulation paradigm to reduce habituation of the nociceptive withdrawal reflex: preliminary results

Authors: C. B. LAURSEN[1], S. GERVASIO[1,2], O. K. ANDERSEN[1], K. HENNINGS[2], *E. G. SPAICH[1];
[1]Aalborg Univ., Aalborg, Denmark; [2]Nordic NeuroSTIM ApS, Aalborg, Denmark

Abstract: The lower limb nociceptive withdrawal reflex (NWR) can be elicited in humans by electrical stimulation of the sole of the foot. A stimulation train consisting of five 1ms-wide pulses delivered at a frequency of 200 Hz, repeated four times at a frequency of 15 Hz, has been used in reflex-based functional electrical therapy for gait rehabilitation in stroke patients. However, repetitive electrical stimulation can result in habituation leading to gradually lower reflex responses. Less habituation can be accomplished by varying the stimulation amplitude and frequency. The aim of this study was to identify a stimulation paradigm that induced the least amount of habituation of the NWR during walking. Three stimulation paradigms were tested: 1) Deterministic paradigm (fixed pulse width, 1 ms, and frequencies, 200 Hz inter-pulse, 15 Hz inter-train), 2) stochastic pulse width paradigm (random pulse width between 0.5-1.5ms), and 3) stochastic frequency paradigm (random inter-pulse frequency between 200-300hz and random inter-train frequency between 10-30hz). The NWR was evoked in healthy subjects (n=7) by stimulating the arch of the foot, every heel-off during 10 minutes of treadmill walking. The three different stimulation paradigms were tested in random order in three different days. To set the stimulation amplitude across experimental days, the same fixed factor (1-1.5) was used as an individual multiplier to the identified pain threshold. The reflex magnitude was assessed from the tibialis anterior muscle as the difference between the root mean square (RMS) of the electromyography (EMG) signal from 250-500 ms after stimulation onset and the RMS in the equivalent window in a control step (no stimulation). The reflex response was normalized to the mean amplitude of the rectified EMG of 30 control steps. To determine the degree of habituation during the course of the stimulation period, the average of the responses to the 10 first stimulations was compared to the average of the responses to the last 10 stimulations. The results showed that by using the deterministic paradigm, the reflex amplitude decreased from 1.32±0.64 (mean±SEM) to -0.10±0.10 after 10 minutes of walking (one-tailed paired t-test, $p<0.03$). For the stochastic pulse width paradigm, the reflex amplitude was 1.21±0.38 at the beginning of the walking period and 0.91±0.48 at the end (N.S.). For the stochastic frequency paradigm, the reflex amplitude was 1.08±0.66 at the beginning of the walking period and 0.25±0.23 at the end (N.S.). These preliminary results suggest that stochastic paradigms result in no significant degree of habituation after 10 minutes of walking.

Disclosures: C.B. Laursen: None. **S. Gervasio:** A. Employment/Salary (full or part-time):; Nordic NeuroSTIM ApS. **O.K. Andersen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nordic NeuroSTIM ApS. **K. Hennings:** A. Employment/Salary (full or part-time):; Nordic NeuroSTIM ApS. **E.G. Spaich:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nordic NeuroSTIM ApS.

Poster

268. Neuron Stimulation Methods

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 268.02/BB75

Topic: G.04. Physiological Methods

Support: UConn Large Grant

Title: High-resolution artifact removal for single and multi-channel electrical stimulation of neural tissue

Authors: A. A. NORRIS[1], H. READ[2], K. DUTTA[3], *M. A. ESCABI[4];
[1]Biomed. Engin., [2]Psychology, [3]Univ. of Connecticut, Storrs, CT; [4]Univ. Connecticut, Storrs Manfld, CT

Abstract: Brain machine interfaces and prosthetic applications, such as cochlear and auditory midbrain implants, increasingly rely on stimulation of neural tissue and concurrent neural recordings to either asses function or provide neural feedback. In such applications passive inductive coupling of the stimulation array and the distant neural recording sites generate artifacts that can obscure neural activity making it difficult to interpret and quantify neural data. This is particularly true in high throughput applications, such as cochlear implants, were stimuli are delivered at a high rate (hundreds of pulses per second) so that the artifacts overlap the delayed neural activity and cannot be easily subtracted via windowing. Here we developed and test the performance of a high-resolution artifact removal algorithm that uses optimal linear filters to predict the stimulation artifacts followed by artifact subtraction. Multi-channel (16 channel neuronexus; 100 kOhms) stimulation arrays were placed in the inferior colliculus of rats along the tonotopic axis and neural recordings were performed concurrently in auditory and surrounding cortical fields of the rat. Biphasic current pulses (200 μsec pulse width) were delivered across the array in a pseudo random spatio-temporal sequence and neural signals were recorded from a cortical array (Neuronexus 4x4 tetrode; 2.5 MOhms). Depending on the stimulation current amplitude (10-50 μA) signal-to-noise ratios (signal=neural activity; noise=current artifact) prior to artifact removal varied approximately between 0 and -10 dB so that neural signals where obscured by the artifacts. For each cortical recording location, we used the multi-channel electrical stimulation signal (input) and the simultaneously recorded cortical signals (output) to estimate a multi-input single output Wiener filter to predict the stimulus artifacts. Predicted artifacts were then subtracted from the neural recordings and the residuals examined. Artifact prediction quality was estimated by cross-validation across multiple trials. Overall there was a substantial enhancement in the recording quality and isolation of the desired neural signals (e.g., isolated action potentials) with a signal-to-noise ratio improvement ranging between 30 and 50 dB.

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Poster

268. Neuron Stimulation Methods

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Topic: G.04. Physiological Methods

Support: NSF GRFP Grant No. 1106401

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NIH Grant 1RO1-NS088674-01

DIBS Incubator Award

Title: Low-frequency repetitive transcranial magnetic stimulation (rTMS) over parietal cortex in rhesus macaques alters neural activity and impairs numerical cognition

Authors: *C. B. DRUCKER[1,2], E. M. BRANNON[3,1], M. L. PLATT[2,1];
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Abstract: Repetitive transcranial magnetic stimulation (rTMS) is used widely for noninvasive neuromodulation in humans for research and treatment purposes. Despite the popularity of this technique, the precise neural mechanisms mediating observed cognitive and behavioral responses to TMS remain unknown. Here we directly probed the impact of rTMS on both neural activity and behavior in rhesus macaques (Macaca mulatta) using a custom coil that permits placement of an electrode within the brain at the site of stimulation. We first examined the impact of low-frequency (1 Hz) rTMS_a stimulation regime thought to suppress neuronal activity_to parietal cortex on the ability of monkeys to order numerical magnitudes and color hues. Stimulation was centered on the intraparietal sulcus, a cortical area strongly implicated in numerical processing and mathematics. Following application of real rTMS, compared with sham stimulation, to parietal cortex, performance on number trials deteriorated to a greater extent than on color trials. To determine the neurophysiological mechanisms underlying these effects, we recorded single- and multi-unit firing as well as local field potentials (LFPs) in parietal cortex prior to and following application of 1 Hz rTMS in both real and sham conditions. We found that single- and multi-unit firing rates decreased and delta band power in the LFP increased following real rTMS compared with sham stimulation. These results suggest that low-frequency rTMS impairs cognition and behavior by dampening neuronal firing while increasing low-frequency oscillatory activity.

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Poster

268. Neuron Stimulation Methods

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Topic: G.04. Physiological Methods

Support: Research Grant, Medtronic, Inc.

Title: Peripheral Nerve Fiber Stimulation - Relevance of angle between electrode and nerve fiber

Authors: *K. S. FRAHM[1], K. HENNINGS[2], L. VERA-PORTOCARRERO[3], P. W. WACNIK[3], C. D. MØRCH[1];
[1]Aalborg Univ., Aalborg, Denmark; [2]Nordic NeuroSTIM Aps, Aalborg, Denmark;
[3]Neuromodulation Res., Medtronic Inc., Minneapolis, MN

Abstract: Introduction Peripheral nerve field stimulation (PNFS) is a novel technique for relieving pain. The method works by subcutaneously implanting a small electrode in the painful area. The electrical stimulation is believed to activate primarily Aβ fibers, which reduces the sensation of pain. When used in the lower back the electrodes are typically implanted either vertically or horizontally in the frontal plane. However, the general direction of the sensory nerves is rather diagonally downwards, originating at the spine. Previous studies have indicated that anodal blocking may block the propagation of an action potential along the nerve fiber. Thus, if anodal blocking occurs during PNFS it may limit the effectiveness of the method. Methods A mathematical model was developed to investigate the importance of the angle between the nerve fiber and stimulating electrode and potential anodal blocking. The model comprised two parts; first a finite element (FE) model was used to simulate the extracellular electrical field; second part, the nerve fiber activation using an active cable model. The FE model comprised of three layers, the skin, the subcutaneous fat and muscle. The stimulating electrode was simulated as a cylinder within the fat layer in a depth of 10mm. The diameter of the electrode was 1.3mm. The nerve fibers were simulated using cable models, with Wesselink membrane kinetics. The nerve fibers were simulated in three different depths; 15, 20 and 25mm below the skin surface and in various directions in relation to the stimulating electrode. Two types of nerve fibers were modeled; Aβ fibers had a diameter of 9μm and Aδ fibers had a diameter of 5μm. Results The results showed that the threshold of the nerve fibers was lowest when the stimulating electrode and nerve fibers were parallel. In contrast the threshold was highest when the nerve fiber and electrode were perpendicular. As the distance between stimulating electrode and nerve fibers decreased so did the activation threshold. Aβ fibers had lower thresholds than Aδ fibers. The lowest Aβ threshold was 0.563V and the lowest Aδ threshold was 0.75V. Most Aβ fibers had thresholds within the therapeutic range of PNFS (<10V), but only Aδ fibers located close to the electrode could be activated. The simulations found no evidence that anodal blocking could occur during PNFS. Conclusion As the results indicated the importance of the placement of the electrode in relation to the targeted nerve fibers, it may therefore be highly relevant to take the overall nerve fiber direction into account when implanting the electrode. This indicates that each PNFS setup should be adapted to each individual patient.

Disclosures: K.S. Frahm: 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.; Medtronic, Inc.. **K. Hennings:** None. **L. Vera-Portocarrero:** A. Employment/Salary (full or part-time):; Medtronic, Inc. **P.W. Wacnik:** A. Employment/Salary (full or part-time):; Medtronic, Inc. **C.D. Mørch:** 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.; Medtronic, Inc..

Poster

268. Neuron Stimulation Methods

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Topic: G.04. Physiological Methods

Support: FRM Postdoctoral Fellowship (SPF20130526842) to A. Kaszas

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ANR PolyProbe (ANR-13-BSV5-0019-01)

FRM (DBS20131128446)

Fondation de l'Avenir

Region PACA

Title: Localized neuron stimulation with organic electrochemical transistors on delaminating depth probes

Authors: *A. KASZAS[1,2], A. WILLIAMSON[1,2], M. FERRO[3], P. LELEUX[4], E. ISMAILOVA[3], T. DOUBLET[1,2], P. P. QUILICHINI[1,2], J. RIVNAY[3], G. KATONA[5], B. RÓZSA[5,6], G. MALLIARAS[3], C. BERNARD[1,2];
[1]Inst. de Neurosciences des Systèmes, Aix-Marseille Univ., Marseille, France; [2]Umr_s 1106, Inserm, Marseille, France; [3]Dept. of Bioelectronics, Ecole Nationale Supérieure des Mines, CMP-EMSE, MOC, Gardanne, France; [4]Microvitae Technologies, Hôtel Technologique, Meyreuil, France; [5]Two-Photon Imaging Ctr., Inst. of Exptl. Med. HAS, Budapest, Hungary; [6]The Fac. of Information Technol. and Bionics, Pázmány Péter Catholic Univ., Budapest, Hungary

Abstract: Stimulating individual neurons in a minimally invasive manner is a requirement for future brain-machine interfaces. Here we report on organic electrochemical transistors (OECTs), which outperform conventional electrodes for stimulating individual neurons. We recently

demonstrated the use of organic electrochemical transistors (OECTs) as high-quality recording devices for *in vivo* applications. However, it is important not only to record but also to stimulate large numbers of neurons. Here we present a minimally invasive and biocompatible device that consists of a channel made of a conducting polymer (PEDOT:PSS) placed in direct contact with an electrolyte. While traditional electrolyte-gated field-effect transistors contain a physical barrier separating ionic charges in the electrolyte from electronic charges in the channel, OECTs use ion transport directly across the electrolyte/channel interface to modulate the doping state of the conducting polymer. The lack of a physical barrier at the interface with the electrolyte creates the opportunity to use the conducting polymer channel as a current source for electrical stimulation, rendering the transistor a bidirectional neural interfacing device. To lessen tissue damage and inflammatory responses caused by the probe, we utilized OECTs implanted on a flexible Parylene-C film that detaches from the rigid shuttle after implantation to reduce the mechanical forces between the probe and the neural tissue. The electrical characteristics of the transistors before and after delamination remain identical when implanted in freely moving rats, and glial scarring was less prominent than for the implanted silicon probe even after 1 month of implantation. The ability of OECTs to deliver stimulation currents was characterized in saline, then in complete extracted hippocampus preparations. An OECT depth probe was implanted in the pyramidal layer to activate Schaffer collaterals, while the responses were followed by 3D two-photon imaging after bolus injection of SR-101 and OGB1-AM. Following stimulation, the devices could be returned to recording mode with no degradation in transistor performance. These results could provide the basis for the application of OECTs in a variety of research and clinical settings as a novel, high performance and minimally invasive transducer for *in vivo* electrophysiology.

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Poster

268. Neuron Stimulation Methods

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Support: CIHR Grant 493054

OBI Grant 493685

Title: Desynchronization prior to seizures is a common feature of electrographic signals in acute and chronic seizure models in rodent and human temporal lobe epilepsy

Authors: *M. T. SALAM[1,2], R. GENOV[3], T. VALIANTE[4,1], J. L. P. VELAZQUEZ[5,6], L. ZHANG[2];
[1]Toronto Western Hosp., Toronto, ON, Canada; [2]Med., [3]Electrical and Computer Engin., [4]Surgery, [5]Paediatrics, Univ. of Toronto, Toronto, ON, Canada; [6]The Hosp. for Sick Children, Toronto, ON, Canada

Abstract: Objective: Electrographic seizures are commonly associated with various synchronization states, from higher to lower than normal synchrony. In this study, we have investigated the dynamic changes of synchronization states and focused on the detection of synchrony changes in the preictal period before the discharge or clinical manifestation of the ictus. Methods: We have investigated the dynamic changes of synchronization states in acute and chronic models of rodent of post-ischemic, cobalt-induced, 4-Aminopyridine (4AP) induced, kindle, and kainic acid (KA) induced EEG discharges, and as well in instances of human temporal lobe epilepsy. Our preictal detection technique relies on the monitoring of the fluctuations in phase synchronization, and detects an upcoming electrographic seizure upon a decrease in synchronization (or, equivalently, an increase in the fluctuations in phase difference) as early as a few seconds to a few minutes before the behavioural and electrographic seizure onset. In an acute (4AP) and a chronic (KA) models, responsive electrical stimulation (150 μA, pulse width 100 μs, frequency 5 Hz, and duration 5 sec) was triggered on the response of decrease in synchronization in order to disrupt the seizure formation. Results: The overall detection performance has been evaluated using EEG discharges from 25 rodents in the five different models (five in each model) and ten patients. The average detection sensitivity and specificity are 86% and 82%, respectively; with ~0.57 false alarms per hour. The mean early detection time is 30 seconds before seizure onset. The responsive stimulation reduced seizure frequency by a 90% in 10 rats, with 83% of the animals rendered seizure-free. Significance: Abnormal synchrony patterns are common features in epilepsy and other neurological and psychiatric syndromes; thus, this type of feedback stimulation paradigm could be a novel therapeutic modality for various neurological and psychiatric disorders.

Disclosures: **M.T. Salam:** A. Employment/Salary (full or part-time):; University of Toronto. **R. Genov:** A. Employment/Salary (full or part-time):; University of Toronto. **T. Valiante:** A. Employment/Salary (full or part-time):; University of Toronto. **J.L.P. Velazquez:** A. Employment/Salary (full or part-time):; University of Toronto. **L. Zhang:** A. Employment/Salary (full or part-time):; University of Toronto.

Poster

268. Neuron Stimulation Methods

Location: Hall A

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Program#/Poster#: 268.07/BB80

Topic: G.04. Physiological Methods

Title: Non invasive brain stimulation techniques for improving cognitive functions in healthy individuals

Authors: *G. B. PATRUDU;
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Abstract: There is an increasing interest in developing novel noninvasive techniques for improving cognitive functions in healthy individuals. Non invasive brain stimulation methods that can have a positive impact on cognitive functions in healthy individuals are TMS (Transcranial magnetic stimulation) , tDCS (Transcranial direct current stimulation), tACS (Transcranial alternating current stimulation) , tRNS (Transcranial random noise stimulation), VNS (Vagal nerve stimulation) and TNS (Trigeminal nerve stimulation) .TMS was shown to improve speed and accuracy in a variety of tasks involving perceptual, motor, executive processing and memory in healthy individuals(Luber & Lisanby, 2014) . Higher frequency repetitive TMS (5-20Hz) can increase cortical excitability (Pascual-Leone et al., 1994; Chen et al., 1997). tDCS is shown to enhance performance across a range of cognitive tasks(Elmasry etal., 2015). It can boost cortical excitability (Boggio et al., 2006, 2007)and improve memory in healthy people(Bennabi etal 2014). There are limited number of studies on the effect tACS on cognitive functions in healthy subjects. tACS in the theta range can improve cognition and in the Alpha range can improve motor performance (Antal & Paulus, 2013).Theta synchronization by tACS significantly improves visual memory-matching reaction times (Polanía etal., 2012), performance on tests of fluid intelligence(Pahor & Jaušovec, 2014), working memory storage and processing functions (Jaušovec etal., 2014). tACS in the alpha range attenuates visual motion adaptation (Kar & Krekelberg , 2014). Santarnecchi etal.,(2013) found that stimulation in the gamma range can improve fluid intelligence. tRNS studies, showed that it positively modulates cortical excitability and improves motor learning in healthy subjects(Terney et al., 2008).It was shown to Improve Neuroplasticity in Perceptual Learning (Fertonani et al., 2011; Cappelletti etal.,2013; Snowball, 2013) and can induce long-term enhancement of cognitive and brain functions (Snowball, 2013; Cappelletti etal., 2013) .Vagal nerve stimulation in healthy individuals showed improvements in cognitive functions like memory (Jacobs, 2015) & response selection during action cascading processes (Steenbergen etal., 2015) .Even though,there are no studies on the effect of TNS on cognitive functions in healthy subjects, drawing from the results of a recent study on the effect of TNS on ADHD patients showing improvements in executive functions, incongruent reaction time and performance on computerized attention network task(McGough etal.,2015) , it has the potential to improve cognitive functions in healthy subjects.

Disclosures: G.B. Patrudu: None.

Poster

268. Neuron Stimulation Methods

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 268.08/BB81

Topic: G.04. Physiological Methods

Title: Engineering mechanoreceptor Piezo1 for fast magneto-genetic neuromodulation

Authors: *G. DURET[1], D. B. MURPHY[2,1], S. POLALI[2,1], E. ANDERSON[3], B. W. AVANTS[1], J. T. ROBINSON[1,3];
[1]Electrical and Computer Engin., [2]Applied Physics, [3]Bioengineering, Rice Univ., Houston, TX

Abstract: Fast remote stimulation of specific neuronal populations would help reveal how the activity of neural circuits deep within the brain affects the behavior of freely moving animals. One method to remotely stimulate these deep brain neurons is to convert magnetic fields that weakly interact with tissues into a force that can activate genetically encoded ion channels. Recent demonstrations of this approach have used paramagnetic nanoparticles to convert an RF magnetic field into heat that in turn activates thermally gated TRPV1 channels. While this approach is an effective and innovative method for remote neuromodulation, magneto-thermal neuromodulation is difficult to control with millisecond timing. Here we report our efforts to develop a temporally precise magneto-genetic neuromodulation method based on converting magnetic fields to a mechanical force that can activate the mechanoreceptor Piezo1. Specifically we have inserted affinity tags in extracellular domains of the cation-selective Piezo1 channel to which we can attach functionalized superparamagnetic nanoparticles. We have identified several binding sites on the Piezo1 protein that are accessible to magnetic nanoparticles and where the protein tag does not disrupt the gating mechanism. We have also demonstrated a high-binding affinity between functionalized superparamagnetic nanoparticles and several tagged variants of Piezo1. Additionally, we have found based on calculations and experiments in HEK293s that a DC magnetic field of only a few hundred mTesla (generated by a permanent magnet or an electromagnetic coil) creates enough force between the nanoparticles to activate Piezo1 and depolarize transfected cells. Ongoing work aims to use this magneto-mechanic ion-channel activation to stimulate action potentials in neurons.

Disclosures: G. Duret: None. **D.B. Murphy:** None. **S. Polali:** None. **E. Anderson:** None. **B.W. Avants:** None. **J.T. Robinson:** None.

Poster

268. Neuron Stimulation Methods

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 268.09/BB82

Topic: G.04. Physiological Methods

Support: MDIC CRADA #199-14

Title: Dissolution of platinum electrodes during electrical stimulation of neural tissue

Authors: D. KUMSA[1,2], D. BARDOT[2], *P. TAKMAKOV[1];
[1]CDRH-OSEL-DBCMS, FDA, Silver Spring, MD; [2]Med. Device Innovation Consortium, St. Louis Park, MN

Abstract: Neuromodulation therapy is designed based on a risk-benefit analysis. One of the risks associated with this therapy is damage to tissue due to high electric current. Current boundaries for safety of electrical stimulation are evaluated based on charge per phase and charge density per phase. One of the possible damaging mechanisms which can be traced to electrochemical processes occurring at the electrode-tissue interface is the formation of soluble platinum salts. In this work, dissolution of platinum electrodes is studied *in vitro* using therapeutic stimulation protocols. Effects of parameters such as current amplitude and duration of stimulation have been investigated. The results show a significant increase in the dissolution of Pt when charge injection levels are exceeded past the currently used boundary of 85 $\mu C/cm^2$ for a 0.00785 cm^2 electrode. Longitudinal data show that Pt dissolution does not plateau, but keeps increasing up to 5 hours. Previous studies showed that stimulation above 85 $\mu C/cm^2$ causes tissue damage. It was also observed that Pt dissolution also significantly increases above this value. Possibility of causation between the two might suggest that minimization of Pt dissolution during electrical stimulation can mitigate tissue damage. The preliminary data quantify platinum dissolution and its correlation to existing electrical stimulation criteria. Future work will assess effects of duration of stimulation, presence of proteins in solution, pulse width, change of polarity of leading pulse, stimulation frequency and absence or presence of oxygen on platinum dissolution.
Acknowledgement: The authors wish to thank and acknowledge Prof. J.T Mortimer for knowledge provided from work conducted by his lab at CWRU under a Sponsored Research Collaboration Agreement on 'Electron Transfer Processes Occurring on Platinum Neural Stimulation Electrodes' that informed the design of the current work. The authors also wish to thank colleagues at CWRU, Dr. Narendra Bhadra ; Prof. Jeff Capadona; Prof. Horst Von Recum; Fred Montague and Kyle Kovach for various useful discussions on data processing and design of experiments. The MDIC wishes to thank and acknowledge the Department of Biomedical Engineering at CWRU for providing research equipment for this work.

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Poster

268. Neuron Stimulation Methods

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 268.10/BB83

Topic: G.04. Physiological Methods

Support: General Stim Inc

Title: Neurostimulation strategy for urinary stress incontinence

Authors: *X. HUANG[1,2], G. E. LOEB[1,2], L. LIAO[3,4];
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Abstract: The majority of patients with stress incontinence have weakness of the external urethral sphincter muscle (EUS) with hypotrophic changes. Kegel exercises are highly effective to treat urinary stress incontinence when done properly and conscientiously. Unfortunately, many patients lack the compliance to obtain full benefit. Electrically stimulated exercise should be able to provide the same effect as voluntary exercise, but previous attempts to use transcutaneous stimulation produced unpleasant sensations from activation of cutaneous nerves. An injectable and wireless microstimulator that can be implanted percutaneously into the pelvic floor muscles should be able to generate strong contractions of the EUS without producing unpleasant sensations or requiring voluntary effort. The pelvic floor muscles are innervated by myelinated motor axons in branches of the deep perineal nerves from the pudendal nerves on each side. In order to investigate the neuromusclar anatomy, percutaneous needle electrodes were used to locate electrical stimulation sites that recruited the EUS as measured by intra-urethral pressure catheter in two female and two male subjects and by recording intramuscular M-waves in two additional female subjects. Perceptual and pressure thresholds were generally concurrent in the range of 0.2-1mA at 0.2ms cathodal stimulation at ~4-5cm depth in female subjects. Unpleasant sensation were reported only in patients with urethral catheters or with high strength stimulations above 7 mA, which may relate to perception of a strong EUS contraction. The precise location of the stimulation electrode had little effect on the ability to achieve strong and widespread muscle contractions in the pelvic floor. Simultaneous bilateral stimulation at levels above unilateral thresholds produced no obvious change in pressure or subjective sensations. The strong EUS activation with unilateral stimulation is consistent with previous studies mapping bilateral spread of M-waves associated with single unit activation. It suggests that a single-channel percutaneous implant can be used to produce clinically effective muscle exercise. Stimulus charge will probably be well below 1uC (5mA x 0.2ms), which should be easily achieved with low voltage circuits (<5V).

Disclosures: **X. Huang:** A. Employment/Salary (full or part-time):; General Stim Inc. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); General Stim Inc. **G.E. Loeb:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); General Stim Inc. F. Consulting Fees (e.g., advisory boards); General Stim Inc. **L.**

Liao: C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); General Stim Inc.

Poster

268. Neuron Stimulation Methods

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Topic: G.04. Physiological Methods

Support: DFG Grant STU 544/2-1

Netherlands Organisation for Scientific Research (NWO)

Title: Temporally precise control of single-neuron spiking by juxtacellular nanostimulation

Authors: *M. C. STUTTGEN[1,2], L. J. P. NONKES[3,2], H. R. A. P. GEIS[2], P. H. TIESINGA[4], A. R. HOUWELING[2];
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Abstract: The temporal patterning of neuronal spike trains affects sensations and activity-dependent neuronal plasticity. Elucidation of the underlying mechanisms requires imposing spike trains with precisely defined patterns, but this has been challenging due to the limitations of existing stimulation techniques. Here we present a new nanostimulation method providing control over the action potential output of individual cortical neurons. Spike induction is realized through the juxtacellular application of short-duration fluctuating currents, allowing for the sub-millisecond precise and reproducible generation of arbitrary patterns of action potentials at all physiologically relevant firing frequencies, including minute-long spike trains recorded in freely moving animals. We systematically compared our method to whole-cell current injection as well as optogenetic stimulation and found that while all three methods achieve high temporal precision, the nanostimulation method presented here is the most easily applied and can be readily performed in awake behaving animals. This new technique promises to be a powerful tool for systematic experimental investigations into the temporal elements of neural codes as well as the mechanisms underlying a wide variety of activity-dependent cellular processes.

Disclosures: M.C. Stuttgen: None. **L.J.P. Nonkes:** None. **H.R.A.P. Geis:** None. **P.H. Tiesinga:** None. **A.R. Houweling:** None.

Poster

268. Neuron Stimulation Methods

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Topic: G.04. Physiological Methods

Support: NAMASEN EC-FP7-PEOPLE ITN 264872

Volkwagen Foundation, Experiment!

Title: Artificial synapse chip: a nanopore array for local chemical modulation of neuronal activity

Authors: *P. D. JONES[1], M. STELZLE[2];
[1]NMI Natural and Med. Sci. Inst. At the, Reutlingen, Germany; [2]NMI Natural and Med. Sci. Inst. at the Univ. of Tuebingen, Reutlingen, Germany

Abstract: We have developed a novel microelectrode array (MEA) with integrated nanofluidics for local chemical modulation and electrophysiological recording of neuronal activity. Neurotransmitters or modulators can be released from single nanopores with high spatial precision. Integration with a network of microfluidic channels enables individual control of each nanopore. This will allow targeting of local populations within neural cultures or tissue slice preparations. Compatibility with standard microelectrode array technology will facilitate simultaneous chemical stimulation and electrophysiological recordings of neural response. State-of-the-art chemical stimulation relies on challenging micropipette-based methods which have remained conceptually unchanged for decades. Using modern microfabrication methods, we have produced a chemical stimulation device, analogous to the advance from single microelectrodes to the microelectrode arrays now widely used in neuroscience research. Microfluidic channels as small as 10 μm were produced by photolithography (SU-8, Microchem Corp., USA; ADEX, DJ DevCorp, USA) on standard MEAs (MCS Multi Channel Systems GmbH, Germany). Nanopores were fabricated by focused ion beam milling in silicon nitride membranes (Silson Ltd, England), which were bonded to the microfluidics by a dry bonding method. Microfluidic channels control fluidic delivery to each nanopore. Chemical release from single nanopores has been confirmed by fluorescence microscopy. Nanopores with diameters below 100 nm enable finer control in comparison to previous chemical release arrays, which have used micropores larger than 1 μm. Our method can be extended to exploit novel nanofluidic effects such as hydrophobic gating, which would prevent leakage by diffusion. This technology will open the possibility for chemical stimulation at a scale previously reserved for advanced electrode arrays, and is a necessary step towards realization of biomimetic neurotransmitter-based neuroprosthetics. We will present the results of proof-of-concept experiments, which will chemically stimulate neurons and measure their electrophysiological response. This technique will be broadly applicable for investigations of localized release of compounds which could include neurotransmitters, drugs, or other pharmacological agents. The adaptation of biological preparations established on normal MEAs, and the implementation of this device in other neuroscience laboratories will be facilitated by its foundation on standard MEA technology.

Disclosures: **P.D. Jones:** None. **M. Stelzle:** None.

Poster

268. Neuron Stimulation Methods

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Program#/Poster#: 268.13/BB86

Topic: G.04. Physiological Methods

Support: AHA Pre-Doctoral Fellowship

Title: Prolonged depressor response induced by transient deep peroneal nerve fasicular stimulation in a hypertensive animal model

Authors: ***A. KANNEGANTI**[1], M. MIZUNO[2], R. DOWNEY[2], M. WIJESUNDHARA[4], S. SMITH[2], M. I. ROMERO-ORTEGA[1,3];
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Abstract: Hypertension affects approximately 70 million American adults, and an estimated half of these cases are not satisfactorily controlled by medication. Uncontrolled hypertension can greatly increase a patient's risk for stroke and myocardial infarction. Pharmacological interventions dominate the treatment options for hypertension, with seven in every 10 patients taking prescription medication despite the diverse side effects and resistivity after prolonged use. Other treatment options, such as transcutaneous or direct electrical stimulation of particular nerves, namely vagus, carotid sinus, and renal sympathetic, have resulted in mixed results among different patient groups. The targeted visceral nerves regulate diverse autonomic functions which may explain the mixed results and the potential side effects due to non-specific targeting. Alternatively, animal studies based on Electroacupuncture (EA), have been shown to attenuate the blood pressure in a hypertensive rat model by enhancing NO/NOS (Nitric oxide/oxide synthase) activity. Direct stimulation of the nerve which underlies a given acupuncture point has been shown to mimic EA efficacy. In this study, we evaluate blood pressure regulation in a spontaneously hypertensive rat (SHR) model via electrical stimulation of the somatic deep peroneal nerve fascicles (corresponding to ST36 acupoint), using a novel Microchannel based Multi-Electrode Array (μCEA). Constant current biphasic pulse stimulation (100μA pulses at 2 Hz for 5 seconds) of the ventral deep peroneal nerve fascicle (vDPN) in SHR rats elicited a significant decrease in mean arterial pressure (MAP) from 177.95 ± 49.20 mmHg, to normotensive levels (152.8 ± 47.8 mmHg; $p \leq 0.05$), as measured from the carotid artery. MAP levels returned to baseline levels within 5-10 minutes post-stimulation. Control WKY (normotensive) rats did not show any changes in MAP (142.6 ± 34.6) during or after electrical stimulation. We are currently investigating whether vDPN stimulation can elicit a prolonged

MAP drop. Preliminary results show that transient (5 minute) electrical stimulation at the motor threshold level results in a significant decrease in MAP from 186.01 ± 29.21 mmHg to 150.04 ± 28.4 mmHg ($p \leq 0.01$) and was sustained up to 3 hours post stimulation. The stimulation currents and duration used for existing somatic neuromodulation based therapies usually range between 2-4 times threshold and require 1 hour of continuous stimulation. Herein we report for the first time, an effective alternative for prolonged hypertension regulation via somatic nerve fascicular neuromodulation with only 5 minutes of stimulation at motor threshold currents.

Disclosures: A. Kanneganti: None. **M. Mizuno:** None. **R. Downey:** None. **M. Wijesundhara:** None. **S. Smith:** None. **M.I. Romero-Ortega:** None.

Poster

268. Neuron Stimulation Methods

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Program#/Poster#: 268.14/BB87

Topic: G.04. Physiological Methods

Title: Comparing ultrasound neuromodulation and ultrasound stimulation in rat motor cortex

Authors: *D. W. GULICK[1], T. LI[2], B. C. TOWE[1], J. A. KLEIM[1];
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Abstract: Ultrasound is known to stimulate motor responses in small animals, and modulate brain activity in large animals and humans. It shows potential for noninvasive neuromodulation more focal than TMS or tDCS. However, the mechanism is unknown and there are unexpected limitations. In rats, ultrasound can evoke movement of hindlimbs and tail but not forelimbs. This experiment sought to clarify the effect of ultrasound on rat motor cortex by testing for modulation effects on electrically-evoked movements. A novel 16-channel epidural cortical stimulation array was implanted. The rat was stimulated under anesthesia, with accelerometers on the contralateral forelimb and hindlimb. Some electrical stimuli were preceded by ultrasound pulses (200 kHz, 60 W/cm^2 SPPA, 300 ms, pulsed at 1 kHz, 50% duty). Responses with vs. without ultrasound were compared. In an analysis for short-term modulation effects, neither limb showed a significant difference (9 trials). This may be due to the 100 ms delay between the end of the ultrasound pulse and the electrical stimulus. There was a significant long-term suppression (over several seconds to minutes) on both forelimb and hindlimb (10% and 13% decrease). In addition to modulating the electrically-evoked response, some ultrasound pulses directly evoked hindlimb movements. This response only occurred when ultrasound pulses were spaced more than ~3 seconds apart. During the refractory period when ultrasound could not evoke movement, the response to electrical stimulation was unaltered. This suggests that ultrasound stimulation has a mechanism separate from that of electrical stimulation.

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Poster

268. Neuron Stimulation Methods

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Topic: G.04. Physiological Methods

Support: BK21

DAPA Grant UD140069ID

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College of Engineering, College of Dentistry, SNU

Title: Efficient stimulation of hypoglossal nerve using cuff electrode

Authors: ***J. SEO**[1], J. PARK[1], H. KIM[2], S. HONG[2], W. LEE[2], J. AHN[2], S. JO[2], S.-H. AHN[1], C. KIM[1], J.-W. KIM[2], S. KIM[1];
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Abstract: In this study, we have evaluated threshold current level for stimulating hypoglossal nerve. A two-channel cuff electrode was designed with polyimide which has biocompatible and flexible characteristics. One of hypoglossal nerves of a rabbit was enclosed by the electrode, which was tightened up with suturing. Subsequently, the electrode was percutaneously connected to a pulse generator which generates a sequence of train-of-pulse with the output current level ranging from 14.4 to 1.84mA. Embletta x100 was used to measure an electromyography and the upper airway movement of the rabbit while stimulated was recorded using C-arm fluoroscopy. The minimum required current level for stimulating the hypoglossal nerve was 41.0with duration 0.376 and rate 0.635. The measured threshold is significantly lower than the previously reported value, several mili-amperes. C-arm fluoroscopy observed that the upper airway had been enlarged when the rabbit was stimulated with the level described above.
While the stimulation with 53.2 current evoked an intense response of the muscle, 41.0 current induced a slight response. The slight muscle response can be applied to treating obstructive sleep apnea during the sleep, since the response is small enough not to interrupt the sleep.
In summary, we have demonstrated that the threshold current level for stimulating the hypoglossal nerve with the cuff electrode is 41.0, which is far less than several mili-amperes described in previous researches. Furthermore, there was the difference in response between 41.0 and 53.2; the former induced slighter muscle response than the latter, which can be used to

stimulate hypoglossal nerve without interrupting the sleep in case of treating obstructive sleep apnea.

Disclosures: **J. Seo:** None. **J. Park:** None. **H. Kim:** None. **S. Hong:** None. **W. Lee:** None. **J. Ahn:** None. **S. Jo:** None. **S. Ahn:** None. **C. Kim:** None. **J. Kim:** None. **S. Kim:** None.

Poster

268. Neuron Stimulation Methods

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Support: CNPq

CAPES

Fundacao Araucaria

Title: Sub-second recording of dopamine release in the nucleus accumbens of awake head-fixed mice by fast-scan cyclic voltammetry

Authors: *C. DA CUNHA[1], A. GÓMEZ-A[1], C. D. BLAHA[2];
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Abstract: In this study we established a new protocol to monitor dopamine (DA) release in the brain of awake head-fixed mice by fast scan cyclic voltammetry (FSCV). This protocol was adapted from a previous protocol established for electrophysiology studies in head-fixed mice (J Neurosci Methods 178:75-79, 2009) and uses the Wireless Instantaneous Neurotransmitter Concentration Sensor (WINCS, Mayo Clinic, USA) system for FSCV recordings of DA at carbon-fiber microelectrodes (J Neurosurg 111:701-711, 2009). Adult male Swiss mice were anesthetized with ketamine-xylazine and mounted in a stereotaxic frame. Two circular openings of 2 mm diameter were drilled above the nucleus accumbens (NAc) and the ventral tegmental area (VTA) in the left frontal and in the parietal bones, respectively. Cylindrical plastic recording chambers (3 mm diameter and 7 mm height) were fitted over the bone around the openings and filled with an antibiotic gel. An Ag/AgCl wire reference electrode was inserted into a smaller hole drilled in the right parietal bone. A V-shaped stainless steel headpost was placed over the skull in the space left between the two chambers and all pieces were fixed to the bone with super glue and dental cement. One or 3 days after surgery the head of the mouse was fixed by the headpost to a frame. This frame supported stereotaxic arms used to lower the recording and stimulation electrodes into the NAc and VTA, respectively. Triangular waveform potentials of -0.4 to +1.0 V versus Ag/AgCl were applied to the recording electrode every 100 ms and

oxidation and reduction currents were recorded. Spontaneous transient increases of DA oxidation current were observed (nearly 6 transients/min). Peaks of DA release were also evoked by electric stimulation of the VTA. Background subtracted voltammograms typical of DA were observed, with an oxidation peak at nearly +0.7 V. When the animals were recorded 24 h after surgery, DA concentration increased nearly 0.3 μM upon stimulation of the VTA. Significantly lower evoked peaks (nearly 0.2 μM) were registered when the animals were recorded 3 days after surgery. Habituation to the head-fixed procedure by handling and maintaining the animals head-fixed for 15 min per day in the 2 days interval between surgery and recording session did not alter the frequency and the magnitude of the DA peaks compared to non-habituated animals. This study showed that this head-fixed mouse preparation can be used for FSCV recordings of DA at carbon-fiber microelectrodes and that the best data can be obtained when measures are performed 1 day after surgery.

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Poster

268. Neuron Stimulation Methods

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The Smart IT Convergence System Research Center funded by the Ministry of Education, Science and Technology as Global Frontier Project (CISS-2012M3A6A6054204)

Title: Extracellular stimulation of rat VTA neurons with sinusoidal waveforms

Authors: *Y. A. CHO[1], Y. LEE[2], J. JANG[2], S. KIM[2], Y. LEE[3], J. LEE[2], S. JUN[2];
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Abstract: Rectangular waveform of electrical current or voltage is conventionally employed for most of neuroprosthetic systems. It was also reported that electrical sinusoidal waveform can be used for stimulating neurons instead of the square wave pulses. Sinusoidal stimulation has the advantage that the stimulation artifact can be effectively removed to identify the evoked neural activity during stimulation. Furthermore, recent studies showed that a large amount of charge injection can cause both electrode corrosion and tissue damage during electrical stimulation with rectangular waveforms. However, most previous studies have been limited in computational simulations or *in vitro* experiments. In this study, we demonstrated that sinusoidal electrical

stimulation on ventral tegmental area (VTA) effectively evoked the neural activities in nucleus accumbens (NAc) by removing the stimulation artifact via a simple bandpass filtering including the corresponding frequency. The VTA region was electrically stimulated with a tungsten stimulating electrode (254 μm diameter), and the neural activities of the nucleus accumbens (NAc) was simultaneously recorded in anesthetized rats. Local field potentials (LFPs) was investigated to show the difference of power discharged between two different stimuli with sinusoidal and rectangular waveforms. As a result, the sinusoidal wave stimulation is expected as an alternative of square wave pulse. In addition, tissue damage was evaluated using immunohistological analysis through long-term stimulation.

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Poster

268. Neuron Stimulation Methods

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Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

Program#/Poster#: 268.18/BB91

Topic: G.04. Physiological Methods

Title: Non-invasive brain stimulation using focused magnetic field produced by magnetic shielding

Authors: ***M. C. CHERRY**[1], Q. MENG[1], E. HONG[2], F.-S. CHOA[1];
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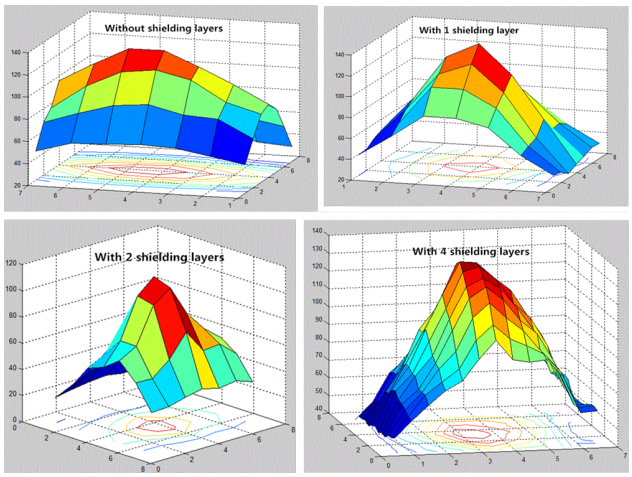
Abstract: Transcranial magnetic stimulation, as one of the most popular brain and nerve stimulation methods, is now widely applied in diagnosis and treatment for many diseases, such as neuropathic pain, treatment-resistant major depressive disorder, and loss of function caused by stroke. Current commercial TMS stimulators are not capable of producing a focused magnetic field beam, particularly when target is located deep and away from the TMS source. The so called deep modulation tools, like e.g. H-coil, are usually spread global modulation tools which cannot target to a specific location in deep brain. In traditional magnetic field focusing methods, a piece of thin magnetic material rod or bar with high relative permeability is used to absorb the magnetic field, making it possible to restrict the magnetic field within a narrow space and realize field concentration. Using a high permeability waveguide would require a rod or bar to sit in between the TMS coil and the targeted treatment site and make it impossible to perform non-invasive stimulation. In this work, we used arrays of copper rings to distort the magnetic field and achieve focusing. The superposition of eddy current induced a field opposing the original magnetic field and produced a final concentrated field distribution. In this work, the stimulator was made of a part of a transformer. It has two poles, aligned in north-and-south arrangement.

The magnetic field was shielded by up to 4 different copper ring arrays. A field probe, reported in previous SfN meeting, was used for field recording at different locations. It was found that when using the shield and under the best condition with a design of 4 layers of ring arrays, the magnetic field spot size was reduced from 2cm down to 0.5cm. The measured results show that with appropriate usage of shielding effect we can distort the field and achieve magnetic focusing. This will be very useful for future targeted noninvasive stimulation applications.



Disclosures: **M.C. Cherry:** None. **Q. Meng:** None. **E. Hong:** None. **F. Choa:** None.

Poster

268. Neuron Stimulation Methods

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Program#/Poster#: 268.19/BB92

Topic: G.04. Physiological Methods

Support: The Sutter Insitute for Medical Research

Title: Gender and high definition transcranial stimulation

Authors: ***M. J. RUSSELL**;
Aaken Res. Inst., Davis, CA

Abstract: Knowledge of what electrical current tissues are receiving is a critical factor in transcranial brain stimulation. Previously we have shown that the 10-20 system or other positioning schemes allow for a great deal of variation in the current received by the brain and do not account for gender differences in cranial bone density. In this report we use MRI tissue resistivity modeling to compare two methods of transcranial electrical stimulation 1) high density electrode positioning and 2) and traditional non-cephalic referencing. Method A combination of MRIs (T1, T2, proton density, and diffusion tensor) was recorded from 14 subjects and then

combined for each individual to obtain models of tissue resistivity. After the models were formed virtual electrodes were placed on each to represent high density and non-cephalic referencing. The amount of current received within a 1 cm sphere placed inside the brain directly under the anodal electrode at a C3 scalp location was estimated for each condition and subject. In the high density stimulation condition 1 anode (+) was modeled with 4 cathode (-) electrodes 1 cm apart on the scalp of the MRI model. In the non-cephalic condition a single electrode was placed at the identical anodal scalp location and non-cephalic cathode electrodes were placed on the subject's trunk below the neck. Comparisons were then made for input current levels of 0.5 mA, 1mA, and 2mA. Result The results were strikingly different between conditions (P>.001) and between genders (P>.03) for the current entering the brain. Women received less current than men in the high definition condition (P>.03). The mean values for the high definition condition were exceptionally low with some individuals receiving less than 1 μA even with the high 2mA input condition. Many of the subjects in the high density condition received less than 1 μA/cm2. Conclusions The cranial bone is a high resistance tissue that prevents most of the electrical energy from entering the brain in the high definition condition when the electrodes are close together. Because female cranial bone is denser then male bone the effect is larger in women. A non-cephalic placement is more consistent for both genders and provides a much higher current density to the brain. Placebo effects should be considered when subjects are receiving less than 3 μA/cm2.

Disclosures: **M.J. Russell:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aaken Laboratories.

Poster

268. Neuron Stimulation Methods

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Support: NSF CAREER award

Epilepsy Foundation Pre-doctoral Award

Title: Neural interfacing hardware for closed-loop stimulation with on-line stimulation artifact removal

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Abstract: Closed-loop control of neural stimulation is a growing discipline in the treatment of neurological and neurodegenerative disorders such as epilepsy or Parkinson's disease. For example, closed-loop control can be used to deliver electrical stimulation when at particular phases of a pathological neural oscillation or during a seizure. Implantable systems incorporating such algorithms determine precise stimulus timing based on features from neural activity. However, the recorded signal following a pulse is corrupted by large stimulation artifacts on recording electrodes, masking underlying neural activity and hindering the performance of the closed-loop algorithm. Furthermore, to enable post-processing stimulation artifact removal, the gain of the amplifier must be reduced so that the stimulus does not saturate the amplifier. Previous studies suggest a variety of post-processing artifact removal algorithms based on large data set analysis but lacking real-time capability necessary for treatment, or limitations on electrode orientation and stimulation characteristics to suppress interference at the recording site. Currently, there are no generalized stimulation artifact removal algorithms for real-time closed-loop neural recording and stimulation that do not constrain electrode configuration or stimulation characteristics. In order to tackle this problem, we have designed and fabricated a neural interfacing integrated circuit for ECoG and LFP signals with a novel adaptive stimulation artifact removal algorithm in real time. The algorithm utilizes an adaptive FIR filter which learns the artifact waveform using a stochastic gradient descent LMS algorithm. The fit stimulation artifact is then subtracted from the recorded signal at the preamplifier to remove the artifact and prevent amplifier saturation. The algorithm learns the artifact waveform within a few stimulation pulses, and can dynamically adapt to changing artifacts over time. To validate the system's feasibility, we have tested the interface circuit in an epileptic rat model. Seizures were induced following injection of 4-aminopyradine unilaterally into the CA3 region of the hippocampus. A recording electrode was placed in the CA1 region of the hippocampus ipsilaterally to the injection region. Stimulation was applied to the ventral hippocampal commissure (VHC) which innervates the CA3 regions bilaterally. We compared neural recordings while stimulating, with and without stimulus artifact removal, during a seizure episode. Our results indicate a large reduction in spectral power and time-domain artifact-related voltage spikes when artifact removal was achieved.

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Poster

268. Neuron Stimulation Methods

Location: Hall A

Time: Sunday, October 18, 2015, 1:00 PM - 5:00 PM

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Topic: G.04. Physiological Methods

Title: TMS waveforms and variability in response to an inhibitory TBS protocol

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Abstract: Background Continuous theta-burst stimulation (cTBS, Huang et al, 2005: Neuron 45(2)) can induce temporary changes in synaptic plasticity of motor cortical neurons in a LTD-like manner. Conventionally, cTBS is applied with biphasic stimulus pulses. Since different directions of monophasic pulse are known to activate separate populations of cortical interneurons, we asked two questions. First, does cTBS using monophasic stimulus pulses applied in the antero-posterior (AP) direction have different effects than TBS applied with posterior-anterior (PA) directions. Second, does cTBS have different effects on test responses evoked by different directions of monophasic stimulation? Experiments 14 healthy volunteers received monophasic cTBS using either AP or PA stimulus pulses to the first dorsal interosseous (FDI) representation of motor cortex. Corticospinal excitability was assessed before and after cTBS via amplitude of MEPs evoked by PA monophasic TMS pulses. A two way ANOVA revealed a significant TIME (pre/post) X DIRECTION (AP or PA) interaction ($F = 2.8$, $P = 0.017$) that was due to the fact that MEPs evoked with monophasic PA pulses were suppressed more by AP cTBS than PA. In a second experiment, 20 healthy volunteers received a "spaced" version of cTBS that has been claimed to be more effective than a single session of cTBS (Goldsworthy et al, 2012: Eur J Neurosci 35(1)) using AP monophasic TMS pulses. Corticospinal excitability was assessed before and after spaced cTBS by measuring the amplitude of MEPs evoked in FDI by PA and AP test pulses. Two way ANOVA of log transformed MEP amplitudes revealed a significant TIME (pre/post) X DIRECTION (AP or PA) interaction ($F = 9.4$; $p= 0.006$) due to the fact that monophasic AP cTBS produced more effective suppression of MEPs evoked by AP than by PA test pulses. For PA test pulses there was a significant correlation ($p=0.028$, $r = .490$) between the variability of an individual's baseline MEPs and the suppressive effect of cTBS. An ongoing experiment is comparing the effectiveness and reliability of AP and PA monophasic spaced cTBS on MEPs evoked by PA and AP test pulses. Discussion cTBS of motor cortex using monophasic AP pulses leads to larger effects on corticospinal excitability than PA-cTBS. The effects are more evident when excitability is tested with AP rather than PA pulses. We suggest that repetitive TMS of motor cortex does not condition all circuits by the same amount. Depending on the direction of stimulation, some circuits can be conditioned while others are relatively unaffected. This could contribute to some of the inter-individual variability that is typical of brain stimulation paradigms.

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Poster

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Title: Closing the loop around firing rate: following dynamic trajectories

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Abstract: Since the advent of optogenetics, there has been ever-growing interest in applying this technology to the control of neural activity. However, factors such as variable opsin expression, differential distribution of light, and spontaneous neural states make robust control of neural activity intractable with open loop stimulation. To overcome these limitations, we have engineered a system for closed-loop control of neural activity which combines single-unit thalamic recording and optogenetic stimulation through real-time interfacing in the rat vibrissa system in-vivo. Beyond allowing the experimenter to overcome unknown factors in the response of an opsin to light, closed loop control allows for decoupling of causally related variables such as firing rate and temporal precision. Previously, we demonstrated successful control of firing rate in single neurons in the ventral posteromedial nucleus of the thalamus (VPm) of the anesthetized rat using PI control. The controller operates on the error between the instantaneous firing rate and a target rate; the resulting control signal is used to modulate the intensity of light (470 nm, LED) conducted to VPM via optic fiber, thereby stimulating opsin-expressing cells in VPm. We have demonstrated the ability to follow target firing rates precisely, decoupling the magnitude of the firing rate from the statistics of the firing in the VPm. Furthermore, we can extend this framework to address the control of more complicated, dynamic firing rate trajectories. Closed loop control of firing rate to follow dynamic trajectories would enable precise characterization of sensory encoding in different neural contexts, such as oscillatory activity. To this end, we have tuned and characterized our system's ability to achieve sinusoidal trajectories. In assessing the capabilities of the original system outside the realm of static trajectories, we first carried out a system identification of the controller experimentally using step inputs, discrete sinusoids, and white noise as targets for the system. We found that the experimental system was limited by the transformation of the firing rate signal from a discrete representation of spikes to a continuous estimate of instantaneous firing rate. To systematically explore controller parameters in an environment that is not limited by experimental recording time, we developed a simulated model of the closed loop controller interacting with a single

neuron. Consistent with the experimental results, a sensitivity analysis of the simulated system parameters implicates the firing rate transformation as a critical parameter affecting the ability to follow arbitrary waveforms.

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Poster

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R01 NS 70872

Title: Neurotransmitter monitoring via boron-doped diamond electrode-based voltammetry during human deep brain stimulation in essential tremor

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Abstract: Introduction: Although it is becoming more evident that neurotransmitter dynamics contribute to therapeutic deep brain stimulation (DBS) mechanism, technologies for human neurotransmitter monitoring still face significant limitations. Previously, we reported acute intraoperative human recordings of neurotransmitter dynamics during DBS using carbon fiber microelectrodes (CFM) together with the Mayo-developed wireless instantaneous neurotransmitter concentration sensor (WINCS) system. One problem with CFM electrodes is their poor durability for long-term neurochemical recording. To overcome this limitation, we developed a durable synthetic diamond-based electrode that is capable of measuring neurotransmitters for longer time periods than a CFM. Here, we demonstrate the use of diamond-based FSCV recording in human essential tremor patients undergoing DBS neurosurgery. Method: Patients undergoing frame-based stereotactic DBS neurosurgery for tremor were recruited to undergo paired pulse voltammetry (PPV) recordings during DBS electrode implantation. Boron-doped diamond microelectrodes were placed 2 mm anterior or posterior to the trajectory of the DBS electrodes, which were placed in the ventralis intermedius nucleus of the thalamus. PPV was applied at 5 Hz (200 ms between two binary pulse sweeps), 400 V/s sweep rate with respect to a reference electrode, from -0.4 V to 1.5 V back to -0.4 V. This study was approved by the Mayo Clinic Institutional Review Board. Results: The detection of

adenosine-like signature was evident both when the recording electrode was advanced and during implantation of the DBS electrode. However, without PPV, the extracellular environmental changes, including pH shift, obscured neurochemical detection. No complications occurred due to boron-doped diamond electrodes and PPV recording in any patients. Conclusion: These results demonstrate that boron-doped diamond microelectrodes in conjunction with PPV can be safely and effectively used for differentiating complex analytes in the human brain and support the potential of wireless PPV monitoring to investigate long-term DBS mechanism of action.

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Poster

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Title: A novel approach to rapidly and effectively perturb the rotation of the eye

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Abstract: Despite recent excitement on the research of extraocular proprioception, validated approaches for studying this sensory signal remain limited. This is because the proprioceptive signal occurs during natural, visually guided eye movements, and the signal inevitably interleaves other visuomotor signals such as corollary discharge and vision. Therefore, separating extraocular proprioception from other sensory-motor signals remains a fundamental challenge. In the present study, we present a non-invasive methodology for probing extraocular proprioception independent of other sensorimotor processing during eye movements. We attached a rare earth magnet to a model eye ball and placed an electromagnet > 10 mm away from the robotic eye. The model eye was real-time controlled to rotate horizontally about a vertical axis. With this approach, we demonstrated that the electromagnet was able to perturb the rotation of the eyes by circumventing the constraints found with conventional approaches like manually tugging the eye ball by a suction lens. The electromagnet could increase or decrease angular displacements and velocities of the model eye. The comparison of the model eye's angular velocities under the presence or absence of electromagnet activation revealed that the

rotation could be rapidly altered within 2 ms of electromagnet activation. In addition, the angular displacement of the model eye was correlated linearly with the relative distance of the electromagnet from the model eye, suggesting that this perturbation can be remotely and safely applied to subjects. The results indicate that the electromagnet serves as an effective means to externally perturbing the rotation of the eyes, making it affordable to separating extraocular proprioception from other visuomotor signals.

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Poster

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Support: Western Michigan University College of Arts and Sciences Interdisciplinary Research Initiative Award

NASA Michigan Space Grant Consortium Undergraduate Research Fellowship

Title: Generating neuron membrane action potentials using optimal input current stimuli in the medicinal leech

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Abstract: A “Reduced Energy Input Stimulus Discovery Method” [1] finds input current stimuli which enable a conductance-based neuron model to track a reference membrane voltage. The technique features an adjustable balance between tracking error and input current stimulus energy reduction. Higher-energy input current stimuli are traditional pulses of constant value, while reduced energy optimal input stimuli have varying values within the stimulation time window. Simulations confirm that variable currents with significantly reduced energies provide excellent tracking of neuron membrane voltages such as those produced with constant valued input currents [1, 2]. This project builds on an initial feasibility study [3] and demonstrates that key features of this method can be employed at a traditional electrophysiology rig. Optimal input current stimuli are computed using conductance-based models with parameters selected to match dynamics of different types of medicinal leech (*Hirudo verbana*) neurons. These optimal current stimuli provide a range of tracking performance; some emphasize reproducing action potentials (at the expense of a higher energy current), while others emphasize energy reduction (sometimes at the expense of action potential timing or generation). The stimuli are used to stimulate leech

neurons using standard sharp electrode electrophysiology techniques. Results for different sensory neuron types show that optimal input current stimuli having significantly reduced energy levels are able to evoke action potentials that closely resemble neuronal responses to non-optimal higher-energy current stimuli. **References** [1] 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. [2] 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. [3] D. A. Miller, M. Ellinger, J. Jellies, A. C. Ferguson, C. L. Linn, and M. E. Koelling, "Pre-Computed Optimal Current Stimuli Evoke Similar Neuron Membrane Voltage Responses as Non-Optimal Stimuli," *Michigan Chapter of the Society for Neuroscience Annual Conference Annual Meeting*, May 11, 2015, Mount Pleasant, MI.

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Poster

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Title: The dose response relationship between single pulse TMS intensity and neuronal activity in cerebral cortex of alert macaques

Authors: *E. M. GRIGSBY[1,6], M. V. SMITH[1], M. J. KOVAL[2], Z.-D. DENG[2,3], M. L. PLATT[4,5], A. V. PETERCHEV[1,2,3], W. M. GRILL[1], M. A. SOMMER[1,4,5];
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Abstract: Transcranial magnetic stimulation (TMS) is a safe, non-invasive method of brain stimulation that induces an electric field in the brain. It has proven to be a valuable tool for cognitive research and clinical practice, despite the fact that little is known about its influence on neuronal activity. We designed a custom TMS coil to produce focal stimulation at the site of neuronal recordings *in vivo*, in combination with amplifier circuits to reduce TMS voltage and current artifacts (Mueller et al. 2014). The methodology permits recordings of neural activity starting a few ms after a TMS pulse. Here we characterize the dose response of single neurons to

single pulse TMS across stimulation intensities in rhesus macaque cerebral cortex (primary motor cortex and frontal eye field, n=3 monkeys). Using a MagStim Rapid2 base unit, we delivered TMS pulses from 10% to 90% of maximum intensity in 10% increments and compared normal TMS ("Stim") to a condition in which instrument currents were reversed to evoke TMS sound and peripheral stimulation but negligible electric field at the recording site ("Sham"). Our hypothesis was that significant, electric-field induced changes in activity occur at and above motor threshold (approx. 60% intensity in our monkeys). We found, first, that responses of individual neurons were heterogeneous, with diverse patterns of TMS-induced excitation and inhibition observed. Second, at the population level, all levels of Sham and <60% levels of Stim TMS caused a burst of activity around 30 ms latency. Third, for Stim TMS at >= 60% intensity, the population response shifted to a distinctly different pattern consisting of short-latency (10 ms) activation followed by long latency inhibition, the duration of which averaged ~100 ms but lengthened slightly with intensity. We conclude that the neuronal activity dose-response relationship to single pulse TMS is non-linear, stepping up near the motor threshold point in support of our hypothesis. At the same time, the Sham and low intensity Stim activations revealed significant effects potentially attributable to sensory afferent responses (e.g. auditory). In summary, single pulse TMS causes a short-latency, excitation-inhibition pattern of neuronal activity in accordance with a non-linear, stepwise dose-response curve that matches motor threshold.

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