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Poster

028. Neuronal Morphology and Cell Death

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 28.01/A1

Topic: A.01. Neurogenesis and Gliogenesis

Title: Characterizing GABAergic interneuron deficit in Holoprosencephaly

Authors: *A. GILANI, J. LIBIEN;
Pathology, SUNY Downstate Med. Ctr., Brooklyn, NY

Abstract: Normal brain function requires an intricate balance of excitation and inhibition. Local interneurons that secrete GABA contribute the major inhibitory input to pyramidal neurons in the cerebral cortex. Cortical inhibitory interneurons show great diversity in their synaptic connectivity, electrophysiological properties and peptide content and may perform distinct functions in the cortical circuit. Not surprisingly, alterations in GABAergic function have been implicated in various psychiatric and neurological conditions, most notably in epilepsy, schizophrenia, and autism. In spite of their potential significance, GABAergic interneurons have been seldom studied in postmortem neuropathological examination of pediatric developmental disorders. A previous study (Fertuzinhos et al, Cerebral Cortex September 2009) showed that in Holoprosencephaly, a neurodevelopmental disorder with incomplete separation between the right and the left hemisphere, specific populations of GABAergic interneurons are reduced. The majority of cortical GABAergic interneurons are produced in the ganglionic eminence (GE), a neuroproliferative zone overlying the future basal ganglia. To further characterize interneuron deficit in Holoprosencephaly, we used immunohistochemical markers that label interneurons produced in the medial (Sox 6) and caudal (COUP-TFII, Calretinin) divisions of the GE. Preliminary results in a small set of fetal human brains with lobar holoprosencephaly indicate a complete absence of Sox 6+ neurons and an abnormal distribution of COUP-TFII and Calretinin+ neurons. These data indicate that different GABAergic proliferative zones are differentially affected in cases of Alobar Holoprosencephaly.

Disclosures: **A. Gilani:** None. **J. Libien:** None.

Poster

028. Neuronal Morphology and Cell Death

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 28.02/A2

Topic: A.01. Neurogenesis and Gliogenesis

Support: Field Neurosciences Institute

Title: Determining the impact of Zika virus on neural stem cells and mature neurons

Authors: *O. V. LOSSIA[1,2,3], M. T. OCEAN[2], E. D. PETERSEN[2,1], B. SRINAGESHWAR[1,3], U. HOCHGESCHWENDER[2,1], G. L. DUNBAR[1,5,4,3], M. J. CONWAY[2], J. ROSSIGNOL[2,3,1]; [1]Neurosci., [2]Col. of Med., [3]Field Neurosciences Inst. for Restorative Neurosci., [4]Psychology, Central Michigan Univ., Mount Pleasant, MI; [5]Field Neurosciences Inst., Saginaw, MI

Abstract: The recent emergence of Zika virus (ZIKV) has been linked to severe nervous system abnormalities in infants, such as microcephaly, and the autoimmune disorder, Guillain-Barré, in adults. The World Health Organization predicts that by the end of this year three to four million people will be infected with the Zika virus, a virus now known to be transmitted by mosquitoes and through sexual contact in humans. Very little is known regarding the basic biology of this virus and its implications in the developing brain, fueling the need to investigate the interactions of ZIKV with neural cells. So far, studies have shown that ZIKV affects the morphology and survival of neural stem cells and neural progenitor cells. In our study, we sought to assess the effect of ZIKV on neural stem cells (NSCs) and mature neurons using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and multielectrode arrays (MEAs), respectively. We were able to determine the impact of infection on the secreted proteome "secretome" of NSCs as well as how the infection impacts the neuronal firing capabilities of mature neurons. Our results show that the ZIKV strain of African lineage is able to replicate in mouse NSCs and leads to a significant downregulation in secretion of proteins into the extracellular environment. These studies provide mechanistic detail on how ZIKV impacts the differentiation, survival, development, and function of neural stem cells and neuronal cultures. *Support for this study was provided by the College of Medicine, the Field Neurosciences Institute, and the John G. Kulhavi Professorship in Neuroscience at CMU.*

Disclosures: O.V. Lossia: None. **M.T. Ocean:** None. **E.D. Petersen:** None. **B. Srinageshwar:** None. **U. Hochgeschwender:** None. **G.L. Dunbar:** None. **M.J. Conway:** None. **J. Rossignol:** None.

Poster

028. Neuronal Morphology and Cell Death

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 28.03/A3

Topic: A.01. Neurogenesis and Gliogenesis

Title: Apoptotic signaling through p75NTR in response to BDNF requires Rab5 and JNK activity

Authors: *C. A. CABEZA, C. ESCUDERO, F. BRONFMAN;
Pontificia Univ. Católica De Chile, Santiago, Chile

Abstract: Introduction: During the development of the sympathetic nervous system, the p75 neurotrophin receptor (p75NTR) triggers apoptosis upon binding to BDNF. Apoptotic signaling through p75 requires activation of the stress kinase, JNK that increases the regulated receptor proteolysis in response to pro-apoptotic ligands. The cleavage facilitated ubiquitination of the DNA binding protein NRIF, a zinc finger protein that interacts with the intracellular domain of p75, resulting in nuclear translocation of NRIF. Moreover, this leads a second wave of activation of JNK. All this is required for a long-term apoptotic signaling of p75. Rab5 regulates the trafficking of receptors by regulating early endosomes dynamics. Results of our lab have shown that p75 transiently interacts with the Rab5 positive endosomes in sympathetic neurons (SCGs) and accumulates in other endocytic organelles. Our aim was to clarify the role of Rab5 positive endosomes on p75 signaling and the relevance of JNK in retrograde apoptotic signaling of p75. **Material and Methods:** We used SCGs prepared from postnatal day one rats. We over-expressed a constitutively active (Rab5CA) or dominant negative (Rab5DN) Rab5 mutants in 6 DIV SCGs and then we treated with BDNF for 30 hrs. We evaluated NRIF translocation and cleaved caspase-3. Moreover, we used compartmentalized culture of SCGs to evaluate JNK activation in axons. In addition, we used SP600125 a JNK inhibitor to evaluate the role of this kinase in the internalization and apoptotic signaling of p75. **Results:** When a Rab5CA mutant was expressed, p75 was accumulated in Rab5 positive organelles and we observed an increase nuclear translocation of NRIF in response to BDNF. Nevertheless, when Rab5 activity was down regulated by expressing a Rab5DN a down-regulation of NRIF nuclear translocation and cleavage of caspase-3 was observed in response to BDNF. Moreover, we evaluated the retrograde activation of JNK by BDNF. Inhibition of JNK activity by SP600125 decreases the internalization and apoptotic signaling of p75. **Discussion:** Our results suggest that although p75 transiently interacts with Rab5 positive endosomes, this interaction is required for BDNF-mediated NRIF nuclear translocation and cleavage of caspase-3. In addition, we determine a new role of JNK in the internalization and retrograde apoptotic signaling of p75.

Disclosures: C.A. Cabeza: None. **C. Escudero:** None. **F. Bronfman:** None.

Poster

028. Neuronal Morphology and Cell Death

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 28.04/A4

Topic: A.01. Neurogenesis and Gliogenesis

Support: Momentum II. Program of the Hungarian Academy of Sciences LP2013-54/2015

National Research, Development and Innovation Office K 116915

Contract of competitiveness and excellence VKSZ_14-1-2015-0155

Wellcome Trust 090946/Z/09/Z

Title: Ventricular zone disassembly and delamination of radial glia progenitors triggers alpha-beta hydrolase domain containing 4 (ABHD4)-mediated cell death in the embryonic neocortex

Authors: *Z. LÁSZLÓ[1,2], Z. LELE[1], Z. BALOGI[1], A. DORNING[1], G. SIMON[3], S. SHU-JUNG HU[4], K. MACKIE[4], B. CRAVATT[3], I. KATONA[1];
[1]Inst. of Exptl. Medicine, HAS, Budapest, Hungary; [2]Sch. of Ph.D. Studies, Semmelweis Univ., Budapest, Hungary; [3]Dept. of Chem. Physiol., The Scripps Res. Inst., La Jolla, CA;
[4]Psychological and Brain Sci., Gill Inst., Bloomington, IN

Abstract: Epithelial-mesenchymal transition (EMT) of epithelial cells and delamination of the non-radial glia-fated daughter cells after asymmetric divisions in the embryonic cortex share several common features. It is presently unclear however, whether an anoikis-like protective apoptosis is also present in the developing cortex and if there is, how non-radial glia-fated daughter cells escape it during their migration? Here, we demonstrate that interference with classic cadherin-dependent adherens junction function in radial glia progenitor cells initiates disassembly of the ventricular zone. In turn, this leads to the abnormal dispersion of Pax6-positive cells into the SVZ, and halts proper cell migration into the cortical plate due to an elevated caspase-dependent cell death response in the electroporated area. Prior studies suggested that endocannabinoid signaling controls cell migration in the developing neocortex, and that cannabinoid ligands regulate cell death processes in various *in vitro* models. Notably, we found that *Abhd4*, a serine-hydrolase, which functions as an N-acyl-phosphatidylethanolamine (NAPE) lipase, and produces a precursor lipid for the biosynthesis of the endocannabinoid anandamide, is highly and specifically expressed in the ventricular zone during cortical development. In addition, expression of *Abhd4* (but not of its hydrolase-dead point mutant version) induces apoptosis *in vitro* in the immortalized neuroepithelial modell NE-4C cells, as well in the generally apoptosis-resistant HEK293 cells. Furthermore, in utero electroporation of Abhd4 into the lateral ventricles triggers a similar cell death response. Most

importantly, however, we also found that loss of Abhd4 eliminates increased neocortical cell death induced by breaking up cadherin connections between radial glia cells. Together, these results demonstrate that Abhd4 is a necessary and sufficient proapoptotic member of the pathway between ventricular zone disassembly due to adherens junction disruption and caspase-dependent apoptosis.

Disclosures: **Z. László:** None. **Z. Lele:** None. **Z. Balogi:** None. **A. Dorning:** None. **G. Simon:** None. **S. Shu-Jung Hu:** None. **K. Mackie:** None. **B. Cravatt:** None. **I. Katona:** None.

Poster

028. Neuronal Morphology and Cell Death

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 28.05/A5

Topic: A.01. Neurogenesis and Gliogenesis

Support: Sigrid Juselius Foundation

Title: Angiopoietin-1 is essential for zebrafish embryonic neurogenesis

Authors: *Y.-C. CHEN, V. MARCHICA, T. S. MARTINS, P. PANULA;
Univ. of Helsinki, Helsinki, Finland

Abstract: Angiopoietin-1 (*angpt1*) is a selective endothelial cell growth factor that binds to its receptor Tie2 and regulates vessel development and vascular stabilization. Besides its crucial role in angiogenesis, it also acts as a proneurogenic peptide involved in neuronal differentiation and affects the brain size in fish. How angpt1 regulates neurogenesis is still largely unknown. The present study focuses on the role of angpt1 during zebrafish development. By quantitative PCR (qPCR), the transcripts of *angpt1*, *angpt2b* and *tie2* were detected at one-cell stage and showed a robust expression at the prim-5 stage (24 hours post-fertilization). The spatiotemporal patterns showed that these angiogenic factors were expressed in the eyes, heart, hypochord and aortic arch arteries in the head and trunk. To elucidate the function of angpt1, we investigated a zebrafish mutant, *angpt1*^{sa14264}, carrying a nonsense mutation resulting in a stop codon at Q261. The *angpt1*mutant embryos showed a smaller size of head and body length compared with the wild type-like siblings, and exhibited cardiac edema, embryonic CNS hemorrhage, poor blood circulation and blood accumulation in the pericardial sac. They did not survive beyond 5 dpf. Additionally, at 3 dpf, a significant reduction of *tyrosine hydroxylase 1* transcript was detected in *angpt1*-deficient fish; however, the mRNA level of *tie1*, *angpt2b*, *tyrosine hydroxylase 2* and *histidine decarboxylase* were unaffected. These results demonstrate that lack of angpt1 leads to aberrant embryonic brain and cardiovascular development.

Disclosures: **Y. Chen:** None. **V. Marchica:** None. **T.S. Martins:** None. **P. Panula:** None.

Poster

028. Neuronal Morphology and Cell Death

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 28.06/A6

Topic: A.01. Neurogenesis and Gliogenesis

Support: CIRM Training Grant TG2-01163

NIH Grant MH101188

Title: Microglial morphology during cortical development

Authors: *J. '. KEITER[1], V. MARTINEZ-CERDEÑO[2], S. NOCTOR[2];
[1]UC Davis, Vacaville, CA; [2]UC Davis, Davis, CA

Abstract: Microglia are resident macrophages of the brain and colonize the cerebral cortex during early development. Recent evidence demonstrates that microglia shape brain development and that disrupting microglial function during early development may be a factor in neurodevelopmental diseases such as Schizophrenia and Autism. The functional role(s) of microglia in the normally developing brain are not well understood. In the healthy adult cerebral cortex microglia are distributed evenly throughout the grey matter of the cerebral cortex, and the cells have a relatively small soma with many finely ramified processes. Microglia displaying this morphology have traditionally been termed 'resting' cells. In contrast, after damage like injury or stroke, and in neuropathological conditions microglia take on an 'activated' morphology characterized by a large soma and a few thick processes, or an amoeboid shape. Activated microglia concentrate in regions of injury or pathology and are a hallmark of pathology. Interestingly, recent work has shown that as microglial cells begin colonizing the normally developing brain they concentrate in distinct regions including cellular proliferative zones, and display the activated morphology that is characteristic of pathology in the adult brain. We have found that microglia display distinct morphological profiles within different structures of the developing cerebral cortex. Here we map out the distribution of microglial cells in the developing brain and correlate morphology with cell location. We show that microglia in the subventricular zone (SVZ) of the developing cerebral cortex are more likely to exhibit an amoeboid shape. Microglia in the SVZ have a large soma, and few short thick processes. In contrast, microglial cells closer to the ventricle in the ventricular zone have a smaller soma and more elaborate processes than microglia in the SVZ. We also note that amoeboid microglia lacking any processes are found in discrete structures. The few microglia that are located within

the developing cortical plate exhibit a “rod” like shape and a radial orientation. Correlating microglial cell morphology with location in the developing brain may offer clues to the varied functions associated these cells in the developing cortex.

Disclosures: J.'. Keiter: None. **V. Martinez-Cerdeño:** None. **S. Noctor:** None.

Poster

028. Neuronal Morphology and Cell Death

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 28.07/A7

Topic: A.01. Neurogenesis and Gliogenesis

Support: NRF Grant No.2012M3A9C6049935

DGIST Convergence Science Center Program 16-BD-04

Title: Mediation of autophagic cell death by type 3 ryanodine receptor (RyR3) in adult hippocampal neural stem cells

Authors: *K. CHUNG, E.-J. JEONG, H. PARK, H.-K. AN, S.-W. YU;
Daegu Gyeongbuk Inst. of Sci. & Technol., Daegu, Korea, Republic of

Abstract: Cytoplasmic Ca^{2+} actively engages in diverse intracellular processes from protein synthesis, folding and trafficking to cell survival and death. Dysregulation of intracellular Ca^{2+} levels is observed in various neuropathological states including Alzheimer’s and Parkinson’s diseases. Ryanodine receptors (RyRs) and IP_3 receptors (IP_3Rs), the main Ca^{2+} release channels located in endoplasmic reticulum (ER) membranes, are known to direct various cellular events such as autophagy and apoptosis. Here we investigated the intracellular Ca^{2+}-mediated regulation of survival and death of adult hippocampal neural stem (HCN) cells utilizing an insulin withdrawal model of autophagic cell death. Despite comparable expression levels of RyR and IP_3R transcripts in HCN cells at normal state, the expression levels of RyRs — especially RyR3 — were markedly upregulated upon insulin withdrawal. While treatment with the RyR agonist caffeine significantly promoted the autophagic death of insulin-deficient HCN cells, treatment with its inhibitor dantrolene prevented the induction of autophagy following insulin withdrawal. Furthermore, CRISPR/Cas9-mediated knockout of the RyR3 gene abolished autophagic cell death of HCN cells. This study delineates a distinct, RyR3-mediated ER Ca^{2+} regulation of autophagy and programmed cell death in neural stem cells. Our findings provide novel insights into the critical, yet understudied mechanisms underlying the regulatory function of ER Ca^{2+} in neural stem cell biology.

Disclosures: **K. Chung:** None. **E. Jeong:** None. **H. Park:** None. **H. An:** None. **S. Yu:** None.

Poster

028. Neuronal Morphology and Cell Death

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 28.08/A8

Topic: A.01. Neurogenesis and Gliogenesis

Support: NSF-DBI-1126118

Title: Exposure of embryonic Xenopus laevis to organophosphate pesticides during development leads to disruption of the cholinergic nervous system

Authors: ***M. BRYSON**[1], F. WATSON[1], E. FRADINGER[2], T. HOLDER[1], M. QUELLHORST[1];
[1]Biol., Washington and Lee Univ., Lexington, VA; [2]Whittier Col., Whittier, CA

Abstract: Organophosphate pesticides (OPs), a subset of acetylcholinesterase-inhibiting chemicals, are widely used to protect crops, homes and businesses from insects. Despite a US ban on residential use of organophosphates, widespread use of these pesticides continues in large scale agriculture. Developing countries have no pesticide restrictions and rely heavily on OPs residentially as well as agriculturally. Because it is difficult to mitigate exposure to these pesticides, it is important to understand both the short and long term effects of exposure to these compounds. Here we examine the effects of chlorpyrifos exposure on the sensory neurons of developing *Xenopus laevis* over a 21-day period. At concentrations above 0.1 μM, we observed reduced survival by day 4. At concentrations above 1 μM, nearly all tadpoles died by day 6. We examined two subpopulations of sensory neurons, dorsal root ganglia and Rohon-Beard neurons, and show increased defects in neural migration at developmental stages 33, 47, and 52. Chlorpyrifos exposure leads to decreased neuronal growth coupled with large scale neural migration defects. Rohon-Beard neurons fail to develop in a linear fashion within the dorsal spinal column, especially in stage 33 tadpoles. Exposure levels of 5 μM and above lead to more severe neural migration defects such that Rohon-Beard neurons often reside outside the spinal column. Dorsal root ganglia neurons also show irregular neural patterns at these higher concentrations. To assess the level of apoptosis, we use Acridine Orange. Preliminary data shows apoptosis occurs within the external skeletal muscle of the tadpoles. This pesticide-induced cell death increases with increasing chlorpyrifos concentrations. Our results provide evidence that OPs have profound neurological effects in developing vertebrates and ongoing studies will investigate and provide a more complete analysis of sensory neuron defects caused by OP exposure.

Disclosures: **M. Bryson:** None. **F. Watson:** None. **E. Fradinger:** None. **T. Holder:** None. **M. Quellhorst:** None.

Poster

028. Neuronal Morphology and Cell Death

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 28.09/A9

Topic: C.05. Neuromuscular Diseases

Support: NHMRC Project Grant 509319

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NHMRC Fellowship (PMB)

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Motor Neurone Disease Research Institute of Australia Grant

Henry Roth Foundation Grant

Motor Neurone Disease Research Institute of Australia Fellowship (JDA)

Title: Mutant SOD1 species induce toxicity in astrocytic cultures: bystander effects occur in a continuum of astrogliosis

Authors: ***R. D. O'SHEA**[1], N. WALLIS[2], C. L. LAU[2], M. A. FARG[3], J. D. ATKIN[4,3], P. M. BEART[2];
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[2]Neurodegeneration, The Florey Inst. of Neurosci. and Mental Hlth., Parkville, Australia;
[3]Biochem., La Trobe Univ., Bundoora, Australia; [4]Biomed. Sci., Macquarie Univ., North Ryde, Australia

Abstract: Astrocytes contribute to the death of motor neurons via non-cell autonomous mechanisms of injury in amyotrophic lateral sclerosis (ALS). Since mutations in the gene encoding Cu/Zn superoxide dismutase (SOD1) underlie the neuropathology of some forms of familial ALS, we explored how expression of mutant SOD1 proteins affected the biology of murine astrocytes in culture. Primary cultures of astrocytes were established from forebrains of C57BL6 mouse pups (postnatal day 1.5; at least n=3 independent cultures), and were subcultured after 10 days into 48-well plates (1 x 10^4 cells/cm^2) and maintained until confluent. Cells were transfected with constructs expressing wild-type SOD1-EGFP, A4V SOD1-EGFP or G85R

SOD1-EGFP, using Transfast according to the manufacturer's instructions. 72 h after transfection, astrocytes expressing A4V SOD1-EGFP displayed impaired mitochondrial activity (~45%, MTT assay) and L-glutamate transporter activity (~25%, [^{3}H]-D-Aspartate uptake), relative to those transfected with wild-type SOD1-EGFP. Astrocytes transfected with A4V SOD1-EGFP also demonstrated altered F-actin and Hoechst staining, indicative of cytoskeletal and nuclear changes, while altered GM130 labelling suggested fragmentation of Golgi apparatus. SOD1 inclusion formation shifted from discrete to "clumpy" over 72 h. A4V SOD1-EGFP more rapidly produced inclusions than G85R SOD1-EGFP and formed more "clumpy" aggregates. A4V SOD1-EGFP, but not wild-type SOD1-EGFP, exerted a substantial, time-dependent effect on GFAP expression, and ~60% of astrocytes were stellate and hypertrophic at 72 h. Spreading toxicity was inferred, since at 72 h ~80% of bystander cells exhibited hypertrophy and stellation. This evidence suggests that mutant SOD1-containing astrocytes release destructive species that alter the biology of adjacent astrocytes. This panoply of mutant SOD1-induced destructive events favours recruitment of astrocytes to non-cell autonomous injury in ALS.

Disclosures: **R.D. O'Shea:** None. **N. Wallis:** None. **C.L. Lau:** None. **M.A. Farg:** None. **J.D. Atkin:** None. **P.M. Beart:** None.

Poster

028. Neuronal Morphology and Cell Death

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 28.10/A10

Topic: C.05. Neuromuscular Diseases

Support: NIH Grant RO1 NS064224

Title: Disruption of nuclear bodies causes activation of DNA damage and repair pathways

Authors: *A. KANNAN[1], K. BHATIA[2], L. GANGWANI[2];
[1]Neurosci., Texas Tech. Univ. Hlth. Sci. Ctr., El Paso, TX; [2]Neurosciences, Texas Tech. Univ. of Hlth. Sci. Ctr., El Paso, TX

Abstract: Nuclear bodies are membraneless subnuclear organelles rich in RNAs and proteins involved in various nuclear processes, including RNA biogenesis (maturation and splicing), stress response, transcription and DNA replication. Integrity of many nuclear bodies such as gems, Cajal bodies (CBs), histone locus bodies (HLBs), promyelocytic leukaemia (PML) bodies is essential for the normal functioning of mammalian cells. Each nuclear body carry out specific function and disruption of these sub-nuclear bodies leads to pathological conditions with human diseases such as neurodegenerative disorders, including spinal muscular atrophy (SMA). SMA is

an autosomal recessive disease caused by the low levels of full length survival motor neuron (SMN) protein. Zinc finger protein ZPR1 interacts with SMN and is required for the accumulation of SMN in nuclear bodies. The interaction of ZPR1 with SMN is disrupted in SMA and SMN fails to accumulate in gems and CBs in cells derived from SMA patients. The severity of SMA disease correlates negatively with the number of SMN containing nuclear bodies. In this study, we have investigated the effect of the low levels of ZPR1 and SMN on the integrity of sub-nuclear bodies such as CBs, HLBs and PML bodies identified by the signatory proteins p80 coilin, LSM11 and PML respectively in cultured cells. Knockdown of ZPR1 and SMN was achieved by using antisense oligonucleotides and RNA interference (RNAi) respectively. We show that the reduced expression of ZPR1 or SMN causes disruption of Cajal, HLB and PML nuclear bodies and marker proteins p80 coilin, LSM11 and PML are mislocalized. We examined the effect of disruption of these nuclear bodies on transcription, replication and integrity of genomic DNA. We report that the disruption of ZPR1 or SMN containing nuclear bodies results in fragmentation of DNA and activation of DNA damage and repair pathways proteins such as γH2AX, 53BP1, pATM, pATR and pDNAPKc. Results from the present study show that the disruption of sub-nuclear bodies causes activation of DNA damage and repair pathways that leads to defects in transcription and DNA replication. This finding suggests that DNA damage may contribute to neurodegenerative disorders, including SMA.

Disclosures: **A. Kannan:** None. **K. Bhatia:** None. **L. Gangwani:** None.

Poster

028. Neuronal Morphology and Cell Death

Location: Halls B-H

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Program#/Poster#: 28.11/A11

Topic: C.05. Neuromuscular Diseases

Support: NIH R21NS094921

NIH R21NS096319

Title: Mechanisms underlying C9orf72 associated toxicity in a *C. elegans* model of disease

Authors: ***S. T. LAMITINA**[1], J. OOSTEN[1], C. SNOZNIK[1], U. PANDEY[2], P. RUDICH[3]; [1]Dept of Pediatrics and Cell Biol., [2]Dept of Neurol., [3]Dept of Pediatrics and Cell Biology, Grad. Program in Cell Biol., Childrens Hosp. of Pittsburgh of UPMC, Pittsburgh, PA

Abstract: Amyotrophic lateral sclerosis (ALS) is a rapidly progressing age-related neurodegenerative disease that affects ~30,000 Americans. ALS causes degeneration of the

upper and lower motor neurons, leading to paralysis. Currently, there are no effective treatments for ALS and only ~50% of patients survive beyond three years after diagnosis. A recently discovered expanded hexanucleotide repeat in the first intron of the C9orf72 gene is the most common known genetic cause of familial and sporadic ALS. The repeat expansion is not thought to cause disease through alteration of C9orf72 function. Rather, the expanded repeat is transcribed in both sense and antisense directions to produce repeat-containing RNAs that are then translated in multiple reading frames to yield up to five distinct dipeptide repeat proteins. This unusual mode of translation is termed Repeat Associated-non-ATG (RAN) translation. It is controversial whether the expanded repeats cause ALS through RNA toxicity, RAN translated dipeptide toxicity, or both. Using codon-varied transgenes, we created a 'dipeptide-only' model in the nematode *C. elegans* to better understand the mechanisms of dipeptide toxicity. The arginine rich dipeptides, PR and GR, were toxic in *C. elegans* when expressed in multiple cell types, including motor neurons. This toxicity was dependent on both the length of the dipeptide as well as its subcellular localization. Genetic inhibition of the insulin pathway, a conserved regulator of ageing, delayed age-onset toxicity caused by PR dipeptides, suggesting that physiological age rather than chronological age is a determinant of PR toxicity. Currently, we are performing RNAseq and using unbiased forward and reverse genetic screens to identify modifiers or arginine-containing dipeptide toxicity. Defining these modifiers will allow us to determine potential mechanisms for dipeptide toxicity and may lead to new disease biomarkers and/or therapies.

Disclosures: S.T. Lamitina: None. **J. Oosten:** None. **C. Snoznik:** None. **U. Pandey:** None. **P. Rudich:** None.

Poster

028. Neuronal Morphology and Cell Death

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 28.12/A12

Topic: C.05. Neuromuscular Diseases

Title: The divergent neuroprotective capacities of *Drosophila* Nmnat in C9orf72 ALS/FTD

Authors: *K. O. RUAN[1], R. ZHAI[2], T. LLOYD[1];
[1]Dept. of Neurol., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Dept. of Mol. and Cell. Pharmacol., Univ. of Miami Miller Sch. of Med., Miami, FL

Abstract: Nicotinamide mononucleotide adenylyltransferase (NMNAT) is a conserved enzyme in the NAD synthetic pathway. It has also been identified as an effective and versatile neuroprotective factor. However, it remains unclear how healthy neurons regulate the dual

functions of NMNAT and achieve self-protection under stress. My previous study has shown that *Drosophila Nmnat* (*DmNmnat*) is alternatively spliced into two mRNA variants, RA and RB, which translate to protein isoforms with divergent neuroprotective capacities against spinocerebellar ataxia 1 (SCA1)-induced neurodegeneration. Isoform PA/PC translated from RA is nuclear-localized with minimal neuroprotective ability, and isoform PB/PD translated from RB is cytoplasmic and has robust neuroprotective capacity. Under stress, RB is preferably spliced in neurons to produce the neuroprotective PB/PD isoforms. The results indicate that alternative splicing functions as a switch that regulates the expression of functionally distinct DmNmnat variants. Neurons respond to stress by driving the splicing switch to produce the neuroprotective variant and therefore achieve self-protection.I am now applying this concept to the study of C9orf72. In this study, I will explore three questions: (1) whether the divergent neuroprotective capacities of NMNAT isoforms also occur in C9orf72 ALS/FTD; (2) whether the protection is cell autonomous; and (3) whether this neuroprotective effect is cell-type specific.

Disclosures: K.O. Ruan: None. **R. Zhai:** None. **T. Lloyd:** None.

Poster

029. Adult Neurogenesis Mammalian Systems

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

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Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant AR047410

Title: Temporal and spatial expression pattern of miRNA and protein expression in peripheral nerve injury

Authors: *V. YEROKHIN, S. DAS, K. MILLER;
Oklahoma State Univ. Ctr. For Hlth. Scienc, Tulsa, OK

Abstract: Peripheral nerve injury (PNI) affects approximately 20 million Americans annually, costing the healthcare system over $150 billion each year. Although current therapies attempt to promote nerve regeneration, only 50% of persons fully regain motor and sensory function, while others retain poor motor control and suffer from life-long, debilitating neuropathic pain. Injured peripheral axons can regenerate, but this is rarely complete due to the slow rate of regeneration. Clearly, a new therapeutic approach for accelerating peripheral nerve regeneration is needed. Although miRNA and anti-miRNA therapy has proved fruitful in normalizing dysregulated protein expression in other diseases, clinical use of this therapeutic modality in pain and PNI has yet to be realized. The absence of translational application of miRNA therapeutics stems mostly

from our limited understanding of the molecular mechanisms underlying nerve injury and regeneration. Because nerve regeneration requires a complex coordination of finely regulated events, understanding these molecular mechanisms is key for designing an effective bio-pharmacological intervention. In this study, we present novel findings of spatial and temporal expression of miRNA let-7a and 23b post-PNI, and elucidate their relationship with Nerve Growth Factor (NGF) and Glutaminase (GLS) expression. Three experimental and three sham surgery groups of male, Sprague-Dawley (SD) rats (n=6 per group) underwent sciatic nerve crush (SNC). The animals were euthanized and the sciatic nerve (SN), the dorsal root ganglia (DRG) and the spinal cord (SC) at the level of SN innervation (L4-L6 levels) were collected at 3 time points: 1) 24 hours (Group 1), 2) 4 days (Group 2) and 3) 7 days (Group 3) post-injury. The SN proximal to, at the location of, and distal to the site of injury was collected. Protein and miRNA expression was measured using Western Blot and RT-PCR. A sham surgical control group (n=6 per group) was paired to each experimental group. The SN was visualized, but not crushed in the sham surgery group. We observed differential expression of the miRNAs and proteins at the different sites following SNC (SN, SC and DRGs). Furthermore, we observed an inverse relationship between the miRNA let-7a and 23b and NGF and GLS expression, respectively. Finally, the differential expression followed a temporal trend. To our knowledge, this study is the first to assess miRNA let-7a and 23b and NGF and GLS expression in sciatic nerve injury model. As such, we provide a novel insight onto possible therapeutic targets in *vivo* for improving functional outcome following nerve injury.

Disclosures: **V. Yerokhin:** None. **S. Das:** None. **K. Miller:** None.

Poster

029. Adult Neurogenesis Mammalian Systems

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 29.02/B1

Topic: A.02. Postnatal Neurogenesis

Support: Chaikin-Wile Foundation

Title: The daily pattern of adult neurogenesis in zebrafish

Authors: *A. STANKIEWICZ[1], V. AKLE[2], L. YU[1], I. ZHDANOVA[1];
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Abstract: The majority of physiological processes follows a circadian pattern and is under direct or indirect control of the intrinsic clock. To investigate whether adult neurogenesis in diurnal

vertebrates, like human, follows a circadian pattern, and the timing of specific phases of the cell cycle, we used zebrafish as a model, in view of its robust circadian clock system, diurnality, and abundant adult neurogenesis. We employed real-time RT-PCR (qPCR) analysis of mRNA expression for D, E, A2 and B2 cyclins involved in specific phases of the cell cycle, and immunohistochemical analysis of markers for S-phase (BrdU and EdU) and G2/M phase (pH3) of the cell cycle. The results demonstrated the presence of a robust daily rhythm of cell proliferation in adult brain and determined that while many cells can repeat the cell cycle the following day, a large proportion of neuronal progenitors can cycle with longer than 24h periods. The number of neuronal progenitors dividing on any given day remains stable, with BrdU/EdU pulse-chase studies revealing gradual decline in the number of older neuronal progenitors being compensated by the emergence of new cells, and reflecting the neuronal stem cell activity. This is a powerful model to determine optimal timing for pharmacological and physical interventions that can promote adult neurogenesis, and to characterize time-dependent adverse impact of diverse agents on adult neurogenesis.

Disclosures: **A. Stankiewicz:** None. **V. Akle:** None. **L. Yu:** None. **I. Zhdanova:** None.

Poster

029. Adult Neurogenesis Mammalian Systems

Location: Halls B-H

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Program#/Poster#: 29.03/B2

Topic: A.02. Postnatal Neurogenesis

Support: Medical Research Council (MRC)

The van Geest Foundation

Research Councils UK (RCUK)

Title: Alterations in human hippocampal neurogenesis predict progression from mild cognitive impairment to Alzheimer's disease

Authors: A. MARUSZAK[1], T. MURPHY[1], A. DOUIRI[2], A. J. NEVADO[3], B. LIU[3], C. DE LUCIA[1], J. PRICE[1], S. LOVESTONE[3], *S. THURET[1];
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Abstract: Introduction: We hypothesized that Alzheimer's disease (AD) systemic milieu (blood/serum) modulates hippocampal neurogenesis (HN). The systemic environment is a

recognized modulator of HN and carries factors implicated in AD. Increasing evidence has demonstrated that adult HN plays an essential role in learning and memory. In addition, altered HN is an early hallmark of AD. Given that HN occurs in one of the earliest affected brain structures in AD, the gradual impairment of memory and learning observed during the disease progression might be linked to changes in HN.
We aimed at establishing how the systemic environment influences HN in AD using an in vitro assay of human hippocampal neurogenesis. Our goal was also to apply our assay in predicting conversion from mild cognitive impairment (MCI) to AD.
Materials and Methods: Human multipotent hippocampal progenitor cell line HPC0A07/03C was exposed to human serum from 1) individuals with MCI that converted to AD (MCI converters) and 2) individuals that were initially diagnosed with MCI but did not develop any disease (MCI non-converters). Serum from each annual follow-up visit was used to model changes in HN with disease progression. Statistical analyses involved linear mixed effects models for repeated measures and stepwise logistic regression.
Results: We determined that decreased proliferation, increased neurogenesis and increased cell death are signatures of disease progression towards conversion to AD. Proliferation of HPC0A07/03C cells in the presence of serum from MCI converters leads to significantly higher proliferation rate and apoptotic cell death as compared to the effects of serum from MCI non-converters. In addition, exposure to serum during both proliferation and differentiation of the HPC0A07/03C cells is associated with increased levels of newborn neurons and elevated apoptotic death when serum donors were MCI converters as compared to MCI non-converters. Finally, we built a statistical model predicting the likelihood of conversion from MCI to AD at baseline. Among the predictors of conversion are education and baseline assay readouts (proliferation rate, average cell count during proliferation, cell death during differentiation). The ROC curve shows that our model is very good at discriminating (AUC=0.9675) who will develop AD, with 92.11% converters correctly classified and 94.12% MCI non-converters correctly discriminated.
Conclusion: Our data highlights the importance of the systemic environment for HN in AD and our assay could be used as a biomarker of disease progression and conversion to AD.

Disclosures: **A. Maruszak:** None. **T. Murphy:** None. **A. Douiri:** None. **A.J. Nevado:** None. **B. Liu:** None. **C. de Lucia:** None. **J. Price:** None. **S. Lovestone:** None. **S. Thuret:** None.

Poster

029. Adult Neurogenesis Mammalian Systems

Location: Halls B-H

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Program#/Poster#: 29.04/B3

Topic: A.02. Postnatal Neurogenesis

Support: NIH/NINDS Grant U54CA163155

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The National Brain Tumor Society

The Loglio Collaborative

The TDC Foundation

The Guggenhime Endowment Fund

The Swedish Research Council (H.S.)

Title: Astrocyte-derived neurogenesis layer II-III of the adult cerebral cortex

Authors: *H. SABELSTROM[1,2], H.-H. TSAI[3,4], O. R. YABUT[2,1], T. FENSTER[1,2], E. YUAN[1,2], E. HUANG[5], T. BJORK-ERIKSSON[7], M. S. BERGER[6], D. H. ROWITCH[4,3], S. PLEASURE[2,1,3], A. I. PERSSON[1,2,6];
[1]Neurol., [2]Sandler Neurosciences Ctr., [3]Eli and Edythe Broad Inst. of Regeneration Med. and Stem Cell Res. and Howard Hughes Med., [4]Pediatrics, [5]Pathology, [6]Neurolog. Surgery and Brain Tumor Res. Ctr., Univ. of California, San Francisco, San Francisco, CA; [7]Oncology, Sahlgrenska Univ. Hosp., Gothenburg, Sweden

Abstract: The cerebral cortex plays a key role for higher-order cortical functions and is traditionally viewed as a non-neurogenic region. However, it remains controversial whether continuous, transient, or no neurogenesis occurs in the adult cerebral cortex. A microcircuit of sensory input from cortical layer IV is projected to layer II-III that in turn drives layer V. Excitation of excitatory neurons in layer II-III is controlled by fewer GABAergic interneurons, providing a rationale for why newborn layer II-III interneurons should contribute to cortical plasticity. Similar to other neurogenic regions, expression of neuroblast markers in cortical layer II-III is regulated by enriched environment, stress, and injuries. Using flow cytometry, we confirmed the presence of neuroblasts antigens in cortical layer II-III of adult mice. Using bromodeoxyuridine (BrdU) pulse-chase and genetic fate-mapping experiments, we demonstrate that glial fibrillary acidic protein-positive astrocytes can give rise to layer II-III neuroblasts during adulthood. Adult-born layer II-III neuroblasts differentiated into GABAergic interneurons in mice. Patients diseased from non-brain related disorders and pulsed with BrdU displayed significant numbers of BrdU+ cells in layer II-III of the cerebral cortex. In conclusion, we identify limited numbers of astrocyte-derived neuroblasts in the adult brain. Identifying cortical astrocytes as a novel source of immature neurons and GABAergic interneurons has wide-spread implications for cortical plasticity, regeneration and brain repair.

Disclosures: **H. Sabelstrom:** None. **H. Tsai:** None. **O.R. Yabut:** None. **T. Fenster:** None. **E. Yuan:** None. **E. Huang:** None. **T. Bjork-Eriksson:** None. **M.S. Berger:** None. **D.H. Rowitch:** None. **S. Pleasure:** None. **A.I. Persson:** None.

Poster

029. Adult Neurogenesis Mammalian Systems

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Program#/Poster#: 29.05/B4

Topic: A.02. Postnatal Neurogenesis

Support: ISF Research Grant 984/12 to T. B-C

ISF Equipment Grant 1764/12 to T. B-C

ICRF - RDCA grant to T. B-C

Title: Multilevel regulation of neural stem cell proliferation and self-renewal by Pros1

Authors: *T. BURSTYN-COHEN[1], K. ZELENTSOVA[1], Z. TALMI[1], G. ABBOUD-JARROUS[1], T. SAPIR[2], T. CAPUTCHA[1];
[1]The Hebrew Univ., Jerusalem, Israel; [2]Dept. of Mol. Genet., Weizmann Inst. for Sci., Rehovot, Israel

Abstract: Revealing the molecular mechanisms underlying neural stem cell self-renewal is a major goal towards understanding adult brain homeostasis. The self-renewing potential of Neural stem cells (NSCs) must be tightly regulated to maintain brain homeostasis. We find the expression of the anticoagulant Protein S (PROS1) in adult hippocampal NSCs, and show that genetic ablation of *Pros1* in neural progenitors increased both NSC proliferation and self-renewal. Mechanistically, we identified the upregulation of Bmi-1 signaling to cell-autonomously promote NSC self-renewal in *Pros1*-ablated cells. Rescuing *Pros1* expression restores Bmi-1 signaling, and reverts the proliferation and enhanced self-renewal phenotypes observed in *Pros1*-deleted cells. Enhanced proliferation in Pros1-deleted NSCs was found to be mediated through dysregulated Notch1 signaling. Our study uniquely uncouples between increased NSC proliferation and depletion of the NSC pool, due to concomitant increased self-renewal. By manipulating PROS1 expression, we provide a novel model in which both Notch1 and Bmi-1 pathways are dysregulated, allowing to investigate the balance between these two key pathways, and their impact on adult neurogenesis. We provide a first indication that the balance between Bmi-1 and Notch signaling pathways is important, and may lead to new outcomes. Conclusion: We identify PROS1 as a novel regulator of NSC biology. PROS1-deleted NSCs are affected at multiple levels: quiescence/proliferation, self-renewal/differentiation and daughter-cell fate determination. Our findings provide novel insight into maintaining brain homeostasis.

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Poster

029. Adult Neurogenesis Mammalian Systems

Location: Halls B-H

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Program#/Poster#: 29.06/B5

Topic: A.02. Postnatal Neurogenesis

Support: NIH Grant R01NS045702

NIH Grant R01HL104173

NIH Grant R01HL128546

IDDRC P30HD40677

NIH Grant R01HD074593

Title: Subnormal neurogenesis and cortical growth in a piglet model of congenital heart disease

Authors: *P. D. MORTON[1], L. KOROTCOVA[2], B. K. LEWIS[6], V. KUMAR[3], F. SHAIKH[4], E. SHORT[2], J. ZHANG[7], S. MORI[7], J. A. FRANK[6], V. GALLO[8], R. A. JONAS[9], N. ISHIBASHI[5];
[1]Cardiovasc. Surgery, Childrens Natl. Med. Ctr., Washington, DC; [2]Children's Natl. Heart Inst. and Ctr. for Neurosci. Res., [3]Children's Natl. Heart Inst. and Ctr. for Neurosci. Research,, [4]1Children's Natl. Heart Inst. and Ctr. for Neurosci. Res., Children's Natl. Med. Ctr., Washington, DC; [5]Children's Natl. Heart Inst. and Ctr. for Neurosci. Res., Children's Natl. Med. Ctr., Washington, DC; [6]Dept. of Radiology and Imaging Sci., NIH, Bethesda, MD; [7]Dept. of Biomed. Engin. and The Russell H. Morgan Dept. of Radiology and Radiologi, Johns Hopkins Sch. of Med., Baltimore, MD; [8]Ctr. for Neurosci. Res., Children's Natl. Med. Center,, Washington, DC; [9]Children's Natl. Heart Inst. and Ctr. for Neurosci. Res., Children's Natl. Med. Ctr., Washington, DC

Abstract: Many patients suffering from severe/complex congenital heart disease (CHD) display significant neurodevelopmental deficits and subnormal cortical development associated with reduced cerebral oxygenation during fetal life and early infancy. Due to technical/ethical barriers, the cellular mechanisms underlying cortical dysmaturation and function in CHD remain elusive and hinder therapeutic advances. Therefore, novel models utilizing gyrencephalic species to recapitulate subnormal cortical brain development in CHD will be essential in determining the cellular mechanisms underlying brain dysmaturation. We have developed a neonatal porcine hypoxia model to address these key issues. Magnetic resonance imaging (MRI) revealed that chronic hypoxia reduced cortical volume and folding of the frontal cortex. Under normal physiological conditions, we found that the porcine subventricular zone (SVZ) shares unique anatomical/structural similarities with the human SVZ; including nearly identical laminar

organization with an astrocyte ribbon. In addition, we identified a subregion of the SVZ with an abundance of multipotent neural stem progenitor cells (NSPCs) that supply the frontal cortex with newborn neurons during early postnatal development. *In vivo* cell labeling demonstrated that chronic hypoxia limits the contribution of SVZ-derived neurons to postnatal cortical development. Finally, a decrease in the number of immature neurons was displayed within the frontal cortices with no changes in apoptosis. These findings suggest that chronic hypoxia reduces the generation of neuronal producing NSPCs in the SVZ which delays/impairs corticogenesis. Since the SVZ generates neural NSPCs capable of replenishing damaged neurons and glia in the brain throughout the human lifespan, novel therapies designed to protect or replenish SVZ-derived NSPCs may restore cortical growth and improve neurological function in patients born with CHD.

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Poster

029. Adult Neurogenesis Mammalian Systems

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 29.07/B6

Topic: A.02. Postnatal Neurogenesis

Support: Training Fellowship, Institutional National Research Service Award TL1 TR001119

Training Fellowship, Institutional National Research Service Award T32 AG021890

Title: Calorie restriction modulates intrinsic and niche factors in the aging murine subventricular zone

Authors: *D. M. APPLE[1], R. SOLANO FONSECA[2], S. MAHESULA[2], E. KOKOVAY[2]; [1]Dept. of Cell. and Structural Biol., Univ. of Texas Hlth. Sci. Ctr. San Antonio, San Antonio, TX; [2]Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX

Abstract: The normal aging process in the brain results in increased susceptibility to damage from brain insults like stroke, inflammation, and degeneration. Calorie restriction (CR) can improve physiological markers of health during aging, including extending lifespan and protecting against age-related damage to the brain. The largest source of neural stem cells in the adult brain is the subventricular zone (SVZ). We sought to determine the effect of long-term CR on neurogenesis and the neural stem cell niche in the SVZ of young and aged mice. Here, we

show that aged mice fed standard control chow have fewer SVZ-derived neurons in the olfactory bulb, indicating that aging impairs neural stem cell function. Long-term CR preserved neural stem cell function and resulted in a significant increase in neurogenesis in aged mice compared with *ad libitum*- fed controls. Confocal imaging and fluorescent staining of SVZ wholemounts revealed an increase in both the total number and reactivity of microglia in the aged control mouse, suggesting increased inflammation in the neural stem cell niche during aging. Remarkably, these age-related inflammatory markers were not observed in the long-term CR aged mice, which appeared no different from young control and young CR mice included in the study. We observed a protective effect of CR on aging related dysregulation of vascular-associated chemoattractants important for stem cell activity. However, CR did not protect against rarefaction of the SVZ vasculature in the aged brain. Altered proliferation profiles in the aged SVZ have suggested a change in cell fate determination, and CR maintains the expression of lin28a, a modulator of stem cell differentiation that declines in the aged brain. The maintenance of lin28a levels in the aged SVZ by CR suggests a potential mechanism by which CR protects the SVZ neural stem cell population in the aging brain.

Disclosures: D.M. Apple: None. **R. Solano Fonseca:** None. **S. Mahesula:** None. **E. Kokovay:** None.

Poster

029. Adult Neurogenesis Mammalian Systems

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 29.08/B7

Topic: A.02. Postnatal Neurogenesis

Support: Kemin Foods LC. Sponsored

Title: The effects of a proprietary spearmint extract on neurogenesis rates in rat hippocampal neurons

Authors: *B. A. FONSECA, K. A. HERRLINGER;
Kemin Human Nutr. and Hlth., Des Moines, IA

Abstract: Decreases in cognitive performance due to aging, stress and/or sleep deprivation can often be associated with specific changes in the brain. An area of the hippocampus known as the dentate gyrus is one of the few areas in the adult human brain that can produce new neurons throughout the lifespan, a process called neurogenesis. Aging, stress and/or sleep deprivation are suggested to decrease the rate of neurogenesis through reduced activity in the dentate gyrus resulting in a corresponding reduction in cognitive performance in healthy adults. Neumentix™

Phenolic Complex K110-42 is a natural extract derived from spearmint containing greater than 66 phenolic constituents that has been shown to improve working memory after 90 days of supplementation in healthy adults with age associated memory impairment. One reason for the observed improvements with Neumentix was hypothesized to be an increase in the rates of neurogenesis in the dentate gyrus of the hippocampus. Therefore, the purpose of this study was to determine if Neumentix could enhance neurogenesis in rat hippocampal cells at physiologically relevant concentrations in a cell culture assay. Four concentrations of Neumentix were tested on primary hippocampal cells treated with the test item, vehicle, or fibroblast growth factor, for a total of 48 h. Digital images were analyzed for the % of Bromdesoxyuridine (BrdU) positive neurons compared to the total number of neurons. Cells, which were labeled by BrdU, NeuN and 4',6-diamidino-2-phenylindole (Dapi), were classified as proliferating neurons. Neurons were defined as cells positive for NeuN and Dapi. Analyses showed that primary embryonic hippocampal neurons responded differentially to various concentrations of Neumentix. Overall, there was a treatment effect as determined by one-way ANOVA ($p=0.0169$). A Fisher's LSD pairwise comparison showed the treated cultures in the lowest dose group (0.02083 mg/L Neumentix) displayed significantly greater levels of neurogenesis than vehicle treated cultures ($p=0.0228$) indicating that Neumentix may support working memory by acting to increase rates of neurogenesis in the adult hippocampus.

Disclosures: B.A. Fonseca: A. Employment/Salary (full or part-time): Kemin Foods, LC. **K.A. Herrlinger:** A. Employment/Salary (full or part-time): Kemin Foods, LC..

Poster

029. Adult Neurogenesis Mammalian Systems

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 29.09/B8

Topic: A.02. Postnatal Neurogenesis

Title: Effects of the Smoothened antagonist MRT-83 on adult neural stem cells quiescence and activation

Authors: L. TIROU[1], M. DAYNAC[1], H. FAURE[1], L. GAUTHIER[2], M.-A. MOUTHON[2], F. BOUSSIN[2], *M. RUAT[1];
[1]NeuroPSI, UMR9197, CNRS, Gif Sur Yvette, France; [2]CEA DSV iRCM SCSR Lab. de Radiopathologie, Fontenay-aux-Roses, France

Abstract: Neural stem cells (NSCs) from the subventricular zone (SVZ) of the lateral ventricle are implicated in adult neurogenesis and brain repair. The Sonic Hedgehog (Shh) pathway is important for regulating adult NSC proliferation, but its roles on particular NSCs (quiescent vs.

activated) and the neurogenic population of the SVZ, are not known (Ruat et al, *Top Med Chem*, 2015). Using immunofluorescence and FACS techniques to distinguish all SVZ neurogenic populations, including quiescent vs activated NSCs, we dug out the role of Shh pathway on the maintenance of adult neurogenesis. We observed that, in adult transgenic Fluorescence Ubiquitination Cell Cycle Indicator (FUCCI)-Green mice that allow tracking the S-G2/M phases with green fluorescence (Sakaue-Sawano et al, *Cell*, 2008), proliferating quiescent NSCs (qNSCs) and activated NSCs (aNSCs) are increased 3 days after intraventricular Shh injections. This effect is specific to qNSCs and aNSCs and not their progeny, the transit amplifying cells or immature neuroblasts. Then, we investigated whether a pharmacological blockade of the Shh pathway could modify the cell proliferation effect observed above. Treatment with the Smo antagonist MRT-83 (1µM) (Roudaut et al*, Mol Pharmacol*, 2011) reduced the number and size of neurospheres *in vitro*, thus mirroring *in vitro* data with Shh. The number of proliferating qNSCs and aNSCs from the SVZ populations was decreased 3 days after intraventricular injection of MRT-83, although not significantly for aNSCs. Thus, blocking of the Shh pathway *in vivo* does not alter significantly NSCs proliferation at short term, which might be related to the low basal expression of the pathway in SVZ cells as investigated by qRT-PCR of *Gli1-3*. Experiments are in progress to further investigate the role of the Shh pathway on qNSCs and aNSCs using a genetic mouse model of Shh activation through the conditional deletion of the Shh receptor Patched in NSCs using the GLAST marker (Ferent et al, *Stem Cell Reports*, 2014). These data contribute to explore how Shh orchestrates the balance between qNSCs and aNSCs in the SVZ with important implications for understanding adult neurogenesis under normal homeostatic conditions or during injury.

Disclosures: L. Tirou: None. **M. Daynac:** None. **H. Faure:** None. **L. Gauthier:** None. **M. Mouthon:** None. **F. Boussin:** None. **M. Ruat:** None.

Poster

029. Adult Neurogenesis Mammalian Systems

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 29.10/B9

Topic: A.02. Postnatal Neurogenesis

Title: Deep brain stimulation regulates diet-responsive hypothalamic neurogenesis in young adult male rats

Authors: *M. P. MEDINA[1], J. SOTELO[2];
[2]Exptl. Neurol. Unit, [1]Inst. Nacional De Neurologia Y Neurocirugia, Ciudad DE Mexico, Mexico

Abstract: Tanycytes of the median eminence are neurogenic progenitors that responds to changes in weight gain and metabolism. In this study we assessed the effects of lateral hypothalamic area (LHA) deep brain stimulation (DBS) on the neurogenesis rate of median eminence tanycytes in a well-characterized rodent model of obesity. We used high frequency DBS to stimulate LHA of male young rats (P45) subjected to high-fat diet (HFD). Tungsten electrodes were implanted within LHA using stereotaxic coordinates, fixed to the skull and connected to subcutaneous pulse generators. Generator parameters were initially adjusted before implantation and included biphasic rectangular pulses of 130 Hz, 60us pulse duration and 1 V of amplitude. Animals received a scheme of daily continuous DBS stimulation during 30 consecutive days. During the first 15 days of LHA DBS, animals were administered with BrdU I.P. injections in order to measure the hypothalamic proliferation rate. Rats were perfused after physical assessments for immunohystochemical staining. Control sham animals showed a significant higher increase in weight gain compared to stimulated partners. Food intake was consistently pronounced in sham animals subjected to HFD compared to animals in ON state with LHA stimulation. Only sham animals with HFD showed differences in weight gain and food-intake when compared to controls with normal chow. BrdU expression in tanycytes within the median eminence was markedly modified by DBS scheme in ON stimulation rats only. Neuronal proliferation rate was positively correlated with gain weight. Interestingly, newborn neurons were positive for POMC and Neuropeptide Y, and were physiologically reactive to intraperitoneal leptin injections. In conclusion, LHA DBS modifies feeding behavior by modulating neuronal proliferation rates within hypothalamic neurogenic niches.

Disclosures: **M.P. Medina:** None. **J. Sotelo:** None.

Poster

029. Adult Neurogenesis Mammalian Systems

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 29.11/B10

Topic: A.02. Postnatal Neurogenesis

Support: Start-up funds of the Chinese government and Harbin Institute of Technology

Title: PINK1-deficient mice show impaired hippocampal neurogenesis and mitochondrial and cell signaling defects in adult neural stem cells

Authors: ***H. BUELER**, S. K. AGNIHOTRI;
Harbin Inst. of Technol., Harbin, China

Abstract: Parkinson's disease (PD) is characterized by a severe movement disability and several non-motor symptoms, including depression, anxiety and cognitive impairments in a sizeable proportion of patients. The latter symptoms are also typical features of affective disorders and have been associated with deficits of adult hippocampal neurogenesis (AHN). Despite of this, studies of neurogenesis in models of PD and, in particular, the importance of mitochondria for AHN are limited. Mutations in the mitochondrial kinase PINK1 cause recessive familial PD. PINK1 and Parkin cooperate to protect neurons against oxidative stress, maintain mitochondrial integrity and promote the selective degradation of depolarized mitochondria through mitophagy. Here we show that adult hippocampal neural stem cells (aNSC) lacking PINK1 display mitochondrial dysfunction and impaired AKT signal transduction, associated with decreased proliferation of aNSC in culture and in the hippocampus of PINK1-deficient mice. Moreover, doublecortin-positive neurons in the dentate gyrus of PINK1-deficient mice show abnormal dendritic morphology and their maturation is impaired. Our results link impaired mitochondrial function and AKT signaling in aNSC with abnormal AHN in a model of recessive Parkinsonism. Taken together, these studies suggest that mitochondrial and cell signaling defects - in particular in PD patients with PINK1 mutations - may lead to abnormal mood and cognition through impairments of AHN. Targeting mitochondria and metabolism of adult NSC to increase neurogenesis in the hippocampus may be a promising strategy for the treatment of affective disorders and the mitigation of related symptoms in PD and other neurodegenerative conditions.

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Poster

029. Adult Neurogenesis Mammalian Systems

Location: Halls B-H

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Topic: A.02. Postnatal Neurogenesis

Support: MOST Grant 105-2320-B-001 -003 -MY2

Title: Regulation of adult neurogenesis in the mouse SGZ by Kv1.1

Authors: S.-M. CHOU[1], C. LI[2], H.-Y. CHEN[1], L. JAN[2], *S.-B. YANG[1];
[1]Inst. of Biomed. Sci., Academia Sinica, Taipei, Taiwan; [2]Howard Hugh Med. Inst. Univ. of California San Francisco, San Francisco, CA

Abstract: In mammals, the sub-granular zone (SGZ) in the dentate gyrus is one of a few brain regions that exhibit extensive adult neurogenesis. The SGZ neurogenesis is tightly regulated by a balance between cell proliferation and apoptosis to keep the total number of neurons constant in

the SGZ. Although the molecular mechanisms that regulate adult neurogenesis have been studied extensively, the role of ion channels and membrane excitabilities in modulating adult neurogenesis remain to be precisely elucidated. Recent studies have found that mice lacking functional Kv1.1 channels have enlarged brain due to excessive adult neurogenesis in the SGZ; however, the molecular and cellular mechanisms by which Kv1.1 suppresses adult neurogenesis are still largely unexplored. We found that, using mosaic analysis with double markers (MADM) in adult mouse SGZ, neurons lacking functional Kv1.1 were more abundant than wild-type neurons that produced from the same progenitor cells; in contrast, astrocytes lacking Kv1.1 did not exhibit proliferation advantage over the wild-type astrocytes. Next, we recorded from Fezf2-GFP expressing neural progenitor cells that were not coupled by gap junctions in the brain slice, and we found a subset of these neural progenitors had a more depolarized potential in the Kv1.1 knockout background. Lastly, we found daily injection of GNF5837, a pan-TrK antagonist, suppressed the over-population of neuronal progenitor cells in the Kv1.1 knockout mice. Our data suggest that Kv1.1 regulates adult neurogenesis in the SGZ in a cell-autonomous manner, as Kv1.1 hyperpolarizes the neuronal progenitor cells and suppresses BDNF-Trk signal in the SGZ.

Disclosures: **S. Chou:** None. **C. Li:** None. **H. Chen:** None. **L. Jan:** None. **S. Yang:** None.

Poster

029. Adult Neurogenesis Mammalian Systems

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 29.13/B12

Topic: A.02. Postnatal Neurogenesis

Support: MH73136

NS28912

P50MS096889

Title: Programming of stress-sensitive neurons via NRSF-dependent epigenetic mechanisms by neonatal experience promotes emotional resilience

Authors: *A. SINGH[1], J. MOLET[3], S. JIANG[2], A. KOROSI[4], J. L. BOLTON[1], Y. NOAM[1], K. SIMEONE[5], J. COPE[1], A. MORTAZAVI[2], T. Z. BARAM[1];
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Abstract: Resilience to stress-related emotional disorders is governed in part by early-life experiences. Here we demonstrate experience-dependent reprogramming of stress-sensitive hypothalamic neurons, which takes place through modification of neuronal gene expression via epigenetic mechanisms. Specifically, we found that augmented maternal care reduced glutamatergic synapses onto stress-sensitive hypothalamic neurons and repressed expression of the stress-sensitive gene, *Crh*. In hypothalamus *in vitro,* reduced glutamatergic neurotransmission recapitulated the repressive effects of augmented maternal care on *Crh*, and this required recruitment of the transcriptional repressor REST/NRSF. Increased NRSF binding to chromatin was accompanied by repressive epigenetic changes *in vitro* and *in vivo*. ChIP-Seq analyses of NRSF targets identified gene networks that, in addition to *Crh*, contributed to the augmented care-induced phenotype, including diminished depression and anxiety. Together, these findings provide the first causal link between enriched neonatal experience, synaptic refinement, and induction of epigenetic processes within specific neurons. They uncover a novel mechanistic pathway from neonatal environment to emotional resilience.

Disclosures: **A. Singh:** None. **J. Molet:** None. **S. Jiang:** None. **A. Korosi:** None. **J.L. Bolton:** None. **Y. Noam:** None. **K. Simeone:** None. **J. Cope:** None. **A. Mortazavi:** None. **T.Z. Baram:** None.

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029. Adult Neurogenesis Mammalian Systems

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 29.14/B13

Topic: A.02. Postnatal Neurogenesis

Support: NINDS 4R01NS078294

NINDS 1F31NS092435

NICHD 5U54HD083092-02 sub 6924

Title: Brain-5, a novel regulator of activity-dependent cellular plasticity and synaptic integration in the adult brain

Authors: *C. K. MCCLARD[1,2,3,4], M. KOCHUKOV[1,2,4], I. HERMAN[1,3,5], Z. LIU[1,4,6], L. HUANG[1,4,7], J. ORTIZ-GUZMAN[1,4,5], D. COLCHADO[8], B. R. ARENKIEL[1,2,4,5,7]; [2]Dept. of Genet., [3]Med. Scientist Training Program, [4]Jan and Dan Duncan Neurolog. Res. Inst., [5]Program in Developmental Biol., [6]Dept. of Pediatrics, [7]Dept. of Neurosci., [1]Baylor Col. of Med., Houston, TX; [8]Rice Univ., Houston, TX

Abstract: The discovery of ongoing neurogenesis in the adult mammalian brain has prompted efforts to investigate the mechanisms of this astonishing plasticity. The mouse olfactory bulb (OB) features lifelong neuronal integration and thus serves as an ideal model system to examine how new cells rewire the adult brain. We discovered a local population of interneurons in OB that secretes the neuropeptide corticotropin releasing hormone (CRH) onto developing neurons, which in turn upregulate expression of the CRH receptor (CRHR1) as they mature. Genetic experiments showed that signaling by local CRH-secreting interneurons is required for normal rates of survival of developing neurons, and is both necessary and sufficient for production of synaptic machinery in development. CRHR1 is a G_S protein-coupled receptor that transcriptionally activates the receptor-expressing cell in the presence of its ligand. We hypothesize that in the adult brain, local CRH binds to CRHR1 to initiate cell-intrinsic programs of survival and synaptogenesis. To determine the identity of these transcriptional programs, we profiled gene expression changes associated with CRHR1 activity in developing neurons of the OB. We found that local CRH signaling dynamically regulates expression of the brain-specific Homeobox-containing transcription factor Brain-5 (BRN5). To test whether expression of BRN5 suffices to enhance normal levels of survival or synaptogenesis, we targeted developing neurons in the adult OB to overexpress BRN5. Neurons that overexpressed BRN5 displayed a higher propensity toward survival and exhibited greater success at recruiting strong functional synaptic connections in the olfactory circuit than controls. Conversely, loss of BRN5 during neuronal development reduced the number of excitatory connections onto adult-born neurons of the OB. These findings suggest that the transcriptional programs directed by BRN5 downstream of local CRH signaling influence neuronal survival and functional development. Together, our studies reveal a novel regulatory pathway by which local neuropeptidergic signaling in the adult brain activates specific genetic programs to govern cell and circuit plasticity.

Disclosures: C.K. McClard: None. **M. Kochukov:** None. **I. Herman:** None. **Z. Liu:** None. **L. Huang:** None. **J. Ortiz-Guzman:** None. **D. Colchado:** None. **B.R. Arenkiel:** None.

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029. Adult Neurogenesis Mammalian Systems

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

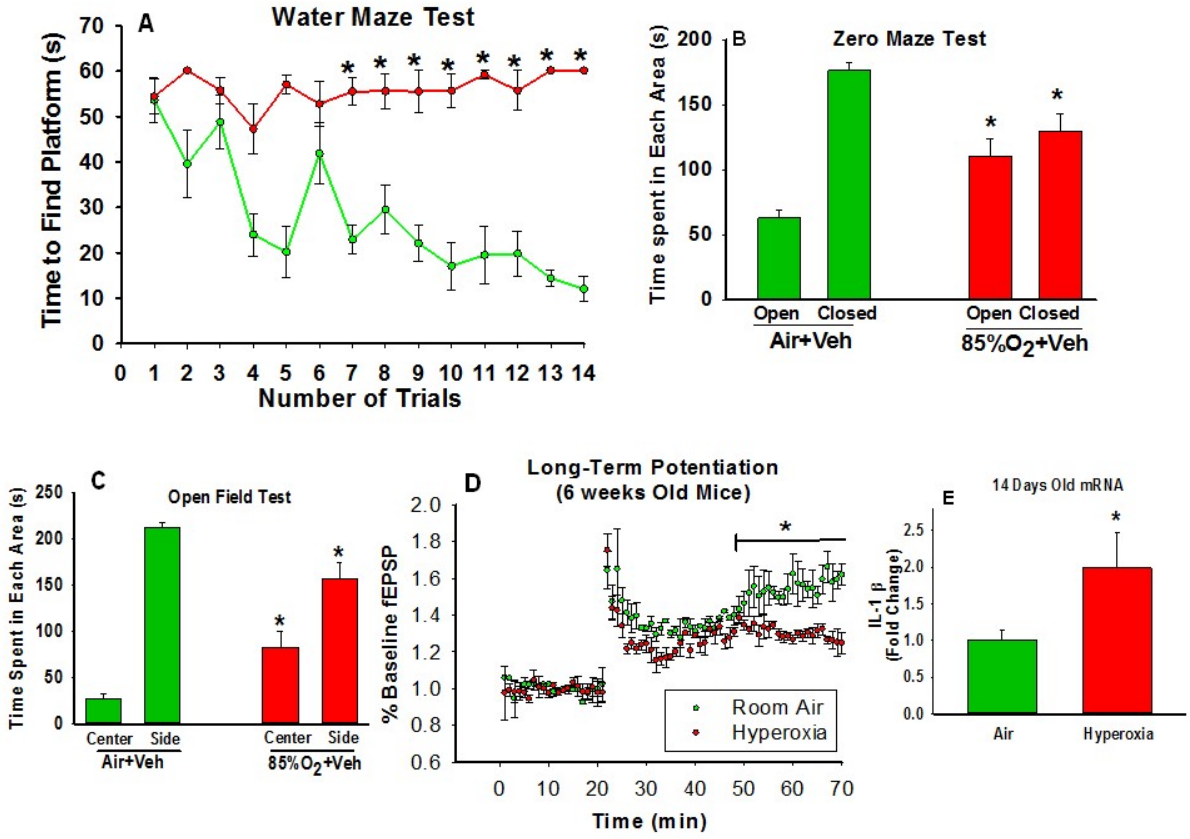
Program#/Poster#: 29.15/B14

Topic: A.02. Postnatal Neurogenesis

Title: Neonatal hyperoxia leads to neurobehavioral impairment in adult mice by impairing synaptic plasticity and increasing inflammation in the hippocampus

Authors: *M. RAMANI[1], N. AMBALAVANAN[1], L. MCMAHON[2];
[1]Pediatrics, [2]Cell Develop. and Intergrative Biol., Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: Background: Very preterm infants are at high risk (30-40%) for developing neurodevelopmental impairment (NDI) even in the absence of obvious brain injury. Many preterm infants with a relatively uncomplicated neonatal intensive care course have poor executive and memory function later in life. The etiology of NDI in children born preterm without apparent brain parenchymal injury is unknown. We recently showed that adult mice exposed to neonatal hyperoxia had deficits in spatial and recognition memory associated with smaller hippocampal volumes. Oxygen is known to increase inflammation and causes cell death in developing brain. Long-term changes in the strength of synapses (long-term potentiation) are critical for the formation and maintenance of memory. We hypothesized that neonatal hyperoxia exposure results in neurobehavioral impairment by (a) deficits in long-term potentiation and/or (b) increase in inflammation. **Methods**: C57BL/6 mouse pups (n = 2 litters per condition; at least 10/group) were exposed to hyperoxia (85% oxygen) or air, from postnatal day 2 to 14 (P2-P14; roughly corresponding from very preterm equivalent to early infancy in human brain development) and then returned to air. We assessed neurobehavioral assessment (14 weeks of age), long-term potentiation studies (6 weeks of age) and hippocampal IL-1β mRNA levels (P14). **Results**: In adult mice, neonatal hyperoxia induced spatial navigation deficits and increased exploratory behavior (Figure A, B, C). Young adult mice (6 weeks of age) exposed to neonatal hyperoxia had deficits in long-term potentiation (Figure D). Neonatal hyperoxia increased hippocampal IL-1 β mRNA levels (Figure E). **Conclusion**: Oxygen exposure during the critical developmental period of the brain increases hippocampal IL-1 β, leading to permanent impairment of long-term potentiation and results in neurobehavioral impairment. This model suggests therapeutic strategies that target the hyperoxia-induced hippocampal inflammation may potentially be of benefit in the prevention of neurodevelopmental impairment in preterm infants.



Disclosures: **M. Ramani:** None. **N. Ambalavanan:** None. **L. McMahon:** None.

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029. Adult Neurogenesis Mammalian Systems

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 29.16/B15

Topic: A.02. Postnatal Neurogenesis

Title: Ependyma derived CCN1 promotes progenitor cell proliferation and neurogenesis in the adult SVZ

Authors: ***Q. SHEN**;
Tsinghua Univ., Beijing, China

Abstract: In adult rodents, the subventricular zone (SVZ) is the largest germinal zone, where new neurons are continuously generated and migrate to the olfactory bulb. Type B neural stem cells (NSCs) in this region give rise to type C transit amplifying cells, which, after several rounds of division, turns into migrating neuroblasts. Apart from various intrinsic mechanisms, this neurogenic process is under tight spatial and temporal control of niche factors. Exploring important factors regulating neural stem cell behavior offers great value for treating nervous system diseases. CCN1 is an extracellular matrix protein, belonging to the CCN family. It is abundant in various cancers. Here we find that CCN1 is specifically expressed by the ependymal cells, the important niche cells in the SVZ, and binds to neural stem and progenitor cells. Conditional ablation of the gene in ependymal cells impairs cell proliferation as well as neurogenesis which involves decreased AKT phosphorylation. In vitro overexpression of CCN1 dramatically increased cell proliferation and decreased neural differentiation. We are interested in exploring the underlying mechanisms as well as increasing our knowledge on adult brain development. We hope that this could be beneficial to neural stem cell therapy and neurological diseases.

Disclosures: Q. Shen: None.

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029. Adult Neurogenesis Mammalian Systems

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 29.17/B16

Topic: A.02. Postnatal Neurogenesis

Title: Diazepam binding inhibitor (DBI): a novel protein for the induction of postnatal neurogenesis in the spinal cord?

Authors: L. NEW, E. TEDFORD, J. SMITH, J. HEE, J. DEUCHARS, *S. A. DEUCHARS; Univ. of Leeds, Leeds, United Kingdom

Abstract: The ependymal cell (EC) layer surrounding the central canal (CC) of the postnatal mammalian spinal cord is considered a neurogenic niche, proliferating extensively after injury and contributing to the development of the glial scar. Understanding the niche, its microenvironment, and how its proliferative behaviour may be manipulated is essential if we are to harness endogenous mechanisms to aid spinal repair. This study examines ways in which GABA and its opposing ‘partner’ DBI may contribute to the balance of proliferation vs. relative quiescence of the ECs. To determine the effects of GABA on spinal cord proliferation, GAD67-GFP mice (4-6 weeks n=3), which have reduced GABA levels as a result of replacing one allele of GAD67 with GFP, and wild type (WT) C57Bl/6 (4-6 weeks n=3) mice were injected I.P with

the thymidine analogue 5-Ethynyl-2'-deoxyuridine (EdU). Animals were anaesthetised (60mg/kg pentobarbital I.P) and perfused transcardially with 4% paraformaldehyde. The thoracolumbar spinal cord was sectioned (40μm) and post hoc fluorescent detection of EdU was performed. EdU+ve cells were counted (n=3 N=36) at both the CC and the grey and white matter. For immunohistochemical (IHC) investigation of DBI and its 'mitochondrial receptor' TSPO in the spinal cord, tissue from WT C57Bl/6 mice (4-6 weeks, n=9 and 15±2 days, n=3) was prepared as above. Tissue was processed for DBI immunoreactivity (IR) and double labelling fluorescence IHC for other cellular markers including; glial fibrillary acid protein, neuN, panQKI, nestin, sox2, Iba1, CD24, S100β. GAD67-GFP mice were used to identify cerebrospinal fluid contacting cells (CSF-cCs). GAD67-GFP animals, which have significantly lower levels of ambient spinal GABA compared to control, (assessed by HPLC $P < 0.05$), exhibited greater levels of proliferation than WT animals at both the CC (0.2778 ± 0.1099 vs. 3.361 ± 0.3589 cells $P < 0.0001$) and across the spinal cord (11.28 ± 1.811 vs. 35.17 ± 2.846 cells, $P < 0.0001$) This is likely due to the reduction in ambient GABA, thus releasing inhibition on proliferation. In both adult and juvenile animals, intense DBI IR was detected within the EC layer. A lack of colocalisation of DBI with GFP in GAD67-GFP mice suggests that DBI is absent from CSF-cCs, whereas sox2, a neural stem cell marker labelling ECs, colocalised with DBI at the CC. These results suggest that DBI is found in the spinal EC population. The EC layer is also TSPO IR. Further experiments will seek to link the two complementary players GABA and DBI together, exploring the ways in which DBI can be used to modulate GABAaR, and therefore influence proliferation and differentiation of ECs within the spinal cord.

Disclosures: L. New: None. **E. Tedford:** None. **J. Smith:** None. **J. Hee:** None. **J. Deuchars:** None. **S.A. Deuchars:** None.

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029. Adult Neurogenesis Mammalian Systems

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 29.18/B17

Topic: A.02. Postnatal Neurogenesis

Support: Anesthesiology department, SUNY Downstate

Title: Early-life sevoflurane exposure targets microRNAs and excitatory-inhibitory balance

Authors: D. LIN[1], J. LIU[2], J. COTTRELL[2], *I. S. KASS[1];
[1]Anesthesiol. and Physiol. and Pharmacol., [2]Anesthesiol., SUNY Downstate, Brooklyn, NY

Abstract: Introduction:
During early brain development, the intricate regulations of neurons include, gene expression, morphology and circuitry establishment within/between regions. Failure to fine-tune these spatiotemporal regulations could have long lasting functional consequences later on in life. Previously, our lab has shown that P7 sevo treated mice have social behavior and cognition deficits later on in life. Subsequently, we identified specific brain microRNAs (miRNAs) to be associated with early-life sevo treatment.
Methods:
Postnatal day 7 (P7) C57/BL6 male pups were exposed to 2-2.3% sevo in a 40% oxygen (O2) 60% nitrogen (N2) gas mixture for 2 hours. The expression of brain miRNAs was examined using the nCounter miRNA Expression Assay (Nanostring Technologies) and validated using Real-Time PCR (BioRad). Morphology experiments were done by using cell cultures from 7 days in vitro (DIV) primary cortical neurons. Paired-pulse extracellular recording was conducted on CA 1 region of the hippocampus slices.
Results:
miRNA profiling from P7 brains identified differentially expressed miRNAs as a result of sevo treatment. We further validated expression changes of miRNA 15a from the hippocampus and miRNA 145 from the whole brain by RT-PCR. At the morphological level, we observed a reduction in axonal growth (as indicated by axon specific GFP) and dendritic branching (as indicated by dendritic specific protein Map 2) after application of miRNA 145 inhibitor in 7 DIV. At the electrophysiological level, paired-pulse tests showed adult sevo treated group has a trend toward increased inhibition compared to control. The opposite was observed in P7 sevo treated groups, a trend toward increased facilitation was shown when recorded at P7 and P10.
Conclusions:
P7 sevo-associated miRNA 145 is critical for early brain development such as dendritic branching and axonal growth. Furthermore, after P7 treatment, hippocampus neurons showed increased excitatory responses when measured at P7 and P10. We are in the process of examining the roles of miRNAs in neuronal excitation/inhibition balance. Our understanding on the role of miRNAs has provided us one of the putative mechanisms of early-life sevo induced neuronal toxicity.

Disclosures: D. Lin: None. **J. Liu:** None. **J. Cottrell:** None. **I.S. Kass:** None.

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029. Adult Neurogenesis Mammalian Systems

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 29.19/B18

Topic: A.02. Postnatal Neurogenesis

Support: NIH/NINDS R01 NS081055.

Title: Neurovascular ligand-receptor systems modulate post-stroke neurogenesis and angiogenesis

Authors: *N. ABDULJAWAD, A. J. BRUMM, M. MACHNICKI, S. T. CARMICHAEL; Neurol., Univ. of California Los Angeles, Los Angeles, CA

Abstract: Ischemic stroke initiates a process of tissue repair that includes the development of new blood vessels (angiogenesis) and the proliferation of neural progenitor cells (NPCs). Previous work has shown that NPCs born in the subventricular zone (SVZ) after stroke localize to angiogenic blood vessels in damaged tissue (peri-infarct), forming a neurovascular unit in which angiogenesis modulates neurogenesis. However, the blood vessel-secreted trophic factors that mediate this targeted migration and neurogenesis remain unknown. Whole-genome expression profiling of peri-infarct vasculature and stroke-responsive neuroblasts 7 days post-stroke, the time point at which post-stroke angiogenesis and neurogenesis reach their peak, reveals upregulation of four ligands of interest in angiogenic vascular cells, along with upregulation of their respective receptors in stroke-responsive neuroblasts: colony stimulating factor 1 (CSF1), thymic stromal lymphopoietin (TSLP), protein S alpha (PROS1), and Wnt5a. To investigate the effects of these molecular signaling systems on post-stroke angiogenesis and neurogenesis, two in vitro culture systems were used. Primary NPCs, isolated from the adult mouse SVZ and expanded as neurospheres for 2 passages, and mouse cerebrovascular endothelial cells (MCVECs) were transduced with lentivirus designed to either overexpress or knockdown each ligand. NPC and MCVEC proliferation in response to each treatment was measured using EdU DNA synthesis assays. NPC differentiation following treatment was measured through immunocytochemical phenotyping of NPCs. Finally, MCVEC development was measured using tube formation assays. Data from these assays are currently being quantified. Results will provide insight into novel molecular signaling systems that potentially regulate endothelial cell and neural progenitor cell function after stroke in vivo.

Disclosures: N. Abduljawad: None. **A.J. Brumm:** None. **M. Machnicki:** None. **S.T. Carmichael:** None.

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029. Adult Neurogenesis Mammalian Systems

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 29.20/B19

Topic: A.02. Postnatal Neurogenesis

Title: The study of sonic hedgehog signaling pathway in development of cerebellar granule cells

Authors: *X. JIAO, N. ASHTARI, K. BAILEY, M. R. BALAEI, S. GHAVAMI, M. DEL BIGIO, H. MARZBAN;
Human anatomy and cell science, Univ. of Manitoba, Winnipeg, MB, Canada

Abstract: Introduction: The cerebellum is responsible for motor control and cognitive functions. The cerebellar cortex can be divided into three layers consist of different cell types, such as granule cells (gcs), Purkinje cells (Pcs) and Bergmann glial cells. During the development, the granule cell precursors arise from the cerebellar rhombic lip and initially migrate rostrolaterally in subpial stream pathway of the cerebellar primordium to form the external germinal zone (egz). Thereafter, the granule cell precursors radially proliferate and then move to granule layer (gl) and differentiate into mature gcs. In this process, sonic hedgehog (Shh) secreted by Pcs is an important promoter of granule cell precursors proliferation. A lysosomal acid phosphatase 2 (Acp2) mutant mouse (*nax*) shows a significant gcs reduction in the cerebellum. We hypothesize that the Shh signaling pathway is interrupted by the Acp2 mutation and causes cerebellar gc neurodevelopmental disorders.
Methods: We used the *nax* and the wild-type sibling mice as control in this study. *In vivo* and *in vitro* immunohistochemistry and Western-blotting are used to detect the molecular expression.
Results: In *nax* mouse the number of gcs is greatly reduced compared with wild-type sibling mice. The Shh expression is down regulated from P12, while the expression of Pax6 by gc precursors in the external germinal zone is down regulated at around P7 and it is maybe due to the decreased number of gcs. The immunostaining shows reduced expression of N-Myc which is an essential downstream effector of Shh pathway in *nax* MEF cells. N-Myc expression is less from P10 to P18 in *nax* as compared to control.
Conclusion: The absence of Acp2 impacts on the gcs proliferation prior to the Shh to cease gcs proliferation during cerebellar development. The significant reduction in the proliferation and probably differentiation of gcs in *nax* mouse reveals that the Shh signalling pathway in gcs is interrupted by Acp2 mutation and causes cerebellar gc neurodevelopmental disorders.

Disclosures: X. Jiao: None. **N. Ashtari:** None. **K. Bailey:** None. **M.R. Balaei:** None. **S. Ghavami:** None. **M. Del Bigio:** None. **H. Marzban:** None.

Poster

029. Adult Neurogenesis Mammalian Systems

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 29.21/B20

Topic: A.02. Postnatal Neurogenesis

Title: Meninges are a niche for neural precursor cells

Authors: I. DECIMO[1], V. BERTON[1], A. PINO[1], S. DOLCI[1], M. MARCHETTO[1], F. PARI[1], *G. F. FUMAGALLI[1], F. BIFARI[2];
[1]Univ. of Verona, Verona, Italy; [2]Univ. of Milan, Milan, Italy

Abstract: Brain and skull developments are tightly synchronized allowing the cranial bones to dynamically adapt to the brain shape. Meninges are the stromal tissue that represent the physical interface between brain and skull. Meningeal cells produce trophic signals necessary for normal corticogenesis and bone development. Different cell populations have been described in meninges including cells that can function as endosteum of the cranial vault. Recently, we and other groups described the presence in meninges of a cell population endowed with neural differentiation potential *in vitro* and, after transplantation, *in vivo*. However, whether meninges may be a niche for neural progenitor cells during embryonic development to adulthood is not known. In this work we provide the first description of the distribution of neural precursor markers in rat meninges during development up to adulthood. We describe that meninges share common properties with the classical neural stem cell niche: i) meninges are unexpected highly proliferative tissue; ii) they contain cells expressing neural precursor markers such as nestin, vimentin, SOX2 and DCX and iii) meningeal tissue is enriched with extracellular matrix components (fractones) known to bind and concentrate growth factors. This study underlines the importance of meninges as a potential niche for endogenous precursor cells during development and in adulthood.

Disclosures: I. Decimo: None. **V. Berton:** None. **A. Pino:** None. **S. Dolci:** None. **M. Marchetto:** None. **F. Pari:** None. **G.F. Fumagalli:** None. **F. Bifari:** None.

Poster

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Program#/Poster#: 29.22/B21

Topic: A.02. Postnatal Neurogenesis

Support: SIP 20161405

CONACYT 168917

Title: Hilus of the hippocampus possess progenitor cells of oligodendrocytes and neurons in the adult brain

Authors: *J. PACHECO-ROSADO[1], Y. GARCÍA-MARTÍNEZ[2], L. FLORES-PÁEZ[2], K. B. SÁNCHEZ-HUERTA[3];
[1]Escuela Nacional de Ciencias Bio, Ciudad DE Mexico, Mexico; [2]Fisiología, Inst. Politécnico Nacional, Mexico City, Mexico; [3]Lab. de Neurociencias, Inst. Nacional de Pediatría, Mexico city, Mexico

Abstract: Adult neurogenesis results in the generation of new neurons or glia cells in the subgranular zone (SGZ) of the dentate gyrus. This process involves both cell populations that dynamically switch between pools of proliferative and quiescent cells, and cells that definitely leave the cell cycle to maturate into neurons or glia cells. Interestingly, the hilus of the hippocampus also has proliferative cells whose mitotic activity responds to environmental signals. It has been shown that seizures or hypothyroidism affect the number of proliferative cells in this region. However, the impact of these changes is poorly understood; since the lineage of proliferative cells and their differentiation process is still unknown. The aim of this work was to characterize the population of proliferative cells present in the hilus of the hippocampus. For this, male Wistar rats (90 days old) were administrated with BrdU (6 times, every 2 hours). Twenty four hours after the last injection, the animals were anesthetized and perfused. Brains were dissected and dorsal hippocampus was serially sectioned. Slices were processed for immunofluorescence. The total number of proliferative cells (BrdU+), precursor cells (Sox2+/BrdU+); neural restricted progenitor cells (NRPs; DCX+/BrdU+); oligodendrocytes progenitor cells (OPCs; NG2+/BrdU+); and proliferative mature astrocytes (PMA; S100β+/BrdU+) were quantified **i**n the hilus and **i**n the SGZ. The results showed that approximately 77% of proliferative cells were precursor cells in both regions studied. Interesting, in the hilus OPCs proliferation (57%) is favored over NRPs (42%); while in the SGZ, NRPs proliferation (66%) is favored over OPCs (10%). Finally, it was found that mature astrocytes do not proliferate in either SGZ or hilus. These results demonstrate the presence of OPCs and NRPs in the hilus of the hippocampus and suggest that this region contributes to the formation of oligodendrocytes and neurons in the adult brain.

Disclosures: J. Pacheco-Rosado: None. **Y. García-Martínez:** None. **L. Flores-Páez:** None. **K.B. Sánchez-Huerta:** None.

Poster

029. Adult Neurogenesis Mammalian Systems

Location: Halls B-H

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Topic: A.02. Postnatal Neurogenesis

Support: Conacyt Grant 239516 (J.S-V)

Title: Differential expression of Gas1 in neural stem cells and in cancer stem cells

Authors: N. AGUIRRE-PINEDA, E. BAUTISTA, J. HERNÁNDEZ, *J. V. SEGOVIA-VILA; Cinvestav-IPN, Mexico 07300 DF, Mexico

Abstract: Growth Arrest Specific 1 (Gas1) is a pleiotropic molecule linked to the outer cell membrane by a glycosylphosphatidylinositol (GPI) anchor. Because of its high structural similarity with the glial cell line derived neurotrophic family receptors (GFRαs), Gas1 is capable of inducing cell cycle arrest and apoptosis of glioma cell lines and of primary cultures of human gliomas by inhibiting the intracellular signaling pathway mediated by GDNF. On the other hand, Gas1 acts as a co-receptor for the morphogen Sonic Hedgehog (SHH) enhancing its signaling. Moreover, Gas1 is expressed in the developing mammalian brain, specifically in progenitor cells, thus indicating a role in the proliferation and differentiation of neural cells. In an effort to understand the function(s) of Gas1 in the neural stem cell (NSC) population we sought to determine its expression in both cancer stem cells (CSC) obtained from the glioma cell lines C6 and U87 MG and in stem cells isolated from primary cultures of the subgranular zone of the hippocampus and the subvbentricular zone of the mouse brain. CSC and NSC were isolated from free floating gliomaspheres and neurospheres, respectively, and characterized by the expression of specific markers, including CD133, nestin, Sox2 and GFAP. We determined by RT-PCR and immunocytochemistry that healthy NSC express Gas1, but that Gas1 is no longer found in CSC. These results indicate that Gas1 has an important role in the control of the cell cycle of NSC and that this regulation is lost in CSC.

Disclosures: N. Aguirre-Pineda: None. **E. Bautista:** None. **J. Hernández:** None. **J.V. Segovia-Vila:** None.

Poster

029. Adult Neurogenesis Mammalian Systems

Location: Halls B-H

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Program#/Poster#: 29.24/B23

Topic: A.02. Postnatal Neurogenesis

Support: NIMH 5P50MH096972

Simms/Mann Chair in Developmental Neurogenetics at CHLA

Title: Characterization of Met receptor tyrosine kinase-expressing serotonergic neurons

Authors: *H.-H. WU[1], R. KAST[2], P. LEVITT[1];
[1]Children's Hosp. Los Angeles, Los Angeles, CA; [2]Neurosci. Grad. Program, USC, Los Angeles, CA

Abstract: There is increasing awareness that the brain serotonin system as a unified system is actually far more complex functionally and structurally. Specific subsets of raphe neurons have different connectivity patterns, electrophysiological properties, and molecular profiles. However molecule profiles that define specific subpopulation are not well studied. We demonstrated that, from late gestation to adulthood in the mouse, MET receptor tyrosine kinase (MET) is specifically expressed in a remarkably small subset of 5-HT neurons that includes the classic B6 subgroup in the dorsal raphe nuclei (DRN). Detailed mapping from embryonic day 16 to postnatal day 14 reveals that MET is expressed almost exclusively in the caudal part of DRN as a paired nucleus situated just below the aqueduct, corresponding to the B6 subgroup. Small population of MET+ neurons is also found in subset of median raphe (B5) 5-HT neurons. Remarkably, the location of MET-expressing 5-HT neurons (5-HT^{MET+}) in the developing rhesus monkey brainstem is highly conserved. This group of unique 5-HT neurons may thus function in a conserved fashion across species. In mice, conditional deletion of *Met* only in the 5-HT neurons causes social behavioral deficits. The connectivity of 5-HT^{MET+} neurons involved in mediating social behavior, and the impact of deleting *Met* on the circuitry of this 5-HT subgroup, is unknown. We set to further characterize this 5-HT^{MET+} subgroup by 1) analyzing its molecular signature using public available database and by 2) determination of their target sites on the forebrain. Using developing mouse brain database in Allen Brain Atlas we found 14 genes, including 4 neurotransmitter/neuropeptide receptors and 2 potassium channels, expressing in the DR region exhibiting a B6-specific manner. Tract tracing experiments shows that almost all the 5-HT axons innervated ventricular/subventricular regions are MET+. Gene expression of candidates is being evaluated in WT and *Pet-1*^{Cre} x *Met*^{fx} mice.

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

Poster

029. Adult Neurogenesis Mammalian Systems

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 29.25/B24

Topic: A.02. Postnatal Neurogenesis

Title: Adult neurogenesis - A comparison between dark Agouti and Wistar rats

Authors: *M. S. RAO[1], F. B. AL HAMDAN[2], J. A. REZQALLA[2], S. SMITHA[1], H. AL HUSSAINI[1];

[1]Dept. of Anat., Jabriya, Kuwait; [2]3rd Year Med. Student, Fac. of Med., Kuwait Univ., Jabriya, Kuwait

Abstract: Adult neurogenesis is a continuous process of addition of new neurons into the nervous system after birth. Newly born neurons in the dentate sub granular zone integrate with dentate gyrus granule neurons and play a role in learning and memory. Adult neurogenesis declines in an age dependent manner in albino rats. However pattern of adult neurogenesis in pigmented Dark Agouti (DA) rats is not known. Objective of the present study was to explore the pattern of neurogenesis at different ages in DA rats and compare it with age matched Wistar rats. DA rats aged 1, 2,4,6,8 and 10 months were euthanized with CO_2 and perfused with saline followed by 4% paraformaldehyde. Brains were dissected and processed for doublecortin (DCX, marker for new neurons) immunostaining. Number of newly born neurons in the dentate gyrus were quantified. Unfixed hippocampal tissues were used for Western blot analysis of DCX content. Hippocampal tissues from age matched Wistar rats were processed in the similar way for comparison. Data were analyzed with one way ANOVA and Bonferroni's test. Neurogenesis declined progressively in both DA and Wistar rats. Number of new neurons in the dentate gyrus of 1 month and 2 months old DA rats were similar to each other as well as to age matched Wistar rats($p>0.05$). However neurogenesis commenced to decline as early as 4 months in DA rats as number of new neurons significantly less in them compared to age matched Wistar rats ($p<0.05$). At 6, 8 and 10 months of age, neurogenesis further declined in DA rats compared to Wistar rats ($p<0.01$). Neuronal count data was supported by Western blot analysis for DCX content in the hippocampus. In conclusion, extent of adult hippocampal neurogenesis in DA rats is significantly less and declines at an earlier age than in Wistar rats. Mechanism, cause, and functional implications of such a decreased neurogenesis at an early age needs to be explored further.

Disclosures: M.S. Rao: None. **F.B. Al Hamdan:** None. **J.A. Rezqalla:** None. **S. Smitha:** None. **H. Al Hussaini:** None.

Poster

030. Genetics of Autism and Autism Models

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 30.01/B25

Topic: A.07. Developmental Disorders

Support: NIH Grant NS034007

NIH Grant NS047384

Title: Assessment of single nucleotide variants in genes encoding eif2α kinases in autism simplex families

Authors: *A. G. VOROBYEVA[1], E. KLANN[1], I. IOSSIFOV[2], T. E. DEVER[3];
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Abstract: Autism spectrum disorder (ASD) is a heritable neurodevelopmental disorder characterized by the early onset of social and communication deficits, repetitive behaviors, and cognitive inflexibility. Multiple lines of evidence suggest that dysregulated translational control is one molecular mechanism underlying ASD neuropathology. Exome sequencing of Simplex families with a child affected by autism revealed numerous heritable and *de novo* autism-associated single nucleotide variations (SNVs) within genes encoding for proteins responsible for maintaining translational homeostasis. We assessed three affected genes; *EIF2AK2, EIF2AK3* and *EIF2AK4*, which encode protein kinase R (PKR), PKR-like ER kinase (PERK), and the evolutionarily conserved general control nonderepressible 2 (GCN2) kinase, respectively. All three kinases are activated under conditions of cellular stress, which results in phosphorylation of eIF2 on its α subunit. Phosphorylated eIF2α terminates global protein synthesis and enhances the translation of mRNAs containing 5'UTRs with unread open reading frames, including the transcription factor ATF4. We hypothesized that heritable and *de novo* missense and nonsense ASD-associated SNVs in genes encoding the eIF2α kinases would alter their protein structure and kinase function, and would significantly dysregulate global protein synthesis leading to abnormal development and cognitive function. We discovered that multiple ASD-associated SNVs in eIF2α kinases result in altered kinase activity as measured by changes in eIF2α phosphorylation and global protein synthesis levels. Consistent with our hypothesis, PERK and GCN2 haploinsufficient mice display atypical cognitive function and ASD-like behaviors. All together our findings suggest that impaired PKR, PERK and GCN2 function occurs in multiple individuals with ASD.

Disclosures: **A.G. Vorobyeva:** None. **E. Klann:** None. **I. Iossifov:** None. **T.E. Dever:** None.

Poster

030. Genetics of Autism and Autism Models

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 30.02/B26

Topic: A.07. Developmental Disorders

Support: National Natural Science Foundation Of China

Title: Functional analysis of the autism gene SHANK3 using iPSCs-derived models of neural development

Authors: *S. GONG[1], L. SHI[2];
[1]Room 819 The Second Sci. and Technol. Buildin, Guangdong, China; [2]Jina Univ., Guangzhou, China

Abstract: Multiple genetic studies have implicated significant roles of exonic deletions in genes coding for pre-postsynaptic complex, such as neurexins, neuroligins and scaffolding protein SHANKs, in the susceptibility to autism spectrum disorders (ASDs). Most previous studies on autism candidate genes were based on animal models or cultured neurons, which do not represent patient-specific models of neurodevelopment. Here we assess the feasibility using urine-derived induced pluripotent stem cells (iPSCs) to build patient-specific models of neural development, and study the functional effects of SHANK3 knockdown. Two inhibitor systems (SB431542, Drosomophorin) were used to induce human urine-derived iPSCs into neural stem cells (NSCs). We then introduced shRNA targeting SHANK3 into NSCs by lentivirus to model SHANK3 deletion, then investigated dynamic activity of developing neuron cells using live cell microscope. We collected fixed cells to analyze the dendrite length and number of branches of early stage neurons, and identified dysregulated molecular pathways by transcriptome sequencing. Urine-derived iPSCs and NSCs can be differentiated into neurons and astrocytes in our in vitro culture system in a highly reproducible manner. After shRNA targeting SHANK3 was introduced into NSCs, SHANK3 expression decreased ~50%. We observed abnormal dynamic activity of neurite outgrowth after SHANK3 KD in the beginning of neural development, where neurites develop into axons much earlier than control. We also found that both the total dendrite length of neuron and the number of branches decreased substantially after SHANK3 knockdown, especially the number of secondary branches. Our results demonstrated that decreased SHANK3 expression affects the fate of neural development, especially axon growth. Our study also suggested that urine can be a feasible and sustainable source for non-invasive collection of patient-specific iPSCs for building in vitro models of neural development.

Disclosures: S. Gong: None. **L. Shi:** None.

Poster

030. Genetics of Autism and Autism Models

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 30.03/C1

Topic: A.07. Developmental Disorders

Title: aberrant mitochondrial autophagy in autistic mouse models

Authors: *H. LI;
Columbia Univ. Med. Ctr., New York City, NY

Abstract: Autism spectrum disorders (ASD) are genetically heterogeneous, but different ASD genes may cluster into a smaller number of pathways, including the protein kinase mammalian target of rapamycin (mTOR). mTOR is a key molecule that regulates protein homeostasis by promoting protein synthesis and inhibiting macroautophagy (autophagy thereafter), a homeostatic degradation process whereby cellular protein and organelles,e.g. damaged mitochondria, are engulfed by autophagosomes, digested in lysosomes and recycled to sustain cellular metabolism. We recently discovered that autophagy deficiency in response to aberrant mTOR hyperactivation contributes to the synapse pathology of ASD. While the precise mechanism driving autophagy-related pathology remains obscure, mitochondrial dysfunction is likely a critical precipitating factor and a downstream mediator of the cellular and physiological responses to faulty autophagy. Here, we examined the effect of mTOR hyperactivation on mitochondrial autophagy (mitophagy) in both Tsc2+/- and Fmr1X-Y ASD mouse models, both of which exhibit excessive mTOR activity. Removal of damaged mitochondria through autophagy requires two steps: induction of general autophagy and priming of damaged mitochondria for selective autophagic recognition. We found impaired general autophagy in both Tsc2+/- and Fmr1X-Y mutant mouse brains, demonstrated by decreased LC3-II and increased p62 protein levels. We analyzed the content of autophagy receptor proteins, including Nbr1, Bnip3, Optineurin and p62, on mitochondrial fraction of each mouse line. These are cargo-recognizing molecules which bind to the surface of damaged mitochondria and regulate the recruitment of the autophagy machinery to the damaged mitochondria, a process called mitochondrial priming. Compared to control mice, both Fmr1X-Y and Tsc2+/- mutants showed a decrease in mitochondria-bound autophagy receptor proteins Bnip3 and p62, suggesting impairment in mitochondrial priming. Our results indicate that mitochondria autophagy is downregulated in both Tsc2+/- and Fmr1X-Y ASD mouse brain due to both reduced autophagy induction and impaired mitochondrial priming.

Disclosures: H. Li: None.

Poster

030. Genetics of Autism and Autism Models

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Topic: A.07. Developmental Disorders

Support: Simons Foundation Autism Research Initiative Grant 305112

SFARI (305112)

NIMH F32MH103949

Title: Identifying a neural circuit of social behavior in an autism mouse model Identifying a neural circuit of social behavior in an autism mouse model

Authors: *J. J. WALSH, G. A. BENDOR, R. C. MALENKA;
Psychiatry, Stanford Univ., Stanford, CA

Abstract: There is an urgent need for effective treatment strategies for autism spectrum disorder (ASD), with a prevalence rate of more than 1 in 100. One of the most common genetic variations found in ASD is a copy number variation (CNV) on human chromosome 16, accounting for approximately 0.5-1% of all ASD cases. While several brain regions have been implicated in ASD, such as the dorsal raphe (DR) and nucleus accumbens (NAc), the neural circuit mechanism and the projection specific pathway underling social impairment in ASD is largely unknown. Studies have implicated the serotonergic system (5HT) in the pathophysiology of ASD, with 5HT neurons in the dorsal raphe (DR) sending projections throughout the forebrain.
Through the use of optogenetic and viral-mediated gene transfer approaches, we found that activation of non-specific, as well as 5HT specific DR neurons and their projection to the NAc increased sociability. Conversely, inhibition of the DR 5HT neurons and their projection to the NAc decreased sociability. Utilizing a mouse line with a conditionally inactive 16p11.2 chromosomal segment, we determined that whole brain deletion and DR specific deletion of 16p11.2 decreased sociability, Furthermore, this deficit was reversed through optogenetic activation of the DR following 16p11.2 deletion, demonstrating the critical nature of DR 5HT activity in social interactions. Future studies will assess how 16p11 deletion in DR 5-HT neurons affects the physiology of these cells and the circuits in which they participate.

Disclosures: J.J. Walsh: None. **G.A. Bendor:** None. **R.C. Malenka:** None.

Poster

030. Genetics of Autism and Autism Models

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Topic: A.07. Developmental Disorders

Support: NIH Grant DA021801

NIH Grant HD036379

Brain and Behavior Foundation

Title: Altered action of 5-HT2a receptor ligands in the 16p11.2 deletion syndrome mouse.

Authors: C. PANZINI[1], *K. G. COMMONS[2];
[1]Boston Children's Hospital/Harvard Med., Boston, MA; [2]Anesthesia, Children's Hosp, Harvard Med., Boston, MA

Abstract: In humans the 16p11.2 deletion syndrome is caused by a loss of a chromosomal segment that encodes about 25 genes. This microdeletion results in variable deficits and features of autism, developmental delay and intellectual disability are common. Mouse models of 16p11.2 deletion syndrome have been developed and these animals have several behavioral abnormalities and are typically hyperactive. Serotonin neurotransmission has long been associated with autism, yet it remains poorly understood if altered serotonin neurotransmission could represent a common deficit in autism generated by distinct genetic or environmental factors. In this study, we studied the function 5-HT2A receptors in 16p11.2 deletion mice (Del) and their wild-type (WT) littermates (Jackson Labs B6129S-Del(7Slx1b-Sept1)4Aam/J; Horev et al., PNAS 2011). To evaluate the status of 5-HT2 receptors we administered the agonist, DOI, and measured head twitch and ear scratch response, both mediated by 5-HT2A receptors. We found that Del mice are relatively insensitive to DOI compared to their WT siblings. Next we examined the effects of the 5-HT2A antagonist M100907. This was done in the context of acute stress (swim), as this stimulus is known to activate the serotonin system. Typically rodents exhibit active coping or escape-directed strategies when initially exposed to the swim, and with time, active coping is gradually replaced by passive coping (floating). This pattern of behavioral adaptation in the swim is evident in WT siblings over a 15 min time-course. However, Del mice are more active initially, and they perseverate with an active coping strategy throughout the test. Immunolocalization of the immediate early gene product Fos showed the relative engagement of the lateral septum and prefrontal cortex and during this task. In WT mice, 0.01 mg/kg M100907 administered prior to the swim test had no significant effect on coping strategy. However dramatically in Del mice 0.01 mg/kg reduced active coping throughout the test, and partially restored the adaptive shift from active to passive strategy, attenuating the behavioral deficit in comparison to their WT siblings. In addition Del mice were hypersensitive to the effects of M100907 on Fos expression compared to WT mice. These effects correlated with regional and cellular distribution of 5-HT2A receptors. Taken together these data reveal alterations in 5-HT2A receptor function in the mouse model of 16p11.2 deletion syndrome.

Disclosures: C. Panzini: None. **K.G. Commons:** None.

Poster

030. Genetics of Autism and Autism Models

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Topic: A.07. Developmental Disorders

Support: NSF GRFP Grant No. 2013162469,

NIH R01MH102603

Title: Brain region-specific contributions of Foxp1 to autism-related phenotypes

Authors: *D. ARAUJO[1], K. TORIUMI[1], C. O. ESCAMILLA[1], M. HARPER[1], A. G. ANDERSON[1], S. BERTO[1], H. O. TUCKER[2], C. POWELL[1], G. KONOPKA[1];
[1]UT Southwestern Med. Ctr., Dallas, TX; [2]UT Austin, Austin, TX

Abstract: Mutations and deletions of the transcription factor *FOXP1* are causative for autism spectrum disorder (ASD) and intellectual disability (ID). Previous work has shown that whole-brain *Foxp1* conditional knockout mice (cKO) display ASD-related phenotypes, and we have identified the brain-region specific transcriptional program of Foxp1 in *Foxp1* heterozygous mice. However, the circuit-specific contribution of Foxp1 to ASD and ID-related phenotypes remains to be determined. In order to answer this question, we crossed *Emx1.Cre*$^{+/-}$ mice with *Foxp1*$^{flox/flox}$ mice to generate *Foxp1* cKO (*Foxp1*^{cKO}) mice with loss of Foxp1 specifically in the pyramidal neurons of the neocortex and hippocampus. Using immunohistochemistry and histology, we find morphological abnormalities in the neocortex and hippocampus of *Foxp1*^{cKO} mice. Correlated with these findings, *Foxp1*^{cKO} mice display alterations in ASD-related behaviors, including deficits in hippocampal-based learning and memory. Future experiments will focus on identifying the molecular and cellular mechanisms underlying these phenotypes.

Disclosures: **D. Araujo:** None. **K. Toriumi:** None. **C.O. Escamilla:** None. **M. Harper:** None. **A.G. Anderson:** None. **S. Berto:** None. **H.O. Tucker:** None. **C. Powell:** None. **G. Konopka:** None.

Poster

030. Genetics of Autism and Autism Models

Location: Halls B-H

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Topic: A.07. Developmental Disorders

Support: King Abdullah II Fund for Development AND King Abdullah II Design and Development Bureau / Scientific Research Department. Research Grant No. [21/2016]

Title: The association between genetic polymorphisms of methionine cycle enzymes and autism in Jordan

Authors: *O. K. HALHOULI[1], M. F. ALKHOUJAH[1], M. ELDAHABI[2], L. ALZGHOUL[2]; [2]Dept. of Physiol. and Biochem., [1]Univ. of Jordan - Fac. of Med., Amman, Jordan

Abstract: Autism spectrum disorder (ASD) is a pervasive developmental disorder characterized by disturbances in social interactions, verbal and non-verbal communication, as well as restricted and repetitive patterns of interest and behavior. Despite the high prevalence of ASD, its etiology and pathophysiology are still largely unknown and heterogeneous. In the last couple of decades, thousands of studies investigating the etiology of ASD have been published and several environmental as well as genetic factors proposed to be associated with ASD when compared to controls. Methionine cycle is an important metabolic cycle in the body, not only because it is the main source for sulfur-containing amino acids, but it also provides the cells with several metabolites that are vital for many cell functions, such as the methylation process and antioxidant activity. Recently, several studies have proposed an association between the pathophysiology of ASD and the alteration in methionine metabolism including increased concentrations of homocysteine (Hcy), decreased DNA methylation, and increased oxidative stress. Genetic studies have demonstrated that genetic polymorphisms in several enzymes functioning in the cycle may alter it. For example, C677T polymorphism of the methylene-tetrahydrofolate reductase (MTHFR) gene coding the enzyme responsible for the conversion of 5,10-methyleneTHF to the 5- methyleneTHF, leads to a 60% decrease in the activity of the MTHFR enzyme and an increase in homocysteine levels, especially in people with low levels of B vitamins. Likewise, genetic polymorphisms of the enzyme methionine synthase reductase (MTRR) and cystathionine b-synthase (CBS) genes, which are also enzymes involved in the methionine cycle may alter the cycle and hence increase the risk of ASD. **Therefore, the goal of this study was to examine the association between the genetic variations in the enzymes of methionine metabolic cycle with ASD.** To do that, the presence of the most common polymorphisms of MTHFR, MTRR, and CBS genes was tested using PCR-RFLP method and compared between 1) a group of Jordanian autistics, 2) their unaffected siblings, and 3) unrelated health controls. Our preliminary data demonstrates a higher frequency of polymorphisms in the

mentioned genes in autistics compared to the unrelated controls. These data indicated that genetic polymorphisms which lead to alteration in the methionine cycle may increase the risk of ASD.

Disclosures: O.K. Halhouli: None. **M.F. Alkhoujah:** None. **M. Eldahabi:** None. **L. Alzghoul:** None.

Poster

030. Genetics of Autism and Autism Models

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

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Topic: A.07. Developmental Disorders

Support: NIH Grant HD055751

Title: The effect of treatment with a partial 5-HT_{1A} receptor agonist on grooming behavior in Shank3 mice

Authors: *J. DUNN, M. E. RAGOZZINO;
Psychology, Univ. of Illinois At Chicago, Chicago, IL

Abstract: Restricted interests and repetitive behaviors (RRBs) are a core diagnostic criteria in autism spectrum disorder (ASD). Phenotypic expression of RRBs encompasses a range of behaviors from motor stereotypy to cognitive rigidity. To date there lacks effective treatments to alleviate various RRBs expressed in ASD. Mouse models of syndromic forms of autism offer an approach to understand the underlying pathophysiology and test potential treatments to alleviate RRBs in ASD. Loss of the SHANK3 gene has been identified as the cause of a monogenic form of autism. Deletion of the SHANK3 gene in mice leads to repetitive behaviors, e.g. self-grooming, that are comparable to that observed in ASD. Therefore, the Shank3$^{tm2Gfng/J}$ (Shank3) mouse can be used to test possible treatments to alleviate RRBs in ASD. A recent study found that a partial 5-HT_{1A} receptor agonist reduces irritability in ASD. It is possible that a similar treatment may also alleviate RRBs in ASD. Therefore, the present study investigated whether the partial 5-HT_{1A} receptor agonist, tandospirone, is effective in reducing self-grooming behavior in male Shank3 mice. Thirty minutes prior a self-grooming test mice received an intraperitoneal injection of sterile water, 1.0 or 3.0 mg/kg tandospirone. Grooming behavior was measured for 10 minutes in an empty plastic cage. As previously reported, Shank3 null mice exhibited elevated grooming behavior. Preliminary results indicate that treatment with tandospirone at the 1 and 3 mg/kg dose reduced grooming behavior in Shank3 null mice. The preliminary results

raise the possibility that treatment with a partial 5-HT_{1A} receptor agonist may be effective in treating RRBs in ASD.

Disclosures: J. Dunn: None. **M.E. Ragozzino:** None.

Poster

030. Genetics of Autism and Autism Models

Location: Halls B-H

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Topic: A.07. Developmental Disorders

Support: NIH Grant 1F05MH097457-01

NIH Grant 4R01MH083728-07

Title: Expression of mutant DISC1 in Purkinje cells increased their spontaneous activity, leading to behavioral phenotypes consistent with aspects of autism spectrum disorders

Authors: *A. V. SHEVELKIN[1,2], B. N. ABAZYAN[2], C. YANG[2], O. A. MYCHKO[2], T. J. KAJSTURA[2], J. C. TRONCOSO[2], D. J. LINDEN[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) are neurons with highest expression of DISC1 in the brain. In order to explore the role of DISC1 in cerebellar physiology and associated ASD-relevant behaviors, we generated a mouse model of inducible and selective expression of dominant-negative form of DISC1 (truncated mutant human DISC1) in PC of anterior lobuli of the cerebellum (II-V and internal side of VI).
We measured the volume of the cerebellum and PCs in mice at postnatal day 21 and assessed the behavioral phenotype in male and female mice of 3-7 months of age using a series of tests relevant to schizophrenia and ASD, including novelty-induced activity, elevated plus maze, Y maze, object and place recognition, fear conditioning and rotarod. Mutant DISC1 male but not female mice demonstrated abnormal social interaction, hyperactivity and deficient novel object recognition, with no group differences in elevated plus maze, spontaneous alteration or spatial recognition in Y maze.
Neither the total number of PC nor the volume of the cerebellum were significantly altered in mutant DISC1 mice. No up-regulation of cellular markers of inflammation was observed in

mutant mice. Whole-cell patch clamp and loose patch recordings in brain slices found larger amplitude and increased frequency of mEPSCs and spontaneous spiking in PCs, but no changes in intrinsic excitability, R_{input} and paired-pulse ratio in mutant DISC1 mice.
Our findings indicate that mutant DISC1 might alter physiology of PC to lead to cognitive and social abnormalities in 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

030. Genetics of Autism and Autism Models

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 30.10/C8

Topic: A.07. Developmental Disorders

Support: NIH Grant P50 MH096972

Department of Cell and Developmental Biology

Title: Changes in thalamocortical projection patterns in a mouse model of autism

Authors: ***J. KRUEGER FISTER**[1], C. D. M. VARGAS[2,3], J. A. MAVITY-HUDSON[8], M. J. ROBSON[3], J. VEENSTRA-VANDERWEELE[10], M. T. WALLACE[4,5,6,9], R. D. BLAKELY[5,3,9,11,12], V. A. CASAGRANDE[8,5,7,6];
[1]Psychology, [2]Interdisciplinary Program in Neurosci., [3]Vanderbilt Conte Ctr., [4]Hearing and Speech Sci., [5]Pharmacol., [6]Vanderbilt Brain Inst., [7]Ophthalmology and Visual Sci., Vanderbilt Univ., Nashville, TN; [8]Cell and Developmental Biol., [9]Psychiatry, Vanderbilt Med. Sch., Nashville, TN; [10]Psychiatry, Columbia Univ., New York, NY; [11]Florida Atlantic Univ. Brain Inst., [12]Col. of Med., Florida Atlantic Univ., Boca Raton, FL

Abstract: Autism Spectrum Disorder (ASD) is a heterogeneous disorder spanning a variety of symptoms involving sensory processing, repetitive behaviors, as well as, deficits in communication and social interactions. The neuromodulator serotonin (5-HT) is known to play an important role in normal brain development and has been implicated in perceptual and behavioral abnormalities in ASD. In fact, 5-HT has been considered a biomarker for ASD in about ~ 25-30% of all cases (Cook, 1990; Veenstra-VanderWeele and Blakely, 2012). Using a mouse model of ASD expressing a human coding variant in the 5-HT transporter (SERT, *SLC6A4*), the present study set out to characterize potential changes in cytoarchitecture and

connectivity patterns of cortical sensory regions that might be linked to alterations in serotonin availability during development. The Gly56Ala substitution mutation at the N-terminus has been shown to produce a hyperactive SERT, clearing away more 5-HT than in the wild type form. With SERT being transiently expressed in thalamocortical axons during embryonic development, our initial analysis examines the anatomical effects of altered 5-HT clearance on the distribution of thalamocortical axons in the adult. Additionally since previous work showed that there is a greater concentration of SERT in projections deriving from unisensory thalamic nuclei than from multisensory nuclei (Lebrand et al., 1996), we expected that differential effects on thalamocortical development might be observable in different regions of cortex depending on their thalamic input source. Primary visual cortex (V1) and multisensory region V2L were subsequently selected for analysis. Utilizing the presence of vesicular glutamate transporter 2 (VGluT2) as a proxy to investigate thalamic projections to these areas, our data reveals a significant difference in the distribution of thalamocortical terminals in the adult knock in (KI) mice. KIs exhibited more broadly distributed VGluT2-positive terminals beyond layer IV in V1 but not in V2L compared to wildtype littermates (129S4/S6). Specifically, thalamic terminals appeared to encroach into supragranular layers suggestive of less precise targeting. Furthermore, since no differences were observed in V2L, alterations in 5-HT availability during development may preferentially impact unisensory regions, potentially leading to unisensory processing deficits. This change may have cascading effects shaping, for example, integration of visual sensory cues with other sensory signals further explaining some of the observable symptoms in ASD.

Disclosures: J. Krueger Fister: None. **C.D.M. Vargas:** None. **J.A. Mavity-Hudson:** None. **M.J. Robson:** None. **J. Veenstra-VanderWeele:** None. **M.T. Wallace:** None. **R.D. Blakely:** None. **V.A. Casagrande:** None.

Poster

030. Genetics of Autism and Autism Models

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 30.11/C9

Topic: A.07. Developmental Disorders

Title: The neurodevelopment of the autistic brain: longitudinal changes of brain connectivity in Fmr1KO and CNTNAP2-KO mice models

Authors: *V. ZERBI[1], M. MARKICEVIC[1], G. D. IELACQUA[2], M. RUDIN[2], N. WENDEROTH[1];

[1]Neural Control of Movement Lab, ETH Zurich, Zurich, Switzerland; [2]Inst. of Pharmacol. and Toxicology, Univ. Zurich, Zurich, Switzerland

Abstract: Motivation. During the postnatal stages the development of functional brain circuits depends critically on the genetic background as well as on neuronal activity driving the formation of new synapses. Alterations of genes regulating migration and synapse formation during development can therefore cause neurodevelopmental disorder like Autism spectrum disorder (ASD). However, how abnormal development at the cell level impacts on functionality at the circuit level is currently unknown. Here we use *Fmr1*KO and *CNTNAP2*-KO mice, two well-established mouse models for ASD, to comprehensively characterize the brain circuits that show delayed or abnormal development in these mice when compared to wild type controls. **Experimental setup.** *Fmr1*KO mice, *CNTNAP2*-KO mice and respective wild type control littermates are longitudinally evaluated during early development (juvenile stage 30 days post-natal, adolescent stage 60 days post-natal, adult stage 120 days post-natal). At each stage, we performed MRI scanning to evaluate structural and functional connectivity changes. The dataset are acquired on a 7T Bruker scanner, equipped with a transmit-receive mouse brain cryogenic coil. Well-established protocols are applied to sedate and monitor mice during the MRI acquisition[1]. Rs-fMRI time series is acquired using gradient echo EPI sequence (2000 repetitions, TR=1s). Thereafter, a multi-shell diffusion-weighted sequence is applied (90 directions, b-values ranging 0-3000). Brain connectivity will be analyzed focusing on specific neural networks. Diffusion-weighted data will be modeled as tensor (DTI) as well as constrained spherical deconvolution (CSD), followed by global tractography[2]. Rs-fMRI will be cleaned from artifacts and analyzed using several established methods[1,3], including independent component analysis (ICA), total correlations and network dynamics analyses. **Expected output and impact.** We will test whether neuroimaging connectivity biomarkers are sufficiently sensitive for detecting differences between the neuro-phenotype of the different mouse models already at young age. This longitudinal experiment will further reveal how circuit development of the autistic brain deviates from normal dynamics depending on the genetic background. This study will provide new insights relevant for developing neuroimaging biomarkers for early diagnosis and monitoring of ASD in human patients. [1] Zerbi, V., et al. NeuroImage 123, 11-21, (2015). [2] Christiaens, D. *et al. NeuroImage* **123**, 89-101, (2015). [3] Nasrallah, F. A., *et al*. *Neuroimage* **86**, 417-424, (2014)

Disclosures: V. Zerbi: None. **M. Markicevic:** None. **G.D. Ielacqua:** None. **M. Rudin:** None. **N. Wenderoth:** None.

Poster

030. Genetics of Autism and Autism Models

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Topic: A.07. Developmental Disorders

Support: NIMH IDDRC 2015

Dup15q Alliance Pilot Award

Title: Spontaneous beta oscillations: an electrophysiological biomarker of Dup15q syndrome

Authors: *S. S. JESTE[1], J. FROHLICH[2], R. SANKAR[3], P. GOLSHANI[4];
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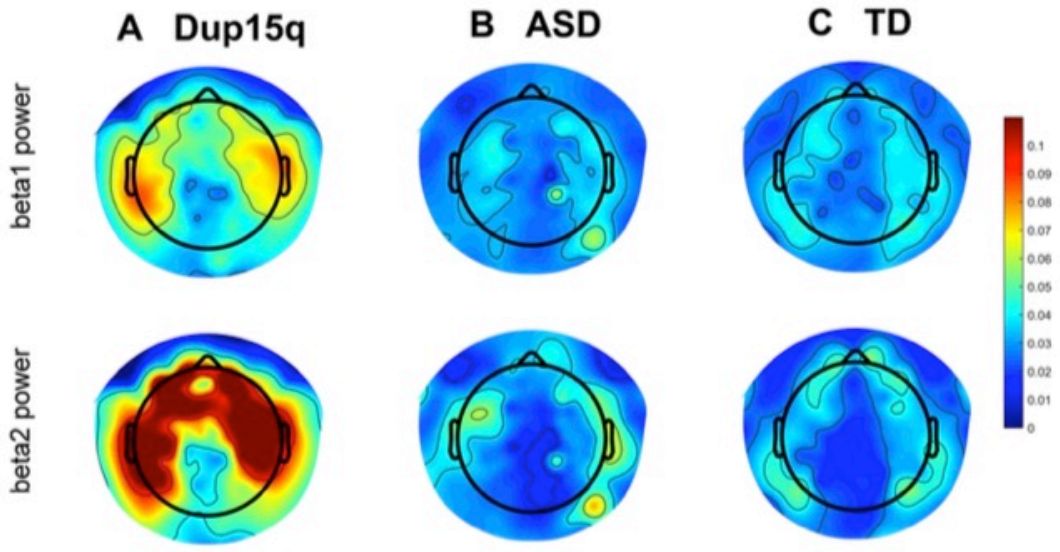
Abstract: Duplications of 15q11.2-q13.1 (Dup15q syndrome) account for 1-3% of autism spectrum disorder (ASD) and also confer a high risk for intellectual disability (ID) and epilepsy. The duplicated region includes three γ-aminobutyric $acid_A$ receptor ($GABA_AR$) subunit genes. Case reports have identified spontaneous beta oscillations (SBOs) in clinical electroencephalogram (EEG) recordings from children with Dup15q syndrome. SBOs resemble beta activity induced by drugs that modulate $GABA_A$, suggesting upregulation of $GABA_AR$ subunit genes.

We measured SBOs from resting-state EEG recordings of children with Dup15q syndrome (n = 11) and compared these recordings to age-matched typically developing (TD) children (n = 9) and age-and-IQ-matched children with nonsyndromic ASD (n = 10). No children in the study were taking benzodiazepine or similar drugs known to induce beta activity. Relative power in delta, theta, alpha, beta1, beta2, and gamma bands was computed for 9 regions of interest and analyzed using repeated measures analysis of variance (ANOVA), with multiple testing corrected for using false discovery rates (FDR). In a larger cohort (n = 27) of children and adults tested across two sites, we examined age, duplication type, and epilepsy status as predictors of beta power using simple linear regressions.

Resting beta1 (12 – 20 Hz) and beta2 (20 – 30 Hz) power was significantly ($p < 0.05$, FDR corrected) stronger in Dup15q syndrome as compared with both control groups. Effect sizes for comparisons of beta2 power as measured with Cohen's d were as follows: d = 1.73, Dup15q – ASD; d = 1.63, Dup15q – TD. Beta2 power was significantly predicted by epilepsy status in Dup15q syndrome ($R^2 = 0.17$, $p < 0.05$).

SBOs are a biomarker of a genetically distinct subgroup of children within the autism spectrum. Quantification of this biomarker could improve diagnosis, prognostication, and measurement of target engagement and outcomes in clinical trials, a model that can inform similar investigations

in the quickly expanding number of high-risk genetic syndromes associated with neurodevelopmental disorders.



Disclosures: **S.S. Jeste:** F. Consulting Fees (e.g., advisory boards); Roche Pharmaceuticals. **J. Frohlich:** None. **R. Sankar:** None. **P. Golshani:** None.

Poster

030. Genetics of Autism and Autism Models

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 30.13/C11

Topic: A.07. Developmental Disorders

Support: NIH Mental Health Grant MH 097236

UC Davis Children's Miracle Network grant

UC Davis MIND Institute

Title: Regulatory small non-coding RNAs in the superior temporal sulcus and primary auditory cortex brain regions of Autism Spectrum Disorders display sexual dimorphism

Authors: *B. STAMOVA[1], B. P. ANDER[2], A. OMANSKA[3], F. R. SHARP[2], C. M. SCHUMANN[3];
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[2]Neurol., [3]Psychiatry and Behavioral Sci., UC Davis Sch. of Med., Sacramento, CA

Abstract: Autism spectrum disorders (ASD) are neurodevelopmental disorders which display social interaction deficits and repetitive behaviors. ASD is sexually dimorphic and more prevalent in boys than girls. Sexually dimorphism in ASD neuropathology has been reported. Thus we investigated whether there is molecular sexual dimorphism in the ASD brain transcriptome. We focused on small non-coding RNAs (sncRNA, including miRNA), which are a major class of regulatory RNAs. We investigated two brain regions: the superior temporal sulcus (STS) and primary auditory cortex (PAC). STS is an association cortex involved in social perception, joint attention, face perception and speech perception and is implicated in ASD. PAC is a primary sensory cortex modulating auditory processing.
We aimed to identify:1) sexually dimorphic and regionally dysregulated sncRNA expression in STS and PAC in postmortem male and female human brains of ASD compared to TD controls; and 2) potentially affected pathways based on predicted sexually-dimorphic dysregulated miRNA targets.
Affymetrix miRNA 3.0 arrays were run on 34 samples (5 ASD Female, 5 ASD Male, 2 TD Female, 6 TD Male; two brain regions – STS, PAC; 4-58 years of age; two of the 18 subjects had only one brain region available). Mixed Regression Model was used to identify sexually dimorphic differentially expressed sncRNA ($p<0.005$, $|\text{fold-change}|>1.2$).
There were 27 and 9 sexually dimorphic sncRNA in ASD STS and PAC, respectively, and 85 sexually dimorphic sncRNA regionally dysregulated in ASD between the two brain regions. Based on the predicted targets of the dysregulated miRNAs, there were many more immune pathways over-represented (Benjamini-Hochberg corrected $p<0.05$) in female (70) than in male (14) in the regional STS vs PAC comparison, with 12 immune pathways in common between male and female. A number of pathways with targets of sexually dimorphic miRNAs have been implicated in ASD, such as axonal guidance, PI3K/Akt Signaling. Moreover, in the common pathways in male and females, there were a number of different mRNA predicted targets. Sexually dimorphic sncRNA expression in ASD brains may contribute to ASD pathophysiology. Even though common pathways may be affected in male and female, different branches of these pathways may be aberrant in the two sexes, which highlights
the necessity for sex-specific treatment and prevention. This study also highlights the importance of the immune system in ASD, and its sexually dimorphic nature. Future studies will need to confirm these findings.

Disclosures: **B. Stamova:** None. **B.P. Ander:** None. **A. Omanska:** None. **F.R. Sharp:** None. **C.M. Schumann:** None.

Poster

030. Genetics of Autism and Autism Models

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NIMH Grant 1R21MH100868

Eunice Kennedy Shriver National Institute of Child Health and Human Development Grant 1R21HD079249

Nancy Lurie Marks Family Foundation

Autism Speaks/National Alliance for Autism Research

Title: Ube3a and seizures impair sociability by down-regulating autism network gene Cbln1 in VTA

Authors: *D. C. STOPPEL, V. KRISHNAN, Y. NONG, M. A. JOHNSON, E. OZKAYNAK, I. NAGAKURA, M. SILVA, M. J. S. NADLER, S. PETERSON, E. M. KASPER, F. MOHAMMAD, R. ARNAOUT, M. P. ANDERSON;
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Abstract: Increased gene copies of *UBE3A* underlie the autism-related behavioral traits observed in individuals with maternally inherited 15q11-13 triplications (idic15). Here we demonstrate that restricting excess UBE3A to the nuclear compartment impairs sociability by repressing the expression of Cbln1, a key node in an autism gene network of interacting proteins that transsynaptically bridges NRXN and GRID family members that are frequently deleted in autism. *Cbln1* mRNA is negatively regulated by neuronal activity and we show that chemically induced epileptic seizures synergize with *UBE3A* dosage to impair sociability through an Ube3a-dependent mechanism that involves glutamatergic neurons located in the ventral tegmental area (VTA). Deleting Cbln1 in VTA glutamate neurons disrupts social behavior by weakening glutamatergic synaptic transmission. Finally using chemogenetics, we demonstrate that normal glutamatergic synaptic transmission emanating from VTA is necessary and sufficient for intact social behavior.

Disclosures: D.C. Stoppel: None. **V. Krishnan:** None. **Y. Nong:** None. **M.A. Johnson:** None. **E. Ozkaynak:** None. **I. Nagakura:** None. **M. Silva:** None. **M.J.S. Nadler:** None. **S. Peterson:** None. **E.M. Kasper:** None. **F. Mohammad:** None. **R. Arnaout:** None. **M.P. Anderson:** None.

Poster

030. Genetics of Autism and Autism Models

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Topic: A.07. Developmental Disorders

Support: NARSAD

BBRF

NSERC

Brain Canada

OBI

Title: OTUD7A is a novel candidate driver gene of neurodevelopmental abnormalities in 15q13.3 microdeletion syndrome

Authors: *B. K. UNDA[1], M. UDDIN[2], S. WHITE[1], N. HOLZAPFEL[1], V. KWAN[1], N. MURTAZA[1], A. FORSINGDAL[3], J. NIELSEN[3], K. HOPE[1], S. W. SCHERER[2], K. SINGH, L8S 4L8[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 is associated with high risk for epilepsy, intellectual disability, schizophrenia and autism spectrum disorder (ASD), and most reported cases are heterozygous for this deletion. However, the neurodevelopmental abnormalities underlying the clinical phenotypes and the gene(s) driving these phenotypes remain unknown. To study this CNV, we are utilizing a heterozygous 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. RNA-sequencing and gene set enrichment analysis (GSEA) of cortical brain tissue from WT and heterozygous mice revealed that differentially expressed genes are highly enriched in forebrain development. We next analyzed postnatal neuronal morphology, which revealed alterations in dendritic arborization and dendritic spine morphology in excitatory cortical pyramidal neurons. Additionally, preliminary biochemical experiments revealed a decrease in post-synaptic density fraction proteins with a concomitant increase in presynaptic fraction proteins in heterozygous mice, further suggesting that synaptic connectivity may be altered in 15q13.3 heterozygous mice. To understand the pathophysiology of 15q13.3 microdeletion

syndrome, we dissected candidate driver genes using whole-genome sequencing (WGS) and brain-critical exon analysis of human transcriptome data. WGS of ASD quartet families identified 4 *De Novo* variants in one of the genes within the deletion, OTUD7A. OTUD7A is also the only gene within the deletion containing a brain-critical exon that is highly enriched in the brain, suggesting a novel role for this gene in brain function. GSEA of human protein expression data revealed that genes that are highly co-expressed with OTUD7A are highly enriched in pathways involved in synaptic connectivity. Additionally, preliminary biochemical experiments showed that OTUD7A is localized to the post-synaptic density of neurons in the mouse brain, further supporting a role for OTUD7A in synaptic connectivity. Future work will focus on further elucidating the role of OTUD7A and associated pathways in brain development and determining whether loss of OTUD7A can account for the observed synaptic defects in the mouse model.

Disclosures: B.K. Unda: None. **M. Uddin:** None. **S. White:** None. **N. Holzapfel:** None. **V. Kwan:** None. **N. Murtaza:** None. **A. Forsingdal:** None. **J. Nielsen:** None. **K. Hope:** None. **S.W. Scherer:** None. **K. Singh:** None.

Poster

030. Genetics of Autism and Autism Models

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 30.16/C14

Topic: A.07. Developmental Disorders

Support: CIHR

Ontario Brain Institute

Title: Loss of lateral asymmetry in the brain of mouse models of autism

Authors: *J. ELLEGOOD[1], B. C. DARWIN[1], M. C. VAN EEDE[1], R. M. HENKELMAN[1,2], J. P. LERCH[1,2];
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Abstract: Background - Lateral asymmetry in the brain is common in most biological systems. Differences in that asymmetry can provide insight into functional and/or behavioural variations in the population. Studies have shown asymmetry differences in autism compared to typically developing controls. Previous work on the mouse highlights several regions of asymmetry in the mouse brain (Spring et al. 2010), including the striatum, hippocampus, and areas within the

thalamus and cortex. **Objectives** - Using 26 different mouse models related to autism as a representative sample of an autistic population, we hypothesized asymmetry differences compared to wild-type (WT) would be found in the autistic population that would be indicative of functional and/or behavioural variations. **Methods** - In total 593 mouse brains were examined from 26 different mouselines related to autism (Ellegood et al. 2015). Imaging was performed using pre-established methods (Lerch et al. 2011). Acquisition images were flipped/mirrored along the midline axis and registered, first to the original image and then all together. Comparisons were made between the flipped and original image to assess asymmetry and across mouselines. Baseline asymmetry was established using 76 C57Bl6/J mice. Areas of significant voxelwise asymmetry were used to form regional asymmetry networks. 11 different areas were identified, and mean effect size differences in asymmetry were calculated in those 11 areas for the WT and mutant in each of the 26 models. Significant differences between the WT and mutant group were calculated using a linear model and multiple comparisons were controlled for using False Discovery Rate (FDR). **Results and Discussion** - Of the 11 regional networks/areas that were examined we found a loss in lateral asymmetry in 5 areas. The strongest losses were found in areas in the hypothalamus and thalamus (p=0.01, FDR=11%) as well as a corpus callosum to motor cortex network (p=0.01, FDR=11%). Additional losses of asymmetry were found in a memory network involving the striatum, hippocampus and amygdala (p=0.03, FDR=13%), and a brainstem network (p=0.03, FDR=13%). The models with the strongest asymmetry loss in those 5 areas were *Cntnap2*, *Nl3*, *En2*, XO and BTBR in that order. The loss of asymmetry in the corpus callosum to motor cortex network could be related to functional deficits as several lines have motor abnormalities (e.g. *En2*, *Gtf2i, Mecp2* etc.). Similarly the loss of asymmetry in the memory network may be related to cognitive deficits in several lines (e.g. *Shank3*, 15q11-13, *Cntnap2* etc.). Overall, we found specific areas where normal lateral asymmetry was lost in the brain of autism related mouse models.

Disclosures: J. Ellegood: None. **B.C. Darwin:** None. **M.C. van Eede:** None. **R.M. Henkelman:** None. **J.P. Lerch:** None.

Poster

030. Genetics of Autism and Autism Models

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Program#/Poster#: 30.17/C15

Topic: A.10. Development and Evolution

Support: NIH R01 MH102416

Title: Understanding the neuronal substrates of neurodevelopmental disorders

Authors: E. ZUCCARO[1,2], *S. LODATO[1,2], A. BYRNES[2], M. STOIBER[3], H.-H. CHEN[1,2], M. ZILLER[4], J. L. RINN[1], B. NEALE[2], P. ARLOTTA[1,2];
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Abstract: Genome-wide association studies (GWAS) have identified many genomic loci associated with the risk of schizophrenia and autism, and additional sequencing efforts are in progress for bipolar disorder and ADHD. The field has a need for strategies to mine this wealth of genetic information to understand disease etiology and progression and to inform treatment strategies. One major obstacle is that the cellular and anatomical substrates of neurodevelopmental disorders are largely not known. We propose that new knowledge of the affected neurons and circuits can be gained by determining whether genomic loci mutated in patients are enriched for genes and pathways characteristic of specific neuronal populations. This requires a more comprehensive understanding of neuronal subtype-specific molecular profiles than currently available. To this end, we are building a critical resource of gene expression data from multiple subtypes of projection neurons isolated from the murine neocortex and, in parallel, from human cortex at both fetal and adult stages.
The approach we used to label neurons does not require genetic labeling and is thus particularly valuable for large-scale transcriptomic studies in human samples, as it allows for the simultaneous isolation of multiple neuronal classes from the same tissue sample. As the number of neuronal classes profiled grows, this type of resource will provide a platform to interface GWAS data with neuronal class-specific expression profiles to facilitate the identification of the classes of neurons most likely affected and to clarify the developmental timing of disease susceptibility.

Disclosures: E. Zuccaro: None. **S. Lodato:** None. **A. Byrnes:** None. **M. Stoiber:** None. **H. Chen:** None. **M. Ziller:** None. **J.L. Rinn:** None. **B. Neale:** None. **P. Arlotta:** None.

Poster

031. Autism Pathogenesis

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

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Topic: A.07. Developmental Disorders

Support: NSF IOS 1353044

NSF Grant DGE 0228243

NIH Grant 2R25GM083270

Title: Matrix metalloproteinase 9 overexpression recapitulates neurophysiological changes induced by early exposure to valproic acid in a Xenopus tadpole model for neurodevelopmental disorders

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

Abstract: Prenatal exposure to valproic acid (VPA), a commonly prescribed antiepileptic medication, is known to greatly increase risk for autism and other neurodevelopmental disorders (NDDs). Prior experiments in our lab had shown that early developmental exposure of Xenopus tadpoles to VPA resulted in behavioral and electrophysiological abnormalities consistent with various NDDs. The etiology of autism, and NDDs, is hypothesized 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 of tadpoles to VPA, caused significantly altered expression MMP9 mRNA in the CNS. Knocking out MMP9 expression, in a Fragile X mouse model, has been shown to rescue the Fragile X phenotype. Here we find that pharmacological inhibition of MMP9 activity recues neurophysiological and behavioral abnormalities induced by early developmental exposure to VPA. Although these findings highlight the potential role of MMP9 in neurodevelopmental disorders it remains unknown if dysregulation of MMP9 is sufficient to cause the abnormalities observed with VPA exposure. Thus, we overexpressed MMP9 in the optic tectum of developing tadpoles and conducted electrophysiological recordings from tectal neurons. We found that MMP9 overexpression caused increased synaptic transmission, elevated network connectivity and excitability, and reduced intrinsic cell excitability. These results recapitulate the observed effects of VPA on the developing neural circuit and suggest a shared mechanism by which genetic, epigenetic, and environmental risk factors may induce neurodevelopmental disorders.

Disclosures: E.J. James: None. **J. Park:** None. **C.D. Aizenman:** None.

Poster

031. Autism Pathogenesis

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 31.02/C17

Topic: A.07. Developmental Disorders

Title: Reduced mGluR5 binding activity and mRNA expression following astrocytes activation

Authors: *T. LEE[1,2], K. J. GREGORY[4], M. DOTTORI[2], C. PANTELIS[3,1,2], A. CHRISTOPOULOS[4], I. P. EVERALL[1], E. SKAFIDAS[2,1], G. CHANA[2,1];
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Abstract: Alterations in excitatory glutamatergic signalling together with increased astrocytic activation and neuroinflammation have been observed in the brain of individuals with autism spectrum disorder (ASD). Within the ASD brain, the metabotropic glutamate receptor 5 (mGluR5) has been found to be downregulated in expression in the DLPFC, with mGluR5 modulators having the potential for clinical benefit for syndromic ASD. At the glutamatergic tripartite synapse, astrocytes play an important role in regulating the pre- and post-synaptic glutamate receptors as well as intercellular glutamate levels. Within the ASD brain elevated levels of excitatory amino acid transporters (EAAT1 and EAAT2) and glia fibrillary associated protein (GFAP) have also been found. For this investigation we utilised human primary foetal and adult human astrocytes to investigate astrocytic glutamatergic signalling in response to a neuroinflammatory insult. Primary human foetal and adult astrocytes were exposed to Polyinosinic:polycytidylic acid (Poly I:C) [100ng/ml] for 24 hours and 1 week to induce acute and chronic activation states. Following on we assessed levels of pro-inflammatory cytokines using enzyme-linked immunosorbent assay (ELISA), mRNA expression of glutamatergic and astrocytic markers using qPCR, and mGluR5 binding activity via [3H] methoxyPEPy radioligand binding assay. Astrocytes exposed to poly I:C showed elevated levels of pro-inflammatory cytokines IL-6 and Rantes versus controls. We also observed a reduction of mGluR5 binding activity in cells exposed to Poly I:C, together with a reduction in mRNA expression of GRM5 (p<0.05), and the main CNS glutamate transporter EAAT2 (p<.001), as well as increased expression of mGluR5 downstream element SHANK3 (p<.001). Finally, we observed a reduction in mRNA expression of the astrocyte cytoskeletal markers ALDH1L1 (p<.005) and GFAP (p<.005). Our findings demonstrate that during astrocytic activation mGluR5 signalling is reduced and is accompanied by a reduction in EAAT2 expression that is suggestive of a reduced capability to clear extracellular glutamate. These findings point to reduced mGluR5 signalling in the ASD potentially associated with abnormal astrocyte functioning and an increased vulnerability to glutamate mediated excitotoxicity, however further post-mortem work is required to confirm this. Our findings of reduced GFAP are to an extent counterintuitive following Poly I:C exposure but may not be reflected at the protein level. Conversely, reduced GFAP and ALDH1 may represent a decrease in structural astrocytic proteins that are indicative of a reduced capacity to carry out normal functioning.

Disclosures: T. Lee: None. **K.J. Gregory:** None. **M. Dottori:** None. **C. Pantelis:** None. **A. Christopoulos:** None. **I.P. Everall:** None. **E. Skafidas:** None. **G. Chana:** None.

Poster

031. Autism Pathogenesis

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Topic: A.07. Developmental Disorders

Support: KAKENHI 26350498

Health Labour Sciences Research Grant

Title: Alteration of Purkinje cells by autism-inducing drugs, and recovery effects with bumetanide or oxytocin administration in developing rat cerebellum

Authors: S. NAKAJIMA[1], T. TOMIDA[1], K. IKAI[1], *Y. FUETA[2], S. UENO[2], N. HOZUMI[1], Y. SEKINO[3], S. YOSHIDA[1];
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Abstract: Autism, a severe neurodevelopmental disorder, becomes increased in young age. In human patients, cerebral and cerebellar developmental abnormalities are reported. Especially, reduction in size and number of Purkinje cells in cerebellum is revealed in both the postmortem human studies and some drug-administrated adult animals. Several chemicals are known to play some roles in onset of autism . Valproate (VPA) is one of the candidates of inducer of autism. Chlorpyrifos (CPF), an organophosphorus agent, is also known as an autistic inducer likewise VPA. In this study, we investigated cerebellar cytological and behavior changes in development with some drug administrations. VPA is known as a HDAC inhibitor. The effects of other HDAC inhibitors, suberoylanilide hydroxamic acid (SAHA), and MS-275, were also investigated. Additionally, CPF or tributyltin (TBT) were tested. Each drug was administrated to embryonic day 16 p.o. (VPA; 600mg/kg, MS-275; 4mg/kg, CPF; 4.3mg/kg, and TBT; 20mg/kg of mother weight, respectively) or i.p. (suberoylanilide hydroxamic acid, SAHA; 50mg/kg of mother weight).
In cerebellar development, the soma of Purkinje cells form a single layer and elongate their dendrites with synapses during the first two weeks. In VPA-administrated rat, we have observed the elongation of Purkinje cell dendrites started earlier and reached all over the molecular layer even in P12. It was observed also in SAHA, or CPF administrated rat, while in MS-275, or TBT administrated rats, it was not. The behavior of VPA- or CPF-administrated animals was observed as same as control animals in the first week, however, VPA-administrated became differentiated from others in the second week. MS-275, or TBT-administrated animals showed unique behavioral development.
After the facilitation of Purkinje cell development in these drugs in the first week, excess gyrus in cerebellar lobules, especially in lobe V to VII, was formed in the second week. During the

developmental progress, this excess gyrus was eliminated with some Purkinje cells.
Recently, some recovery effects from autism with bumetanide, or oxytocin administration have been reported. We investigated the neuronal effects of these drugs to the VPA-induced autistic model animals. We suggest that drug-induced autistic model rat would become useful to evaluate the recovery effect of a drug.

Disclosures: **S. Nakajima:** None. **T. Tomida:** None. **K. Ikai:** None. **Y. Fueta:** None. **S. Ueno:** None. **N. Hozumi:** None. **Y. Sekino:** None. **S. Yoshida:** None.

Poster

031. Autism Pathogenesis

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Program#/Poster#: 31.04/C19

Topic: A.07. Developmental Disorders

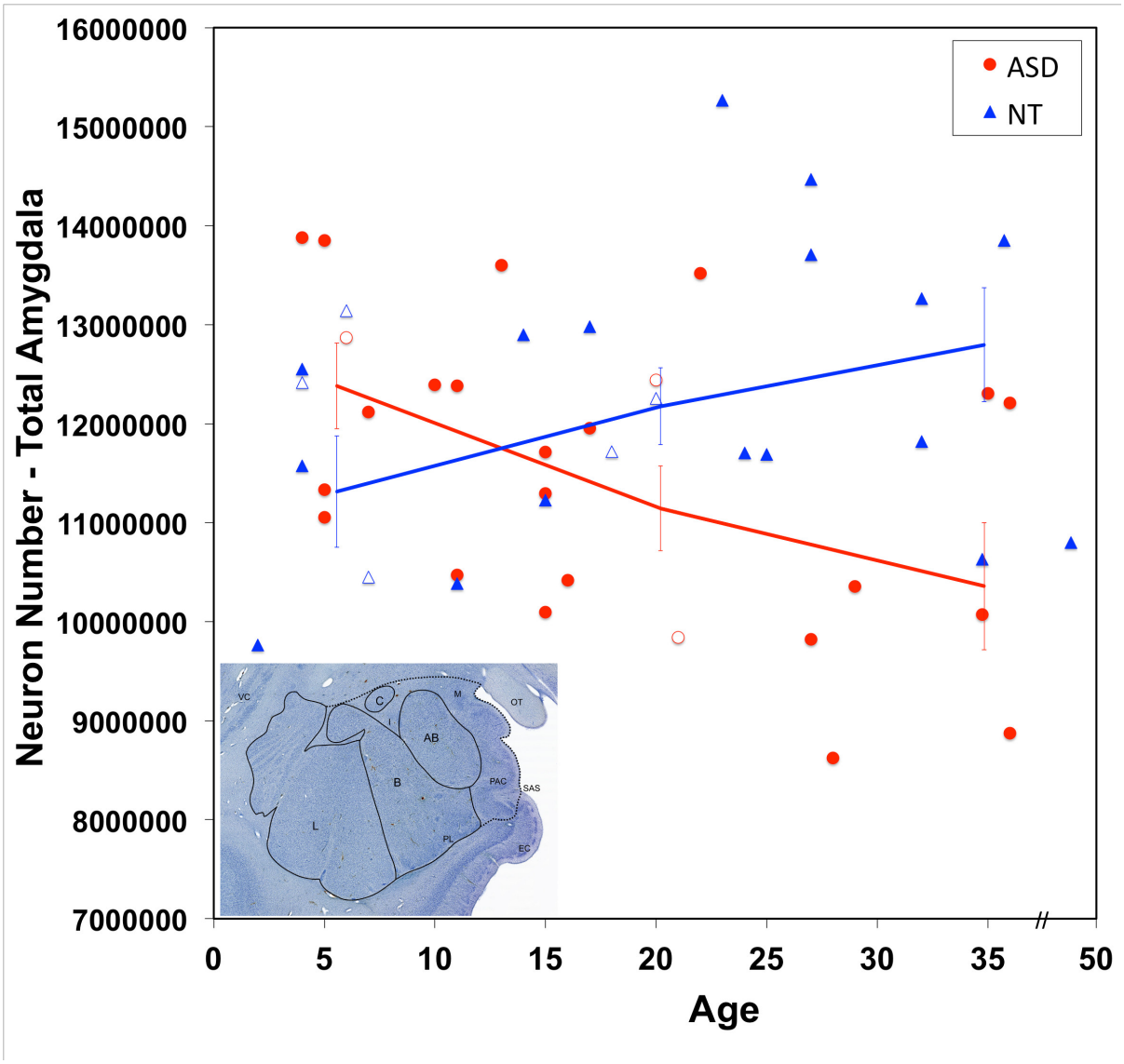
Support: R01MH097236-01

Title: Lifelong trajectory of human amygdala neuronal development is disrupted in autism

Authors: *T. A. AVINO, N. BARGER, M. VARGAS, M. D. BAUMAN, D. G. AMARAL, C. M. SCHUMANN;
Psychiatry & Behavioral Sci., UC Davis MIND Inst., Sacramento, CA

Abstract: The amygdala is a complex cluster of nuclei located in the rostral portion of the temporal lobe. It plays a prominent role in modulating socioemotional processing, yet little is known about the postnatal neuronal development of this structure in humans. Understanding these developmental processes is critical for untangling a number of neuropsychiatric disorders that have been associated with amygdala dysfunction such as anxiety disorder, depression, and autism spectrum disorder (ASD). Structural and functional imaging studies have demonstrated an altered age-related growth trajectory and activation of the amygdala across the lifespan in individuals with autism. The cellular substrates of these lifelong alterations are not well understood. We performed a stereological analysis of neuron number and size in subregions of the amygdala utilizing postmortem tissue from 50 human brains (28 ASD, 22 Typical) representing the largest study of its kind to date. In typical development, the number of neurons in the amygdala increases with age from childhood through adulthood by 23% in the basal nucleus and 15% in the accessory basal nucleus. In contrast, individuals with autism on average show an initial 15% increase in neuron number during childhood in the basal, accessory basal, and central nuclei relative to age-matched typically-developed brains. This initial increase is followed by a pronounced reduction in neuron number in autism with increasing age in all

amygdala subregions, including lateral, basal, accessory basal, and central nuclei, such that by adulthood there is, on average, a 17% decrease relative to the typical adult brain. These findings provide the first evidence of the normal pattern of lifelong neuronal development and maturation in the postnatal human amygdala and demonstrate that these typical processes are disrupted in autism, resulting in an age-related loss of neurons across amygdala nuclei in adults with ASD.



Disclosures: **T.A. Avino:** None. **N. Barger:** None. **M. Vargas:** None. **M.D. Bauman:** None. **D.G. Amaral:** None. **C.M. Schumann:** None.

Poster

031. Autism Pathogenesis

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Program#/Poster#: 31.05/C20

Topic: A.07. Developmental Disorders

Title: Abnormal phosphorylation of AMPA receptor subunit GluR1 in the basolateral amygdala of the valproic acid-exposed rat model of autism following extinction learning

Authors: *S. H.-M. CAVALIER[1], K. GRIFFIN[2], A. ALVAREZ-DIEPPA[2], C. MCINTYRE[2]; [1]Univ. of Texas At Dallas, Dallas, TX; [2]Univ. of Texas at Dallas, Dallas, TX

Abstract: This work compares the expression of synaptic plasticity-associated protein GluR1, a subunit of the AMPA receptor, in the basolateral complex of the amygdala (BLA) in the valproic acid (VPA) rat model of autism spectrum disorder vs. saline-treated controls, after fear conditioning and extinction. Fear extinction is a process in which a conditioned fear response is suppressed by the formation of a new memory that competitively opposes the fear-association. The BLA plays a vital role in extinction of conditioned fear response and is heavily modulated by reciprocal connections with the infralimbic cortex (IL). Because extinction is a form of new learning, it involves both memory acquisition and consolidation and is therefore dependent on the cellular and molecular processes that mediate synaptic plasticity. Extinction of conditioned fear is impaired in rats prenatally exposed to VPA. Fear extinction learning in neurotypical animals promotes increased GluR1 synaptic expression in the IL and extinction learning related to cocaine withdrawal promotes increased synaptic GluR1 in the IL and decreased synaptic GluR1 in the BLA; however, little is known about the molecular and synaptic state of the BLA in VPA exposed rats, and how potential synaptic dysfunction in this region might contribute to extinction impairment. To examine the effect of prenatal exposure to VPA on the expression of synaptic GluR1 in the BLA, 500 mg/kg VPA or saline were injected (i.p.) to pregnant dams on embryonic day 12.5. Two month-old male offspring were subjected to auditory fear-conditioning followed 24 hours later by extinction training. VPA exposed rats showed extinction deficits when tested on day three ($F(1,14)=12.89$; $p=0.003$). Animals were sacrificed 45 minutes following retention testing. Tissue punches were taken from the BLA for western blot analysis of phosphorylated GluR1 at the Ser831 and Ser845 sites. No difference in phosphorylated levels of GluR1 at Ser831 was seen. Expression levels of phosphorylated GluR1 at the Ser845 site were significantly increased in the BLA of VPA exposed rats compared to saline controls ($t(15)=2.13$; $p=0.02$). Phosphorylation of Ser845 is associated with increased GluR1 subunit synaptic trafficking, which could alter the ion selectivity of resultant AMPARs and affect plasticity. These results indicate that the VPA animal model of ASD presents normal acquisition but impaired extinction memory consolidation, and suggest that this impairment is associated with abnormal AMPA receptor subunit trafficking in the BLA.

Disclosures: **S.H. Cavalier:** None. **K. Griffin:** None. **A. Alvarez-Dieppa:** None. **C. McIntyre:** None.

Poster

031. Autism Pathogenesis

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 31.06/C21

Topic: A.07. Developmental Disorders

Support: NIH FX Center grant 1U54HD082013

Title: Dysregulated protein synthesis in autism and fragile X syndrome patient iPSC-derived neural progenitor cells

Authors: ***N. RAJ**, G. J. BASSELL;
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Abstract: Autism spectrum disorders (ASDs) are complex neurodevelopmental disorders characterized by stereotyped behaviors, and impairments in social interaction and communication. While a majority of autism is idiopathic, several genetic and environmental factors have been shown to be involved in its diverse etiology and symptoms, posing a serious challenge to the development of therapeutic strategies. Many symptoms of idiopathic autism are also seen in other neurodevelopmental disorders, suggesting that these symptoms may converge upon a unifying molecular nexus – the phosphoinositide-3 kinase (PI3K) pathway. PI3K is a key regulator of signaling and protein synthesis and is implicated in several monogenic forms of autism including fragile X syndrome (FXS), the most common known genetic cause of autism. We have recently shown that a prefrontal cortex-selective genetic knockdown of the PI3K catalytic subunit, p110β, rescues specific cognitive deficits in a FXS mouse model. We have also found that a p110β subunit selective inhibitor ameliorates the increased protein synthesis rate in synaptoneurosomes from the *Fmr1* knockout mouse model and human FXS patient cells. Dysregulated protein synthesis has been shown to be a key feature of several neurodevelopmental disorders; however our knowledge of the molecular pathogenesis in human neurons has, until recently, been largely limited to studies conducted in post-mortem tissue from patients. In this study, we extend our investigation of the therapeutic value of targeting overactive PI3K signaling in fragile X syndrome and autism to a more translationally relevant model, using human patient induced pluripotent stem cells (iPSCs). Using both BONCAT and a flow cytometry-based assay in multiple control and patient iPSC-derived neural progenitor cells (NPCs) to measure novel protein synthesis, we have found that patient-derived NPCs exhibit increased global protein synthesis compared to control NPCs. Furthermore, treatment with a

p110β inhibitor reduced the exaggerated protein synthesis rate in FXS patient-derived NPCs. Thus, this work suggests that patient iPSC-derived neural progenitor cells recapitulate molecular phenotypes seen in the mouse model, and can be used as valid model for critical pre-clinical testing and comparison of therapeutic strategies.

Disclosures: **N. Raj:** None. **G.J. Bassell:** None.

Poster

031. Autism Pathogenesis

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Topic: A.07. Developmental Disorders

Support: Recruitment Program of High-end Foreign Experts of the State Administration of Foreign Experts Affairs (GDT20144400031)

Title: Neurobiological basis of Oxytocin on pro-social behaviors

Authors: ***G.-Y. WU**[1,2], Y.-J. HO[1], Z.-Y. HU[1], Q.-W. HUO[1], G. CHEN[2];
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Abstract: The neuropeptide, oxytocin has attracted tremendous attention as potential therapeutic treatment for various social deficits in recent years. However, the neurobiological basis of oxytocin on pro-social behaviors is still poorly understood. Here we report an activity-dependent regulation of oxytocin receptor (OXTR) stability and trafficking in cultured neurons. When neuronal activity is increased, the steady-state level of OXTR is rapidly increased by up-regulation of protein synthesis and down-regulation of ubiquitin-proteasome (UPS)-dependent degradation. Conversely, when neuronal activity is decreased, the steady-state level of OXTR is decreased by up-regulation of receptor internalization and UPS-dependent degradation. Furthermore, we have established a stable multi-site *in vivo* recording system of single-unit spikes from different brain regions simultaneously, permitting better exploring relevant neuronal circuitry underlying oxytocin's effect on pro-social behavior. In a preliminary study, we found that intravenous application of oxytocin alters neuronal firing pattern from continuous into burst activities in several brain regions implicated in social circuitry. We are currently exploring how this is relevant to social behaviors, and determining the molecular mechanisms underlying OXTR trafficking and degradation.

Disclosures: **G. Wu:** None. **Y. Ho:** None. **Z. Hu:** None. **Q. Huo:** None. **G. Chen:** None.

Poster

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Topic: A.07. Developmental Disorders

Support: NSERC

CIHR

CIHR- Vanier CGS

CIHR- Neuroinflammation training program

Title: Maternal immune activation disrupts synaptic pruning in the mouse offspring brain

Authors: *L. FERNÁNDEZ DE COSSÍO GÓMEZ, A. GUZMAN, G. N. LUHESHI;
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Abstract: Background: Environmental challenges to the maternal immune system during pregnancy increase the likelihood of neurodevelopmental disorders, such as Autism, appearing in the progeny. While previous studies show that the mother's inflammatory response is implicated, how inflammation during pregnancy can disturb brain development remains unknown. Microglia, the brain's resident immune-cells, have been recently shown to be critically involved in normal brain development, shaping connections between neurons by pruning superfluous synaptic spines. Objective: To show that maternal immune activation (MIA) alters microglia's spine pruning function and lead to behavioral disturbances. Methods: Using our established mouse model of MIA induced by bacterial Lipopolysaccharide (LPS), we quantified spines and assessed alterations in some molecular signals involved in pruning, within the offspring's brain. Additionally we assayed the offspring's behaviour during development. Results: Prenatal LPS resulted in a significant increase in the number of spines in dentate gyrus neurons. In addition, we show reduced hippocampal expression of fractalkine and its microglial receptor (CX3CR1), which are involved in mediating the pruning process. In both cases these changes were only noted in the male offspring of LPS challenged dams. Interestingly, C3, a compliment protein involved in tagging spines for microglia pruning, was less expressed in male pups independent of treatment. We further found an LPS effect in the offspring's duration of ultrasonic vocalizations. Conclusions: Our results provide an early indicator that microglial function is altered in the brain of maternally challenged progeny and that the effects in the brain appear to be specific along sex lines. In addition, we find early-onset alterations in communication in the offspring of infected mothers, a hallmark of disorders like Autism.

Disclosures: **L. Fernández De Cossío Gómez:** None. **A. Guzman:** None. **G.N. Luheshi:** None.

Poster

031. Autism Pathogenesis

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Topic: A.07. Developmental Disorders

Support: NIH Grant MH100717-01

Title: Zinc and Shank regulation of AMPAR during neuronal development

Authors: ***H. T. HA**[1,2], S. A. KIM[2], C. C. GARNER[3], J. R. HUGUENARD[2];
[1]Neurosci., [2]Neurol. and Neurolog. Sci., Stanford Univ., Palo Alto, CA; [3]German Ctr. for Neurodegenerative Dis., Berlin, Germany

Abstract: Autism Spectrum Disorders (ASDs) affects 1 out of 68 children in the US. Despite the growing number of diagnosed children worldwide, the underlying biological mechanisms are unknown, and there are no effective medications. Mutations in the **Shank family of proteins** are a highly prevalent and penetrant genetic risk factor for ASDs given their role as essential scaffolding molecules at synapses. While the genetic heritability of autism is high, environmental factors (such as prenatal **zinc deficiency**) might promote the onset and manifestation of ASDs. Considering the role of the zinc ion as a signaling molecule in the brain and its ability to bind and activate Shank3, it could offer a potential mechanism for gene/environment interaction. Identifying specific downstream targets of zinc deficiency and *Shank* mutations will facilitate the development of new treatments for ASDs. One such potential molecule implicated in previous Shank and zinc studies is **AMPAR** (the major group of glutamate receptors in the nervous system). *Here, we ask how zinc, Shank and their interaction regulate AMPAR* by using a combination of electrophysiology and imaging approaches in dissociated neuronal culture. We found that AMPAR-mediated synaptic current is strengthened by zinc addition during early synaptic development, particularly relevant since ASDs manifest at a young age when the brain is highly vulnerable to genetic mutations or environmental insults. The effect of zinc on AMPAR activity is subunit-specific, suggesting the involvement of this signaling pathway in particular phases of plasticity. Interestingly, Shank2 and Shank3 display different synaptic expression pattern over neuron development, which hints at their potential contribution to the developmental dependence of AMPAR's zinc sensitivity. This work demonstrates that zinc regulates the function of AMPAR early in development potentially through its interaction with Shank3. It also

identifies modulation of AMPAR function as a potential therapeutic strategy for autism patients with history of prenatal zinc deficiency.

Disclosures: **H.T. Ha:** None. **S.A. Kim:** None. **C.C. Garner:** None. **J.R. Huguenard:** None.

Poster

031. Autism Pathogenesis

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Program#/Poster#: 31.10/C25

Topic: A.07. Developmental Disorders

Support: Alberta Children's Hospital Research Foundation

Title: Regulating ERK signaling improves vocal communication in a mouse model of autism

Authors: ***N. CHENG**, M. KHANBABAEI, E. HUGHES, K. MURARI, J. M. RHO;
Univ. of Calgary, Calgary, AB, Canada

Abstract: Language delays and deficits have been considered a defining feature of autism spectrum disorder (ASD). Currently, only co-morbid manifestations of the disorder can be alleviated, but not the core symptoms. Notably, the most common genetic linkages to ASD include ones that alter the extracellular-signal-regulated kinase (ERK) pathway. Here, we hypothesized that ERK signaling is dysregulated in ASD and that interventions aimed at normalizing this pathway can alleviate the core symptoms of the disorder. We tested this hypothesis in the BTBR mouse model because it robustly exhibits all of the core behavioural features of ASD. We found that the levels of both active MEK and ERK proteins were significantly increased in the neocortex of juvenile (postnatal day 35, or P35) BTBR mice compared with age-matched B6 animals. To test if pharmacological inhibition of ERK signaling would mitigate ASD-like behaviour in BTBR mice, we injected i.p. both B6 and BTBR mice from P24 to P35 with U0126 (12.5 mg/kg/day), a highly selective and potent MEK inhibitor. Using liquid chromatography-tandem mass spectrometry, we first confirmed that the concentration of U0126 in neocortex homogenate after i.p. injection was above its IC_{50}. In addition, i.p. injection of U0126 reduced pERK levels in the neocortex of BTBR mice. Behavioural testing revealed that while U0126 treatment did not affect body weight or motor activity of either BTBR or B6 animals compared with vehicle-injected controls, it increased the number of ultrasonic vocalizations (USVs) emitted by BTBR mice during male-female encounters, without altering the average duration, peak frequency, or maximum amplitude. Further analysis showed that similar to what has been reported in neonatal mice, USVs in juvenile B6 mice were mostly clustered into strings of calls separated by relatively long pauses.

In contrast, the USVs from BTBR mice showed only modest string organization, with larger proportion of calls not associated with a string structure, as well as fewer total number of strings and calls per string during the test. U0126 treatment increased the proportion of calls associated with string structure and the total number of strings emitted from BTBR mice. Thus, U0126 treatment improved both the volume and structure of vocalizations from BTBR mice. In addition, we also examined the effect of U0126 treatment in the 3-chamber sociability assay, and found that the BTBR mice showed a trend toward improvement. Together, our data indicate that regulating ERK signalling ameliorates deficits in vocal communication of the BTBR model of ASD, suggesting that targeting this pathway could have potential therapeutic benefit.

Disclosures: N. Cheng: None. **M. Khanbabaei:** None. **E. Hughes:** None. **K. Murari:** None. **J.M. Rho:** None.

Poster

031. Autism Pathogenesis

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Topic: A.07. Developmental Disorders

Support: Supported by Gates Foundation OPP1119489

Title: Maternal plasma fetal neuronal exosomes: a non-invasive tool to interrogate in-utero neuronal injury

Authors: *L. GOETZL[1,2,3], E. J. GOETZL[4], N. MERABOVA[3], E. LAURETTI[3], G. TATEVOSIAN[3], D. MARTIROSYAN[3], N. DARBINIAN[3];
[1]Shriners Hosp. Pediatric Res. Ctr., Temple Univ. Sch. of Med., Philadelphia, PA; [2]Obstetrics & Gynecology, [3]Ctr. for Neural Repair and Rehabilitation, Shriners Hosp. Pediatric Res. Ctr., Lewis Katz Sch. of Med. at Temple Univ., Philadelphia, PA; [4]Univ. of California, San Francisco, CA

Abstract: **Introduction**: We have recently reported that fetally derived neuronal exosomes (FNEs) can be isolated from maternal plasma and their proteins analyzed as a non-invasive method for evaluating fetal neurodevelopment. We have also found that, in some cases, response to a toxin such as ethanol (EtOH) results in more dramatic reductions in markers of synaptic function and neuronal survival in FNEs than in paired fetal synaptosomes (SYNAPs). This may be due to homeostatically reduced loading of these key proteins into exosomes to preserve levels in damaged neurons. We hypothesized that biomarker levels would remain highly correlated between FNEs and paired SYNAPs. **Methods:** We performed an IRB-approved, matched case-

control study in women 9 -19 weeks' gestation (GA). Heavy EtOH cases were compared to controls matched for GA and fetal gender (n=10/group). Maternal plasma exosomes were precipitated (ExoQuick) and the fetal subset enriched (anti-Contactin-2/TAG1 streptavidin bead absorption). Synaptosomes (SYNPs) were prepared from fetal brain tissue and protein levels quantified by ELISA. **Results**: The majority of markers of synaptic function and neuronal survival showed moderate to strong correlation between protein level in SNYPs and FNEs (**Table 1**). We previously identified SNP, REST, NG and Synapsin-2 as highly discriminatory in FNEs with little or no overlap between cases and controls. Three of these 4 most promising biomarkers had highly significant FNE-SNYP correlations. SNYP NG may correlate poorly due to its post-synaptic role. **Conclusions**: These results represent additional evidence that developing neurons release exosomes into the fetal circulation from where they are able cross the placenta into the maternal circulation. Our novel methods for isolating FNEs from maternal plasma provide potentially clinically relevant biomarkers of acute and/or chronic neurotoxic exposures between 9 and 19 weeks GA. Both EtOH and viral infections may ultimately result in fetal microcephaly through an imbalance between progenitor cell production/differentiation and cell death. Therefore FNE-based assays may ultimately prove useful in predicting microcephaly in an earlier gestational age window than fetal ultrasound and magnetic resonance imaging.

Table 1. Correlations between Median Protein Levels in FNEs and SYNAPs (Spearman's ρ, non-parametric)

Marker	Rho	p value
Synaptophysin (SNP)	0.61	0.004
Synaptotagmin-2	.008	0.98
Restriction element-1 silencing transcription factor (REST)	0.55	0.01
Growth Associated Protein 43 (GAP43)	0.18	0.44
Neurogranin (NG)	0.21	0.38
Synaptopodin	0.47	0.04
Brain-derived neurotrophic factor (BDNF)	0.56	0.01
Synapsin-2 total	0.86	<0.001

Disclosures: **L. Goetzl:** Other; Patent application. **E.J. Goetzl:** Other; Patent application. **N. Merabova:** None. **E. Lauretti:** None. **G. Tatevosian:** None. **D. Martirosyan:** None. **N. Darbinian:** None.

Poster

031. Autism Pathogenesis

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Topic: A.07. Developmental Disorders

Support: NIH 1U54HD082008

Title: Mmp-9 deletion rescues developmental abnormalities in the auditory cortex of fragile x syndrome mouse model

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

Abstract: Fragile X Syndrome (FXS), a leading genetic cause of autism and intellectual disability, is characterized by language deficiencies, impaired social reciprocity and auditory processing deficits. The Fmr1 knockout (KO) mice replicate most of these FXS-associated auditory deficits which are likely the result of an impaired excitatory-inhibitory (E/I) balance due to reduced excitatory drive onto inhibitory cells, a reduced number of PV interneurons or increased firing rate of excitatory neurons and their high firing probability during UP states in KO mice. In this study, we analyzed the density of parvalbumin (PV)-positive inhibitory interneurons in developing auditory cortex of KO mice. We also investigated the formation of perineuronal nets (PNNs), which are thought to regulate the functions of PV interneurons. We found a significant increase in PV cell density in both wt and KO mouse auditory cortex from P14 to P60. PNN formation was also observed during the same period with a major increase in PNN-positive cell density at P21 and P30. PNNs were mostly observed in layer 4 but not layers 2/3 or 1 of the auditory cortex. Further analysis showed lower PV cell density, PNN density, and lower numbers of PV cells enwrapped with PNNs in layer 4 of KO mice as compared to WT animals at P21 but not P30. Matrix metalloproteinase-9 (Mmp-9), a protein that is a target of Fmrp transcriptional regulation, also regulates enzymatic cleavage of the extracellular matrix, in particular PNNs around PV-positive interneurons. We found abnormally high Mmp-9 levels in the developing auditory cortex of KO mice which may explain impaired PNN formation observed at P21. We therefore investigated the effect of full and partial deletion of Mmp-9 and found a restoration of PV and PNN cell density in the auditory cortex of Fmr1/mmp-9 double KO mice at P21. Density of PV/PNN positive neurons and the percentage of PV cells enwrapped with PNN was also restored to WT levels. We further investigated whether irregular PV cell maturation in the KO contributed to auditory cortex deficits by analyzing the expression of neuropeptide Y (NPY), a marker that is expressed in immature PV cells. We found increased expression of NPY in PV cells in the KO auditory cortex and an increased propensity of NPY cells to be enwrapped by PNNs at P30, which was not observed in wt or double KO mice. These

findings indicate that elevated Mmp-9 levels may be responsible for the abnormal development and maturation of PV/PNN in the auditory cortex of Fmr1 KO mice leading to impaired excitatory-inhibitory balance and the development of auditory processing deficits. This work was supported by grants from FRAXA Research Foundation and NIH 1U54HD082008.

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Poster

031. Autism Pathogenesis

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Topic: A.07. Developmental Disorders

Support: NIH Grant R00MH087628

Title: Time-delimited MET receptor tyrosine kinase signaling controls glutamatergic circuits formation and refinement in the developing forebrain

Authors: ***X. MA**[1], Z. LU[2], G. LI[2], L. ZHANG[2], M. PIECHOWICZ[2], J. WU[3], S. QIU[2]; [1]Dept. of Basic Med. Sci., The Univ. of Arizona Col. of Med., Phoenix, AZ; [2]Univ. of Arizona COM-PHX, Phoenix, AZ; [3]Barrow Neurolog. Inst., Phoenix, AZ

Abstract: The human *MET* gene encodes the MET receptor tyrosine kinase, which a replicated risk for autism spectrum disorder (ASD) and is implicated in the structural and functional brain development. *MET* plays a pleiotropic role in embryogenesis and modifies a large number of neurodevelopmental events. In the mouse forebrain, MET expression in the dorsal pallium is turned on at late embryonic stage, peaks during the first two postnatal weeks, and precipitously down-regulated after the third postnatal week. Our previous works suggest that MET signaling controls synaptogenesis and the time of maturation in the developing hippocampus and the prefrontal circuits. Here, using a transgenic mouse model with controllable MET over-expression, we show that continued MET expression after the third postnatal week in mouse forebrain leads to excessive neurite growth, and profuse generation of small, immature dendritic spines. These morphological and functional impairments may be caused by enhanced neurite growth, and/or impaired dendrite/spine developmental pruning. In addition, two photon in vivo imaging in MET over-expression neurons suggest impaired synapse pruning. Therefore, normal time-delimited MET signaling, particularly the timely downregulation of MET expression is critical in regulating the timing of neuronal growth, glutamatergic synapse maturation and cortical circuit function. Dysregulated MET signaling may lead to pathological changes in

forebrain maturation and connectivity, and thus contribute to the emergence of neurological symptoms associated with ASD.

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Poster

031. Autism Pathogenesis

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Topic: A.07. Developmental Disorders

Support: NIMH Grant MH102244

Title: Synaptic protein interaction network analysis of seven autism mouse models

Authors: ***S. E. SMITH**[1], A. A. WILLIAMS[2], E. A. BROWN[2], S. C. NEIER[3], A. G. SCHRUM[3];
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Abstract: Autism genetic studies have identified multiple genetic risk factors with protein products localizing to the glutamate synapse. Moreover, mouse models of autism often show disruptions of glutamatergic transmission and excitatory/inhibitory balance, even when the gene product under study is not localized to the synapse. These data suggest that impaired glutamatergic transmission may be a central component of many different genetic and environmental causes of autism. To test this 'synaptic hypothesis of autism', we have assembled a quantitative multiplex immunoprecipitation (QMI) assay to measure dynamic protein-protein interaction networks at the glutamate synapse in mouse models of autism. The QMI assay immunoprecipitates a given protein onto a microbead substrate, and quantifies the abundance of co-immunoprecipitated proteins in shared complexes using fluorescently tagged antibodies read by a flow cytometer. Using this principle, we can simultaneously measure 100+ binary protein combinations of autism-linked and related gene products using Luminex addressed beads. We have analyzed seven independent genetic and environmental mouse models of autism and quantified the differences in the synaptic protein interaction network in the hippocampus and frontal cortex. Our results show that when one synaptic protein is genetically altered (e.g. Shank3), the abundance of other, seemingly unrelated protein complexes is affected (e.g. a complex containing Homer1 and PSD95). By comparing protein network changes in different mouse models using graph-theory-based techniques, we are beginning to identify a set of

proteins in shared complexes that are altered in multiple models of autism. We predict that a better understanding of both levels of individual protein abundance and, critically, the amount of interaction among synaptic proteins, will enable the identification of convergent molecular pathways shared among different models of autism and allow the classification of biologically relevant sub-groups of the disorder.

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Poster

031. Autism Pathogenesis

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Topic: A.07. Developmental Disorders

Title: S100B protein accumulation alters intracellular trace metal homeostasis and affect autism associated signaling cascades in the central nervous system

Authors: *S. HAGMEYER[1], J. S. CRISTÓVÃO[2], T. M. BOECKERS[3], C. M. GOMES[2], A. M. GRABRUCKER[4];
[1]WG Mol. Analysis of Synaptopathies, Neurol. Department, Neurocenter of Ul, Univ. Ulm, Ulm, Germany; [2]Chem. and Biochem. Dept., BioISI – Biosystems & Integrative Sci. Inst., Lisbon, Portugal; [3]Inst. for Anat. and Cell Biol., Ulm, Germany; [4]WG Mol. Analysis of Synaptopathies, Neurol. Dept., Neurocenter of Ulm University, Ulm, Germany, Inst. for Anat. and Cell Biol., Ulm, Germany

Abstract: An increasing number of studies have reported the implication of an altered trace metal homeostasis, in particular abnormal zinc and copper levels, in several neurological disorders including Autism Spectrum Disorders (ASD) and Alzheimer´s disease (AD). This non-genetic risk factor may unfold not only in response to nutritional deficiencies, but may also be caused by the occurrence of abnormal accumulations of trace metal binding proteins that induce local changes in trace metal homeostasis. S100B, an EF hand calcium binding protein of the S100 protein family, has been recently reported to be prone to aggregation. S100B is mostly expressed in the brain where it accounts for 0.5% of soluble proteins and mainly exists as a homodimer. Besides its calcium-binding property, S100B proteins also bind two zinc ions per dimer. Elevated serum levels of S100B have been reported in cases of ASD as well as AD, both disorders that also show alterations in metal ion homeostasis and neuro-inflammation. Taken this into account, we investigated the effects of abnormal S100B protein aggregates on neuronal metal ion homeostasis and down-stream effects on synapse formation, composition and function

at different developmental stages in *in vitro* cell culture systems and *in vivo* in mouse models. We could show that S100B binds zinc under physiological conditions *in vitro*. After the exposure of neurons to elevated levels of dimeric or tetrameric S100B, indeed intracellular zinc levels were affected. Rescue experiments confirmed that the effects were caused directly by zinc-binding to S100B. The reduction in zinc levels leads to impaired postsynaptic scaffold formation affecting proteins of the SHANK family, such as SHANK2 and SHANK3 *in vitro*. SHANK2 and SHANK3 proteins have previously been identified as candidate genes for ASD. Intriguingly, a dysregulation of S100B has been reported in other cases of ASD. We conclude that an altered trace metal homeostasis can be caused by abnormal protein aggregations of S100B and might lead to synaptic dysfunction.

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Poster

031. Autism Pathogenesis

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Topic: A.07. Developmental Disorders

Support: NYU Challenge Grant 20142015

Title: Pericyte initiation of Intussusceptive (splitting) angiogenesis in the postmortem cortex of autism spectrum disorder

Authors: *X. F. JIA, E. C. AZMITIA, Z. T. SACCOMANO, K. N. LATCHA;
biology, New York Univ., New York, NY

Abstract: Autism Spectrum Disorder (ASD) is a pervasive developmental disorder. We previously detected persistent angiogenesis in ASD brains that had many of the characteristics of intussusceptive angiogenesis (IA; Azmitia et al, 2016). We now report on further studies aimed at exploring the details of this form of splitting angiogenesis that involves proliferating pericytes and recruitment of precursor endothelial cells. The present work used postmortem brains of 10 children/young adults with ASD and their age matched controls. Specific mouse monoclonal antibodies against membrane-associated proteins selected for study were Ulex Europaeus Agglutinin lectin (UEA-1) (Vector Laboratories, Burlingame, CA), CD34 (Abcam Cambridge, MA) and nestin (Millipore, Temecula, CA). UEA-1 showed pleiotropic binding of endothelial cells, while nestin identified proliferating pericytes and CD34 labeled precursor endothelial cells. The cellular labeling of vascular cells was found in all layers of the gray and white matter of the

STC in both pre-capillary arterioles and capillary vessels. We confirmed our previous observation of nestin labeling pericytes seen principally in ASD brains of all ages (2.8-28 years) but only in young controls (<2.1 years). UEA-1 labeling endothelial cells in both ASD and control brains, however, compared to control brains where the staining along the fiber length of endothelial cells was homogenous, in ASD brains branch points (nodules) were more heavily stained at all ages (control: 2.1-30 years, ASD: 2.8-28 years). CD34 labeling endothelial cells were seen principally in ASD brains of all ages (2.8-28 years) but only in young controls (<2.1 years). As seen with UEA-1, the CD34 label showed a non-homogeneous distribution of the label along the fiber length. In ASD brains, CD34 labeling along the fiber was especially dense at branch points, thin branches and terminal endpoints of the vessel. In cortex from autism patients, co-localization of proliferating pericytes (nestin) and endothelial cells (UEA-1) in branching blood vessels were identified, whereas in control brains only UEA-1 labeling was seen throughout cortex Interestingly, we documented pericyte vessels devoid of endothelial cells branching off both large arterioles and capillaries in ASD. These results are in support of previous in vitro studies (Nehls et al., 1992; Balabanov. 1998; Otani et al., 2000; Witmer et al., 2004) suggesting an important role of proliferating pericytes in leading and recruiting precursor endothelial cells in the formation of intussuceptive angiogenesis.

Disclosures: **X.F. Jia:** None. **E.C. Azmitia:** None. **Z.T. Saccomano:** None. **K.N. Latcha:** None.

Poster

031. Autism Pathogenesis

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 31.17/C32

Topic: A.07. Developmental Disorders

Support: NIH Grant NIMH 096816

NIH Grant NINDS 076708

Title: Microbial reconstitution reverses maternal diet-induced social and synaptic deficits in offspring

Authors: *S. A. BUFFINGTON[1,2], G. VIANA DI PRISCO[1,2], J. F. PETROSINO[3,4], M. COSTA-MATTIOLI[1,2];
[1]Neurosci., [2]Memory and Brain Res. Ctr., [3]Mol. Virology and Microbiology, [4]Ctr. for Metagenomics and Microbiome Res., Baylor Col. of Med., Houston, TX

Abstract: Maternal obesity during pregnancy has been associated with increased risk of neurodevelopmental disorders, including autism spectrum disorder, in offspring. Here we report that maternal high fat diet (MHFD) induces a shift in microbial ecology that has a negative impact on offspring social behavior. Social deficits and gut microbiota dysbiosis in MHFD offspring are prevented by co-housing with offspring of mothers on a regular diet (MRD) and transferable to germ-free mice. In addition, social interaction induces synaptic potentiation (LTP) in the ventral tegmental area (VTA) of MRD, but not in MHFD offspring. Moreover, MHFD offspring had fewer oxytocin immunoreactive neurons in the hypothalamus. Using metagenomics and precision microbiota reconstitution, we identified a single commensal strain that corrects oxytocin levels, LTP, and social deficits in MHFD offspring. Our findings causally link maternal diet, gut microbial imbalance, VTA plasticity and behavior, and suggest that probiotic treatment may relieve specific behavioral abnormalities associated with neurodevelopmental disorders.

Disclosures: S.A. Buffington: None. **G. Viana Di Prisco:** None. **J.F. Petrosino:** None. **M. Costa-Mattioli:** None.

Poster

031. Autism Pathogenesis

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 31.18/C33

Topic: A.07. Developmental Disorders

Support: NIH MH097236

Title: Greater amygdala spine density in young ASD brains

Authors: *R. K. WEIR[1], M. D. BAUMAN[2], C. M. SCHUMANN[2];
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Abstract: The amygdala, a medial temporal lobe structure implicated in social and emotional regulation, undergoes an aberrant growth trajectory in individuals with autism spectrum disorder (ASD), which includes precocious growth early in childhood followed by cell loss in adulthood. The goal of this study is to determine if the aberrant amygdala growth in children with ASD may be attributable to alterations in neuronal dendritic morphology.
Tissue from 34 postmortem human brains (age range 4-46) was assembled from the NIH NeuroBioBank at the University of Maryland, the BEARS program at UC Davis MIND Institute, Autism BrainNet and the Harvard Brain Tissue Resource Center. Blocks of amygdala tissue approximately 1.5x1.5x0.5cm were excised from the temporal lobe and neurons were visualized

using a modified Golgi-kopsch staining technique. After dehydration and embedding in parlodion, 150µm sections were cut on a sliding microtome. 10 lateral nucleus neurons per case were selected (fully impregnated, central within the slice and free from obscurities) to be traced using Neurolucida software (MBF Biosciences). Spine counts were collected from one dendrite per neuron. Measures of dendrite morphology such as total dendritic length, segment count and spine density were analyzed using Neurolucida Explorer and statistical analyses conducted in SPSS.
The success of Golgi-kopsch staining in post-mortem human tissue was in line with previous studies (Rosoklija et al., 2014) allowing complete data collection from 13 TD and 14 ASD cases. There was a positive correlation between total dendritic arborization and age in both TD and ASD amygdala. There was no difference in dendritic arborization between diagnostic groups. However, spine density was significantly different between diagnostic groups across age ($F_{(3,26)}$=4.36, P=0.014). Younger cases (≤16 years old) of ASD have a greater spine density than TD controls. While spine density remains relatively constant in the amygdala into adulthood in TD, there is a significance decrease in spine density as people with ASD age.
This study shows a clear difference in spine density between young ASD and TD cases, which possibly represents an underlying difference in amygdala innervation or pruning mechanisms in ASD brains that may contribute to social and emotional impairments into adulthood.

Disclosures: R.K. Weir: None. **M.D. Bauman:** None. **C.M. Schumann:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 32.01/C34

Topic: A.07. Developmental Disorders

Title: Deletion of CTNNB1 in inhibitory circuitry contributes to autism-associated behavioral defects

Authors: *F. DONG[1], J. JIANG[1], C. MCSWEENY[1], D. ZOU[1,2], L. LIU[1,3], Y. MAO[1];
[1]Biol., Penn State Univ., University Park, PA; [2]First Affiliated Hospital, Guangxi Med. University,, Nanning, China; [3]Dept. of Chem. and Biology, Col. of Science, Natl. Univ. of Def. Technol., Changsha,, China

Abstract: Mutations in β-catenin (CTNNB1) have been implicated in cancer and mental disorders. Recently, loss-of-function mutations of CTNNB1 were linked to intellectual disability (ID), and rare mutations were identified in patients with autism spectrum disorder (ASD). As a key regulator of the canonical Wnt pathway, CTNNB1 has an essential role in

neurodevelopment. However, the function of CTNNB1 in specific neuronal subtypes is unclear. To understand how CTNNB1 deficiency contributes to ASD, we generated CTNNB1 conditional knockout (cKO) mice in parvalbumin interneurons. The cKO mice had increased anxiety, but had no overall change in motor function. Interestingly, CTNNB1 cKO in PV-interneurons significantly impaired object recognition and social interactions and elevated repetitive behaviors, which mimic the core symptoms of patients with ASD. Surprisingly, deleting CTNNB1 in parvalbumin-interneurons enhanced spatial memory. To determine the effect of CTNNB1 KO in overall neuronal activity, we found that c-Fos was significantly reduced in the cortex, but not in the dentate gyrus and the amygdala. Our findings revealed a cell type-specific role of CTNNB1 gene in regulation of cognitive and autistic-like behaviors. Thus, this study has important implications for development of therapies for ASDs carrying the CTNNB1 mutation or other ASDs that are associated with mutations in the Wnt pathway. In addition, our study contributes to a broader understanding of the regulation of the inhibitory circuitry.

Disclosures: **F. Dong:** None. **J. Jiang:** None. **C. McSweeny:** None. **D. Zou:** None. **L. Liu:** None. **Y. Mao:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 32.02/D1

Topic: A.07. Developmental Disorders

Support: Telethon# GGP13187

CARIPLO foundation grant #2013 0879

Title: Protocadherin 19 downregulation affects cortical development and autism-related behaviors in rats.

Authors: *A. W. CWETSCH[1], L. PERLINI[1], S. BASSANI[2], M. PASSAFARO[2], L. CANCEDDA[1];
[1]Dept. of Neurosci. and Brain Technologies, Inst. Italiano Di Tecnologia, Genova, Italy; [2]Dept. of BIOMETRA, CNR Inst. of Neurosci., Milan, Italy

Abstract: Epilepsy and mental retardation limited to females (EFMR) is a genetic disorder caused by mutations in the protocadherin 19 (PCDH19). The clinical characteristics of EFMR include seizures, fever sensitivity, as well as varying degree of intellectual disability and autistic behaviors. PCDH19 has been implicated in cell adhesion and cell-cell interactions *in vitro*, but its

role during *in vivo* development has not been characterized yet. Here, we found that PCDH19 expression is increased during early development while fading in adulthood in the rat hippocampus and cortex. By three-electrode *in utero* electroporation coupled with RNA interference (siRNA), we downregulated PCDH19 expression in pyramidal neurons migrating to both somatosensory or motor cortex *in vivo*. We found that PCDH19 siRNA-treated animals presented ectopic positioning of neurons. Furthermore, PCDH19 siRNA-treated animals also showed an impaired morphology. Moreover, we found that downregulation of PCDH19 *in utero* in the somatosensory cortex resulted in decreased number of ultrasonic vocalizations in pups at P9. We are currently investigating the physical and functional interaction of PCDH19 with other adhesion molecules and signaling pathways previously implicated in autism. These data suggest that PCDH19 has a crucial role in the correct development of the cortex *in vivo* that may relate to autism-related behaviors in PCDH19-downregulated animals.

Disclosures: A.W. Cwetsch: None. **L. Perlini:** None. **S. Bassani:** None. **M. Passafaro:** None. **L. Cancedda:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

Location: Halls B-H

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Program#/Poster#: 32.03/D2

Topic: A.07. Developmental Disorders

Support: NIH IRACDA/INSPIRE Grant: K12GM093854

NJ Governor's Council on Medical Research and Treatment of Autism

Title: Autism animal model exhibits abnormal norepinephrine innervation and increased stress circuit activity following forced swim.

Authors: *J. W. LUNDEN[1], M. GENESTINE[2], C. C. PENG[3], V. MIRABELLA[1], S. PREM[1], J. MILLONIG[1], E. DICICCO-BLOOM[1];
[1]Neurosci. and Cell Biol., Rutgers - Robert Wood Johnson Med. Sch., Piscataway, NJ;
[2]Columbia Univ., New York, NY; [3]Rutgers Univ., Piscataway, NJ

Abstract: Autism spectrum disorder (ASD) is a pervasive neurodevelopmental disorder characterized by impairments in social interaction 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 abnormalities in

social interaction, depression related tasks (forced swim), fear conditioning and spatial learning, all associated with diminished hippocampal neurogenesis, norepinephrine (NE) levels, and NE fiber innervation. While these deficits in neurogenesis and NE systems suggest abnormalities in the stress system, nothing is known about NE fiber innervation and neural activity in other limbic regions (amygdala and paraventricular nucleus of the hypothalamus, PVN), and responses to acute stress. To examine this issue, postnatal day 60-70 wild type (WT) and KO mice (N=3-6/genotype) were assessed for protein levels of NE transporter (NET) and tyrosine hydroxylase (TH, the rate-limiting enzyme for NE biosynthesis) using western blotting. Immunohistochemistry was used to quantify NET positive fibers in the amygdala and PVN as well as c-fos expression before and after swim stress. En2 KO mice exhibited a 1.7-fold increase in TH protein levels ($p<0.02$) and 1.5-fold increase in NET levels ($p<0.002$) in the amygdala compared to WT controls. NET fiber counts were also increased, by 2.6-fold in the basolateral amygdala ($p<0.004$) and 1.7-fold in the PVN ($p<0.016$) in KO mice. Finally following swim stress, KO mice exhibited a 2.25-fold increase in c-fos in the PVN ($p<0.006$), while there were no changes in the ventral hippocampus. These observations indicate that NE fiber innervation is increased in some En2-KO limbic system regions, a result that contrasts with reduced NE fibers in the dorsally localized hippocampus. We tentatively conclude that region-specific dysregulation of NE fibers in the En2-KO leads to increased PVN and decreased hippocampal neural activities that in turn impact 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.

Disclosures: J.W. Lunden: None. **M. Genestine:** None. **C.C. Peng:** None. **V. Mirabella:** None. **S. Prem:** None. **J. Millonig:** None. **E. DiCicco-Bloom:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 32.04/D3

Topic: A.07. Developmental Disorders

Support: CONACyT scholarship 212825 (to AAPL)

Title: Decreased expression of β_2 subunit of $GABA_A$ receptor in the hippocampus and cerebellum in a rat model of autism

Authors: *A. PUIG-LAGUNES[1], E. VELASCO-CERCAS[2], I. ZAMORA-BELLO[2], A. PUIG-NOLASCO[3], R. TOLEDO-CÁRDENAS[2], C. PÉREZ-ESTUDILLO[2], C. MORGADO-VALLE[2],

L. LÓPEZ-MERAZ[2];
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Abstract: Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the brain; its fast inhibitory action is mediated through $GABA_A$ receptors ($GABA_A$-R). Several studies in patients with autism spectrum disorder (ASD) have demonstrated abnormalities in $GABA_A$-R expression in different brain areas. Alterations in the GABAergic neurotransmission have also been reported in experimental models of ASD. This evidence suggests that a dysregulation in this system could be involved in ASD pathogenesis. The main goal of this study was to evaluate the expression of β_2 subunit of the $GABA_A$-R ($GABA_A$-R β_2) in the hippocampus and cerebellum from developing rats prenatally exposed to valproic acid (VPA). Wistar pregnant females were injected with VPA (600 mg/Kg, i.p.) during the twelfth embryonic day (E12); control rats were injected with saline solution. On the fourteen postnatal day, rats from VPA (3 females and 11 males) and control (7 females and 7 males) groups were anesthetized, perfused with 4% phosphate-buffered paraformaldehyde and 40-µm-thick brain sections were obtained at the level of the dorsal hippocampus (coronal sections) and the cerebellar vermis (sagittal sections). Colorimetric immunohistochemistry was performed in order to detect $GABA_A$-R β_2 in CA1, CA2, CA3 fields (oriens, pyramidal and radiatum strata) and dentate gyrus (granule layer and hilus) from hippocampus, as well in the ten lobes of the medial vermis (molecular and granular layers). Presence of $GABA_A$-R β_2 in the brain was determined semi-quantitatively by a densitometrical analysis. Results showed a significant reduction of $GABA_A$-R β_2 inmunoreactivity in the pyramidal layer of CA2 region in the VPA group compared with the control group. A similar decreased was detected when VPA males were compared with control males. No statistical differences in $GABA_A$-R β_2 expression were observed between experimental groups at any lobe from cerebellar vermis. Nevertheless, VPA females had lower $GABA_A$-R β_2 inmunoreactivity in the molecular layer of lobes III and IX and in the granular layer from lobes IV and IX compared with control females. Furthermore, VPA females showed a lower $GABA_A$-R β_2 expression in the granular layer from lobes I, III, IV and X and in the molecular layer from lobes II, III, V and X in comparison with VPA males. These data demonstrate that prenatal exposure to VPA reduces the expression of $GABA_A$-R β_2 in the hippocampus and support that a GABAergic dysfunction could be implicated in the pathogenesis of ASD. Additionally, prenatal VPA exposure produces changes in cerebellar $GABA_A$-R β_2 expression depending on the gender.

Disclosures: **A. Puig-Lagunes:** None. **E. Velasco-Cercas:** None. **I. Zamora-Bello:** None. **A. Puig-Nolasco:** None. **R. Toledo-Cárdenas:** None. **C. Pérez-Estudillo:** None. **C. Morgado-Valle:** None. **L. López-Meraz:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 32.05/D4

Topic: A.07. Developmental Disorders

Title: Epilepsy in toll like receptor 3, 7, 9 deficient mice

Authors: *J. CORDERO, O. ARIAS-CARRION;
Unidad de Trastornos del Movimiento y Sueño, Hosp. Gen. Dr. Manuel Gea Gonzalez, Ciudad DE Mexico, Mexico

Abstract: The role of the innate immune system in the defence against exogenous pathogens is well established. In contrast, its role in the central nervous system needs further investigation. Immunological analysis revealed that mice with a triple deficiency of the nucleic acid recognizing Toll-like receptor (TLR)-3, 7 and 9 lose control of endogenous retroviruses of the MuLV type in the hematopoietic system. Surprisingly, we noticed that a significant number of these triple TLR deficient mice exhibited spontaneous seizures. Although disorders of the innate immune system have been implicated in the etiology of epilepsy, convincing evidence for a causal role has yet to be reported. Like most patients with epilepsy TLR379 deficient mice appear behaviourally normal between seizure episodes. Here, we show that cFos, an immediate early gene that is upregulated by seizures, was up regulated in the hippocampus. This suggests that these mice experience seizures that involve temporal structures, e.g. hippocampus. Therefore, these mice may prove to be a novel model of temporal lobe epilepsy, providing insight into the mechanisms underlying the role of viral infections or impaired innate immunity in neuronal diseases, such as epilepsy.

Disclosures: J. Cordero: None. **O. Arias-Carrion:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 32.06/D5

Topic: A.07. Developmental Disorders

Support: PCDH19 allianche

InsiemeperPCDH19-Cute syndrome

Cariplo

Title: Protocadherin-19 downregulation affects the surface expression of associated $GABA_A$Rs and increases seizure susceptibility in rats

Authors: S. BASSANI[1], L. GEROSA[1], A. CWETSCH[2], L. CANCEDDA[2], *M. PASSAFARO[3]; [1]CNR -Institute of Neurosci., Milan, Italy; [2]Dept. of Neurosci. and Brain Technologies, Inst. Italiano di Tecnologia, Genova, Italy; [3]DTI-CNR-IN Sect Cell, Milan, Italy

Abstract: PCDH19-Female Limited Epilepsy (PCDH19-FLE) is a debilitating neurological condition characterized by early onset seizures, intellectual disability and autistic features. PCDH19 (Xq22) encodes for the cell-adhesion protein protocadherin-19 (pcdh19) whose function is largely unknown. Our data indicate that PCDH19 is highly expressed in the rat brain, especially in the cortex and hippocampus. Consistent with a cell-adhesion role, pcdh19 is expressed on the plasma membrane and is recruited at cell-cell contacts in cultured hippocampal neurons. Furthermore, pcdh19 shows a synaptic distribution and pcdh19 levels affect the expression of synaptic markers, suggesting pcdh19 involvement in neuronal wiring. By yeast two-hybrid screening we found that pcdh19 interacts with the alpha1 subunit of the $GABA_A$ receptor ($GABA_A$R). Pcdh19 and $GABA_A$R coimmunoprecipitate in neurons and pcdh19 shRNA-mediated downregulation reduces $GABA_A$R surface expression, suggesting the involvement of pcdh19 in $GABA_A$R trafficking. Consistently with a GABAergic defect, pcdh19 shRNA-mediated downregulation *in vivo* by *in utero* electroporation affects neuronal migration and morphology. Furthermore rats expressing pcdh19 shRNA in the hippocampus show higher susceptibility to-pentylentetrazol-induced seizures at postnatal day 7 (P7) compared to control animals. We hypothesize a role for PCDH19 in the regulation of neuronal excitability both at the circuit level, through its adhesive properties, as well as at the cell-autonomous level, through the regulation of $GABA_A$R trafficking and hence inhibitory transmission.

Disclosures: S. Bassani: None. **L. Gerosa:** None. **A. Cwetsch:** None. **L. Cancedda:** None. **M. Passafaro:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 32.07/D6

Topic: A.07. Developmental Disorders

Support: Hope 4 Harper

Title: Heightened neuronal network excitability in a mouse model of CDKL5 disorder

Authors: *M. YENNAWAR[1], H. SUN[2], Z. ZHOU[2], F. E. JENSEN[2];
[1]Pharmacol., [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: CDKL5 disorder frequently appears as a pattern of neurological deficits including severe early-life epilepsy, intellectual disability, and autism. It results from loss-of-function mutations in cyclin dependent kinase-like 5 (CDKL5), a kinase that is enriched in the brain and upregulated postnatally. The onset of seizures and intellectual disability in CDKL5 disorder occur during a developmental period where the excitation-inhibition (E-I) balance is already shifted to favor excitation. Normally, heightened excitation during this time facilitates synaptogenesis and neural circuit formation. However, when this sensitive balance is dysregulated it can result in epilepsy and autism. To understand alterations in the E-I balance in CDKL5 disorder, we investigated neuronal network activity and plasticity during development a CDKL5 knock-in (KI) mouse model using slice electrophysiology. The CDKL5 KI mouse model is based off of a human mutation that causes truncation of the protein in the kinase domain, rendering the protein nonfunctional. The mice are grossly similar to their WT litter mates although their weights are significantly lower than WT at postnatal day (P)21 ($n=14$, $p=0.0176$). Slice recordings for all experiments were done at CA3-CA1 synapses. At P21, input-output curves obtained from CDKL5 KI and WT litter mates indicate that CDKL5 KI mice have significantly greater network excitability ($n=10$, $p<0.0001$). Additionally, after a repetitive stimulation paradigm (20 pulses, 100 Hz), the CDKL5 KI mice exhibited heightened short-term plasticity. There was no difference in paired pulse facilitation at P21, indicating that presynaptic function is not altered in CDKL5 KI mice. No animals were observed to be spontaneously seizing. These results show that CDKL5 KI mice have significantly elevated network activity and heightened plasticity due to alterations in postsynaptic function. Ongoing studies are evaluating differences in excitatory and inhibitory postsynaptic receptor expression and function.

Disclosures: **M. Yennawar:** None. **H. Sun:** None. **Z. Zhou:** None. **F.E. Jensen:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 32.08/D7

Topic: A.07. Developmental Disorders

Support: Cure Sanfilippo Foundation

Title: Synaptic dysfunction in Sanfilippo Syndrome: the affects of lysosomal heparan sulfate accumulation on the cell surface glycocalyx

Authors: *C. WRIGHT DWYER[1], S. L. SCUDDER[2], Y. LIN[1], L. DOZIER[2], N. J. ALLEN[3], G. N. PATRICK[2], J. D. ESKO[1];
[1]Cell. and Mol. Med., [2]Neurobio., Univ. of California San Diego, LA Jolla, CA; [3]Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: Sanfilippo Syndromes, MPSIII A-D, result from deficits in lysosomal enzymes that specifically degrade heparan sulfate, a type of sulfated glycosaminoglycan. In humans, MPSIII typically presents during early childhood following a period of developmental delay, accompanied by hyperactivity and autistic-like social behaviors. The appearance of these behaviors in MPSIIIA mouse models arises by the third postnatal week. Heparan sulfate accumulation and lysosomal expansion occurs in the developing MPSIIIA brain. In fact, excitatory synaptic function is reduced in the cerebral cortex of developing MPSIIIA mice, which correlates with the onset of behavioral symptoms. Current work is focused on ascertaining the mechanism causing reduced excitatory synaptic function in the developing MPSIIIA brain. MPSIIIA mice exhibit synaptic deficits and behavioral phenotypes similar to mice deficient in heparan sulfate biosynthesis, suggesting changes in extracellular heparan sulfate may cause synaptic dysfunction in the developing MPSIIIA brain. Our work investigates the role of heparan sulfate fine structure on excitatory synaptic function and contributions to synaptic pathology in MPSIIIA mice.

Disclosures: C. Wright Dwyer: None. **S.L. Scudder:** None. **Y. Lin:** None. **L. Dozier:** None. **N.J. Allen:** None. **G.N. Patrick:** None. **J.D. Esko:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); TEGA Therapeutics Inc..

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

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Program#/Poster#: 32.09/D8

Topic: A.07. Developmental Disorders

Support: NIH Grant R01MH99660

Title: Elevated doses of 22q11.2 genes arrest the developmental maturation of working memory capacity and adult neurogenesis

Authors: *S. BOKU[1,2], S. ABE[2], T. IZUMI[2], T. TAKAHASHI[2], T. HIRAMOTO[2], Y. NAKA[2], H. NOMARU[2], A. NISHI[2], G. KANG[2], A. HISHIMOTO[2], G. DURAN-TORRES[3], K. TANIGAKI[4], J. ZHANG[2], K. YE[2], S. KATO[5], K. KOBAYASHI[5], P. T. MÄNNISTÖ[3], N. HIROI[2];
[1]Kobe Univ. Grad. Sch. of Med., Kobe, Japan; [2]Albert Einstein Col. of Med., Bronx, NY; [3]Univ. of Helsinki, Helsinki, Finland; [4]Shiga Med. Ctr., Moriyama, Japan; [5]Fukushima Med. Univ., Fukushima, Japan

Abstract: Working memory capacity, a critical component of executive function, developmentally expands from childhood to adulthood. This developmental process halts its upward trajectory during adolescence and increasingly lags behind toward adulthood in individuals with autism spectrum disorder (ASD), suggesting that this process is functionally relevant to the developmental trajectory of neuropsychiatric disorders. However, the cellular and neuronal substrates contributing to this process are not understood. As duplication/triplication of human chromosome 22q11.2 is one of the copy number variants (CNVs) consistently and robustly associated with developmental neuropsychiatric disorders, we used this genetic variant as an entry point to delve into the cellular substrates for this developmental atypicality. Using a region- and cell-specific gene expression approach, we over-expressed catechol-O-methyl-transferase (COMT) or Tbx1, two 22q11.2 CNV-encoded genes, in adult neural stem/progenitor cells in the hippocampus of C57BL/6J mice. Mice were tested for spontaneous alternation in a T-maze, a measure of spatial working memory, at either 1 month or 2 months of age. Following behavioral analysis, mice were sacrificed and the location of gene-transduced cells within the granule cell layer of the hippocampal dentate gyrus was examined. In a separate experiment, cells were taken from the hippocampus of C57BL/6J mice and cultured and passaged to isolate adult neural stem/progenitor cells. Cells in culture were then tranfected with plasmid carrying either COMT or Tbx1, and the rate of proliferation and apoptosis was examined. Mice increased working memory capacity from 1 to 2 months of age, corresponding to adolescence to young adulthood. Over-expression of COMT in adult neural stem/progenitor cells reduced the maximum working memory capacity at 2 months, but not at 1 month of age. Similarly, over-expression of Tbx1 in the same cell population at 2 months reduced working memory capacity. Moreover, over-expression of COMT or Tbx1 reduced the rate of migration of progenies of adult neural stem/progenitor cells in the hippocampus granule cell layer. When COMT or Tbx1 was over-expressed in adult neural stem/progenitor cells *in vitro*, their proliferation was reduced without elevating the rate of apoptosis. Our data provide evidence for the novel notion that elevated levels of these 22q11.2 genes deter the typical developmental maturation of working memory capacity via an altered rate of proliferation--and migration of progenies--of adult neural stem/progenitor cells in the hippocampus.

Disclosures: **S. Boku:** None. **S. Abe:** None. **T. Izumi:** None. **T. Takahashi:** None. **T. HIramoto:** None. **Y. Naka:** None. **H. Nomaru:** None. **A. Nishi:** None. **G. Kang:** None. **A. Hishimoto:** None. **G. Duran-Torres:** None. **K. Tanigaki:** None. **J. Zhang:** None. **K. Ye:** None. **S. Kato:** None. **K. Kobayashi:** None. **P.T. Männistö:** None. **N. Hiroi:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 32.10/D9

Topic: A.07. Developmental Disorders

Support: Childrens Research Institute

Title: A novel mouse model of neurosteroid insufficiency: the AKR1c14 floxed mouse

Authors: *D. BAKALAR[1], H. LACAILLE[1], J. J. O'REILLY[2], A. A. PENN[1,3];
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Abstract: A major consequence of premature birth is the early loss of the placenta and its hormonal contributions to development. Less dramatic, but more common, placental dysfunction also alters placental endocrine function, including the placental progesterone derivative allopregnanolone (ALLO). ALLO, a critical neurosteroid that acts as a potent $GABA_A$ receptor allosteric modulator, has been implicated in many neurodevelopment processes (neurogenesis, outgrowth, and survival, synapse stabilization). ALLO may also be neuroprotective: It inhibits toxin-induced cell death, protects against excitotoxicity, and increases in vivo in response to immune challenge and hypoxia. Although ALLO can be produced de novo in fetal brain, the major source of fetal ALLO is the placenta. Despite the importance of placental ALLO, until now, limited tools were available for direct investigation of the effects of placental ALLO alterations on neurodevelopment and behavior. We therefore generated a mouse model in which ALLO production is suppressed only in the placenta using a placenta-specific lentiviral knockdown of the enzyme responsible for ALLO production, 3αHSD. In this model, we showed a specific reduction in the number of cortical intermediate progenitor cells in the subventricular zone, validating the importance of placental ALLO production. We have now produced a new mouse model (*AKR1c14*$^{fl/fl}$), with LoxP sites surrounding the *AKR1c14* gene site (encoding 3αHSD) allowing inexpensive, efficient and flexible ALLO manipulation. Upon crossing with a placenta-specific Cyp19-CRE mouse, we show that recombination occurs in placenta but not brain, and *AKR1c14* mRNA is significantly reduced in placenta but not in brain. We also show a compensatory increase in Cyp11a1 (the rate-limiting enzyme in all neurosteroid synthesis from cholesterol) in both brain and placenta in the *AKR1c14*$^{fl/fl}$. This increase has been observed in preeclamptic human placentas, supporting use of this mouse as a preclinical model. We are currently investigating the effects of 3αHSD knockout on developing and adult cortical phenotypes and on behavior. ALLO and its synthetic analogs will be used to target developmental and behavioral deficits. Better understanding of the role of placental ALLO may

lead to therapeutic treatments to improve developmental outcomes in high-risk fetuses and infants.

Disclosures: **D. Bakalar:** None. **H. Lacaille:** None. **J.J. O'Reilly:** None. **A.A. Penn:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

Location: Halls B-H

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Program#/Poster#: 32.11/D10

Topic: A.07. Developmental Disorders

Support: New Life Foundation 14GM19

Title: Elucidation of the molecular mechanisms of cognitive impairment in Bardet- Biedl syndrome

Authors: ***N. HAQ**[1], C. SCHMIDT-HIEBER[2], M. HÄUSSER[2], P. BEALES[1], S. CHRISTOU-SAVINA[1];
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Abstract: Bardet-Biedl syndrome (BBS) is a genetically heterogeneous, autosomal recessive disorder. The phenotype includes severe retinal degeneration, early onset obesity, cognitive impairment, polydactyly and renal malformation. Approximately half of all individuals with Bardet-Biedl syndrome experience developmental disabilities ranging from mild impairment or delayed emotional development to severe mental retardation. Investigation of BBS proteins prove to be undeniably important to the field of molecular genetics and molecular biology as it led to emergence of a new class of human disorders, the ciliopathies, disorders of primary cilia. Cilia, a membranous antenna like organelle, were found on almost all cells including neurons. It is believed now that primary cilia is involved in various cellular functions by modulating cell signalling via specialised receptors integrated into its membrane that includes somatostatin receptor 3, melanin-concentrating hormone receptor 1, serotonin receptor 6, dopamine receptor type 5. Loss of BBS proteins has been proved to be vital for the function of cilia. Recent MRI study in patients affected with BBS revealed a high rate of unilateral or bilateral hippocampal dysgenesis as well as reduced volume of hippocampus. Specifically Bbs4 models reveal ventriculomegaly of the lateral and third ventricles, thinning of the cerebral cortex and reduced volume of hippocampus. Our study aims to investigate the mechanisms of how loss of ciliary Bbs proteins leads to cognitive impairment and learning difficulties. We hypothesise that impaired neuroplasticity may contribute to development of cognitive impairment in BBS.

Despite the varied genetic and nongenetic factors that contribute to intellectual disability, this condition has been consistently associated with changes in dendrite and dendritic spine structure. We have analysed the morphology of dentate gyrus of hippocampal neurons and have found 50% reduction in dendritic spine density across the whole dendritic tree. We have also found that loss of spines is not restricted to hippocampus but affects other brain structures such as frontal cortex and basolateral amygdala. Interestingly, we have found that the spine loss occurs within the first 3 postnatal weeks and it results from the increased autophagy and impaired mTOR signalling during this period. Notably, aerobic exercise can partly reverse this "loss of spine" phenotype in BBS mice models.

Disclosures: N. Haq: None. **C. Schmidt-Hieber:** None. **M. Häusser:** None. **P. Beales:** None. **S. Christou-Savina:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

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Program#/Poster#: 32.12/D11

Topic: A.07. Developmental Disorders

Support: NIH R00 NS076661

Title: The Noonan Syndrome-linked Raf1^{L613V} mutation drives increased glial number and alterations in learning

Authors: *M. HOLTER[1], L. T. HEWITT[1,3], S. V. KOEBELE[2,3], J. JUDD[2], C. WEDWICK[1], H. A. BIMONTE-NELSON[2], C. D. CONRAD[2], B. G. NEEL[4], T. ARAKI[4], W. D. SNIDER[5], J. M. NEWBERN[1];
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Abstract: RASopathies are a family of related syndromes caused by perturbations in the RAS/RAF/MEK/ERK signaling cascade that typically exhibit neurological deficits, such as intellectual disability, motor delay, and epilepsy. The precise role of aberrant ERK/MAPK signaling or mutation-specific alterations in nervous system pathogenesis remains poorly understood. RASopathy mutations in upstream MAPK components, such as *Nf1*, *Shp2/Ptpn11*, and *Ras* have been well-studied, whereas less is known about the precise deficits associated with downstream mutations. Here, we assessed the contributions of a Noonan Syndrome-linked,

RAF1 gain-of-function mutation, *Raf1*$^{L613V+/-}$, to cellular and behavioral phenotypes in the mouse forebrain. We report that *Raf1*$^{L613V+/-}$ mutants do not exhibit a significantly altered number of NEUN/RBFOX3-labeled neurons in the cortex. In contrast, we observed a statistically significant increase in the number of GFAP^{+} astrocytes in the adult mutant cortex and hippocampus. No significant changes in the number of activated microglia labeled with IBA1 were noted. OLIG2^{+} oligodendrocytes were increased in number in the adult cortex but not in the hippocampus, providing evidence that responses to RAF1 mutations are region-specific. Analysis of myelin sheath thickness in the corpus callosum revealed no change in average g-ratio or the proportion of myelinated to unmyelinated axons. To better understand the behavioral ramifications of altered nervous system development, we tested learning and memory in *Raf1*$^{L613V+/-}$ mutants using the Morris water maze and cued fear conditioning. Significant changes in locomotor or anxiety-like behavior were not detected. However, following five days of Morris water maze testing, we found that *Raf1*$^{L613V+/-}$ mutants performed better than controls by exhibiting decreased swim distance to the hidden platform by day three. In fear conditioning, mutants and controls showed similar increases in freezing to tone during three tone-footshock pairings. Following exposure to cued fear conditioning, mutants exhibited a significant increase in freezing to tone 24 hrs later and impaired extinction to tone when compared to controls. Overall, these data suggest that the observed alterations in forebrain glial development are not sufficient to diminish learning in *Raf1*$^{L613V+/-}$ mutants and highlight the importance of mutation-specific mechanisms in the RASopathic nervous system.

Disclosures: **M. Holter:** None. **L.T. Hewitt:** None. **S.V. Koebele:** None. **J. Judd:** None. **C. Wedwick:** None. **H.A. Bimonte-Nelson:** None. **C.D. Conrad:** None. **B.G. Neel:** None. **T. Araki:** None. **W.D. Snider:** None. **J.M. Newbern:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 32.13/D12

Topic: A.07. Developmental Disorders

Title: Mutation in a tubulin gene reveals a novel model of leukodystrophy

Authors: *I. D. DUNCAN[1], A. B. RADCLIFF[1], J. J. MORAN[2], J. P. SVAREN[2];
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Abstract: The *taiep* rat is a well characterized autosomal recessive mutant that has a unique myelin disorder with early hypomyelination followed by severe demyelination of white matter in the brain and certain tracts of the spinal cord. We have shown that a unique disturbance of

oligodendrocytes occurs with the progressive accumulation of microtubules (MTs) in the cell body and processes leading to a defect in the transport of MBP mRNA. In addition, changes in the polarity of MTs in oligodendrocyte processes has been suggested to disrupt myelination and the maintenance of the myelin sheath.
Previous mapping studies on the taiep rat had identified a candidate interval for the causative gene on chromosome 9. Since the defect was observed in oligodendrocytes, it seemed likely that the gene may be regulated by the Sox10 transcription factor, which is required for multiple facets of oligodendrocyte development. Therefore, gene expression profiling data and Sox10 binding site maps in rat spinal cord by ChIP-seq were used to identify candidate causative genes in the chromosome 9 interval. From this analysis, one primary candidate gene was selected, Tubb4a, since point mutations in this gene have been identified in humans with a white matter disorder. Sequencing cDNA from taiep spinal cord identified a homozygous mutation in Ala353 in the Tubb4a gene, which was absent in wild type littermates. Mutations in flanking residues (352 and 354) have been found in humans with MRI evidence of hypomyelination. The affected residue is positioned within the binding interface between beta-tubulin and alpha-tubulin.
Mutations in the Tubb4 gene in humans result in an incompletely characterized leukodystrophy known as Hypomyelination and Atrophy of the Basal Ganglia and Cerebellum (H-ABC). The *taiep* rat may provide an ideal model in which to study the putative CNS changes in this rare human disorder.

Disclosures: I.D. Duncan: None. **A.B. Radcliff:** None. **J.J. Moran:** None. **J.P. Svaren:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

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Topic: A.07. Developmental Disorders

Support: NSF IGERT Grant 1144399

NIH P01HD057853

Title: Evaluation of visual motion perception ability in mice with knockout of the dyslexia candidate susceptibility gene Dcdc2

Authors: *P. A. PERRINO[1], A. R. RENDALL[1], J. J. LOTURCO[2], R. H. FITCH[1];
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Abstract: Dyslexia is a common neurodevelopmental disorder, affecting over 3 million individuals per year in the US. Dyslexia is characterized by difficulties in learning to read and write despite typical intelligence and educational opportunity. The etiology indicates a strong but complex genetic basis, with heritability estimates ranging from 40% to 80%. Although the neurobiological and genetic mechanisms underlying dyslexia remain poorly understood, several dyslexia candidate risk genes have been identified. One of these candidate risk genes - doublecortin domain containing 2 (*DCDC2*) - has been shown to play a major role in neuronal migration, as well as cilia function. Disruption of these functions during neurodevelopment could contribute to the dyslexic phenotype. At a behavioral level, variants of *DCDC2* have been associated not only with dyslexia *per se*, but also intermediate phenotypes such as impairments in phonological processing and working memory. To further explore the relationship between *DCDC2* and dyslexia, a genetic knockout (KO) of the rodent homolog of *DCDC2* (*Dcdc2*) was created. Initial studies showed that cortical pyramidal neurons from *Dcdc2* KO mice exhibit increased excitability and decreased temporal firing precision (Che et al., 2015). Behavioral assessments also showed that *Dcdc2* KOs display deficits in auditory processing, as well as working memory (Truong et al., 2015). These findings parallel observations from the clinical population. More recently, a specific mutation in *DCDC2* has been strongly linked to deficits in motion perception - a skill subserving reading success. It has been suggested that the decreased temporal precision of AP firing and spontaneous NMDAR-mediated activity in KO mice may contribute to the link between *DCDC2* and motion perception deficits. Since motion perception skills have not yet been assessed in the *Dcdc2* KO mouse model, the current study was designed to evaluate the association between *DCDC2* and motion perception. Specifically, we developed a novel motion perception task based on published human assessments, utilizing touchscreen technology and operant conditioning in a modified Pairwise Discrimination task. Subjects were exposed to visual stimuli known as Gabors (adjusted for mouse vision to provide higher contrast and longer display), as used to assess individuals with dyslexia. Our overall goal was to replicate the clinical finding that *DCDC2* plays a role not only in auditory processing and working memory, but also in visual motion perception. Abnormalities in these processes could relate to impairments in the acquisition of reading skills.

Disclosures: **P.A. Perrino:** None. **A.R. Rendall:** None. **J.J. LoTurco:** None. **R.H. Fitch:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

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Topic: A.07. Developmental Disorders

Support: NSF IGERT Grant 1144399

NIH Grant P01HD057853

Title: Behavioral and neuroanatomical evaluation of the Ts2-neo mouse model of Timothy syndrome, a rare genetic disorder associated with autism spectrum disorders

Authors: *A. R. RENDALL, A. L. FORD, P. A. PERRINO, R. H. FITCH;
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Abstract: Autism Spectrum Disorders (ASD) is a set of neurodevelopmental disorders characterized by a complex behavioral phenotype, encompassing deficits in social and cognitive domains. Accepted core symptoms are heterogeneous, and range from atypical social interactions and language impairments to repetitive behaviors. To date, causal mechanisms underlying ASD remain poorly understood, but likely include a complex combination of polygenic and environmental risk factors. The genetic influence in ASD is strong, with heritability rates ranging from 70-90%, however over 1,000 risk genes have been identified making the underlying genetic architecture complex. One of these genes, calcium voltage-gated channel subunit alpha1 C (*CACNA1C*) has been recently associated to ASD. Specifically, a single *de novo* missense mutation to the 8A exon of *CACNA1C* gene, which codes for the voltage-gated L-type Ca2+ channel (Cav1.2), results in a rare disorder known as Timothy syndrome (TS) (Splawski et al. 2004). TS is strongly associated with cardiac arrthytmias, ASD and neurological dysfunction such as language impairments, seizures and intellectual disability. A genetically engineered knock-in mouse with a heterogeneous TS2 (G406R) mutation in the L-type calcium channel containing a neomycin resistance cassette was developed to study. This mouse model (Ts2-neo) provides us with a platform to investigate the role of calcium channel inactivation and calcium signaling related to brain development and ASD. Previous behavioral studies that examined Ts2-neo mice found that these animals exhibit normal general health and anxiety level but, display restricted respective behavior, altered social behavior and ultrasonic vocalizations as well as enhanced tone-cued and contextual memory following fear conditioning (Bader et al., 2011). The purpose of the current study is to further behaviorally characterize Ts2-neo mice by assessing their performance on a wide variety of paradigms. Results indicate that the loss of Ca.V1.2 inactivation in this mouse model affects motor learning, auditory processing, social and repetitive behaviors but does not have an impact on spatial learning and memory. These results further validate the role of CaV1.2 channels are playing in ASD and support prior work done with this model. Following behavioral examination, all subjects' brains were extracted and underwent a neuroanatomical Nissl-stain assessment of various white matter and cortical structures.

Disclosures: **A.R. Rendall:** None. **A.L. Ford:** None. **P.A. Perrino:** None. **R.H. Fitch:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

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Topic: A.07. Developmental Disorders

Support: NIDA Grant DA021801

NICHD Grant HD036379

BCH Anesthesia Research Distinguished Trailblazer Award

Title: Disrupted Cav1.2 L-type calcium channel function and expression alters behavior and ascending serotonin system activity

Authors: *D. G. EHLINGER[1,2,3], C. M. PANZINI[2,3], K. G. COMMONS[2,3];
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Abstract: Human genetic variation in the gene *CACNA1C* altering expression of the Cav1.2 L-type calcium channel (LTCC) has been strongly associated with enhanced risk for the development of neuropsychiatric disorders including major depression, bipolar and schizophrenia. Furthermore, a missense mutation (G406R) of the Cav1.2 LTCC that results in reduced channel inactivation associated with Timothy Syndrome has been modeled in the TS2-neo mouse model of autism spectrum disorders (ASD). Here, we ask whether alterations in Cav1.2 LTCC function and expression influence a common neural circuit in the ascending serotonin (5HT) system. First, we assessed 5HT system abnormalities in the TS2-neo mouse. Following an acute stressor (forced-swim), behavioral analyses and immunofluorescent co-labeling of Tph2 and C-Fos reveals that TS2-neo mice exhibit enhanced active coping behavior, enhanced 5HT neuron activity, and altered 5HT1A-dependent feedback inhibition in both caudal and rostral subregions of the dorsal raphe nucleus (DRN). These alterations are accompanied by enhanced 5HT and 5HIAA content in forebrain regions including the orbitofrontal cortex, dorsal striatum and dorsal hippocampus. Next, we sought to determine whether temporally controlled knock-out of 5HT neuron Cav1.2 LTCCs also produces behavioral alteration and changes in 5HT neuron activity. To this end, we crossed Tph2-icre/ERT2 mice with Cav1.2-loxP/Ai14-TdTomato mice (Tph2-Cav1.2KO). Following tamoxifen treatment, preliminary results suggest that Tph2-Cav1.2KO mice display altered behavior on the forced-swim and open-field paradigms. Additional behavioral characterization and analysis of 5HT neuron activity within the DRN will provide context regarding these deficits. Collectively, our results suggest that disruptions of Cav1.2 LTCC function in the TS2-neo mouse and Cav1.2 LTCC expression within 5HT neurons may alter a common neural circuit in the ascending 5HT system. This

provides a potential neurological mechanism through which alterations in the gene *CACNA1C* may enhance risk for development of a broad range of psychiatric and developmental disorders.

Disclosures: D.G. Ehlinger: None. **C.M. Panzini:** None. **K.G. Commons:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

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Topic: A.07. Developmental Disorders

Support: NIH Grant NS082761-01

Epilepsy Foundation 367394

Title: Ablation of arx in mature interneurons impaired network functions via disruption of calcium homeostasis in the mouse hippocampus

Authors: D. J. JOSEPH[1], A. J. MCCOY[1], R. RISBUD[1], *J. G. JACKSON[2], E. D. MARSH[1,3]; [1]Neurol., [2]Neurosci., Children's Hosp. of Philadelphia, Philadelphia, PA; [3]Neurosci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Transcription factors (TFs) establish molecular codes that orchestrate the assembly of neural circuits. The Aristaless Related homeobox (*Arx*) protein is a TF within such regulatory network controlling early stages of GABAergic interneurons development. However, *Arx* remains expressed in mature interneurons, suggesting a divergent, yet unknown, function. We previously showed that postnatal ablation of *Arx*, in interneurons, resulted in abnormal EEG, altered synaptic transmission, and impaired long term plasticity (LTP) in the hippocampus. Here, we begin to investigate the molecular basis for the synaptic impairments induced by postnatal ablation of *Arx*.

We temporally controlled *Arx* expression by crossing a floxed *Arx* mouse ($Arx^{fl/fl}$) with a tamoxifen-inducible Cre^{ER}- mouse, resulting in complete loss of *Arx* 30 days after injection. To identify the mechanisms by which *Arx* impaired synaptic transmission and plasticity, we targeted intracellular Ca^{2+} handling given its implication in limiting plasticity at CA2 synapses. We reasoned that if achieving and maintaining homeostatic Ca^{2+} levels are the factors limiting LTP expression, then elevation of external Ca^{2+} or chelation of excess intracellular Ca^{2+} could reverse these deficits and rescue LTP. To that end, we recorded field excitatory postsynaptic potentials (fEPSPs) at the CA1 synapse in response to Schaffer collaterals (SC) stimulation in $Arx^{+/y}$ and $Arx^{-/y}$; Cre^{ER} mice in normal recording solution or in solution containing high extracellular Ca^{2+}

or BAPTA-AM. We measured the input-output (I/O) coupling of fEPSPs to assess synaptic transmission under those recording conditions. To measure LTP, baseline fEPSPs were recorded for 30 min in normal recording solution or in high Ca^{2+} or BAPTA-AM for the last 5 min of baseline recordings and post-tetanus fEPSPs were recorded in normal conditions.
Our results demonstrated that *Arx*$^{-/y}$; Cre^{ER} mice had reduced LTP and perfusion with higher external [Ca^{2+}] during induction partially restored CA1 LTP to *Arx*$^{-/y}$ slices. This restoration of CA1 LTP in *Arx*$^{-/y}$; Cre^{ER} mice was also noted when Ca^{2+} influx was partially blocked by reducing the extracellular Ca^{2+}/Mg^{2+} ratio. Application of BAPTA-AM partially attenuated the impairment in I/O coupling of fEPSP and completely restored CA1 LTP to *Arx*$^{-/y}$ slices.
Our results suggest that the cellular machinery required for LTP expression remains intact following loss of *Arx* but this postnatal loss of *Arx* resulted in elevation of postsynaptic Ca^{2+} that limits SC-CA1 LTP. These data suggest that modulation of Ca^{2+} buffering capacity may be temporally targeted for therapy in patients with *Arx* mutations and more broadly in epilepsy.

Disclosures: D.J. Joseph: None. **A.J. McCoy:** None. **R. Risbud:** None. **J.G. Jackson:** None. **E.D. Marsh:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

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Topic: A.07. Developmental Disorders

Support: NIH Grant F31 MH098636

NIH Grant NS047700

Tourette Syndrome Association

Title: EphrinA5$^{-/-}$ mice display behavioral abnormalities and exhibit altered striatal organization and synaptic function

Authors: *L. F. KROMER[1], R. WURZMAN[2], S. VICINI[3], J. G. PARTRIDGE[3];
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Abstract: Disruptions in striatal organization and dysfunction in corticostriatal circuits are implicated in multiple neurodevelopmental spectrum disorders, such as OCD, ADHD, and autism. Currently, we have limited understanding of the molecular and cellular events that

contribute to the development of shared versus unique neuropsychiatric behaviors associated with these disorders. To address this issue, we have begun studying mice with Eph receptor or ephrin deletions (E/E) as models for investigating how disruptions in the function of cell-cell signaling molecules that regulate neuronal migration, axon guidance, and synaptogenesis produce corticostriatal circuit abnormalities and spectrum disorder behavioral endophenotypes. During development, E/E expression is tightly regulated temporally and regionally in striatum (matrix neurons express EphA4 and matrisome neurons express EphA7 receptors) and cortex (EphA7 is highly expressed in specific cortical subregions). Moreover, there is an inverse correlation in the topographic organization of corticothalamic and corticostriatal axons expressing high levels of ephrinA5 with thalamic and striatal regions expressing high EphA7 levels. Although mice with ephrinA5 gene deletions have abnormal corticothalamic and thalamocortical projections, it is not known whether there are disruptions in corticostriatal projections or what behavioral abnormalities these mice exhibit. Thus, the present study behaviorally characterized ephrinA5$^{-/-}$ mice and examined whether these mice have altered striatal compartment organization and corticostriatal synaptic function. Using immunohistochemical procedures, we determined that ephrinA5$^{-/-}$ mice possess a normal striosome/matrix organization but exhibit expansion of the matrisome compartment compared to controls. Behavioral analyses indicated that ephrinA5$^{-/-}$ mice exhibit hyperactivity in both the open field and elevated plus maze and have impaired learning in the Morris water maze and accelerating rotarod task. Using whole-cell voltage clamp and traditional stimulating techniques in acute brain slices from BAC-*drd2*-eGFP:ephrinA5$^{-/-}$ mice, we detected several measures of synaptic transmission that differed between control and ephrinA5$^{-/-}$ mice. This included the ratio of AMPA- and NMDA-mediated components of synaptic transmission on spiny projection neurons and glutamate release probability. Together, these results support a developmental role for ephrinA5 in striatal organization and corticostriatal synaptic functions involved in regulating important cognitive and motor behaviors affected in neurodevelopmental spectrum disorders.

Disclosures: **L.F. Kromer:** None. **R. Wurzman:** None. **S. Vicini:** None. **J.G. Partridge:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

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Topic: A.07. Developmental Disorders

Support: Alamaya Foundation

Title: Deletion of the NR2A subunit of NMDAR increases susceptibility to redox dysregulation causing long-term impairment of prefrontal parvalbumin interneurons and perineuronal net.

Authors: *P. STEULLET[1], R. CARDIS[2], J.-H. CABUNGCAL[3], K. Q. DO[3];
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Abstract: De-novo mutations in GRIN2A gene, coding for the NR2A subunit of NMDA receptors (NMDAR), are linked to developmental brain disorders such as mental retardation, epilepsy, autism, and schizophrenia. A large scale GWAS study has revealed GRIN2A as a risk gene for schizophrenia. NR2A plays a critical role during early postnatal brain development as it undergoes an increase in expression in parallel with a decrease in expression of NR2B. While administration of general NMDAR antagonists during the second postnatal week affects durably prefrontal parvalbumin interneurons via oxidative stress, specific NR2A antagonists also impact the maturation of these neurons. The goal of the study was to assess the effect of a lack of NR2A (GRIN2A KO) on parvalbumin interneurons in the medial prefrontal cortex, and whether the observed anomalies of this type of interneurons in GRIN2A KO mice are the consequence of an enhanced susceptibility to redox dysregulation. While the number of parvalbumin immunoreactive (IR) neurons and the perineuronal net (PNN) surrounding many of these interneurons were normal in young adult (P60) KO mice, the PNN was less developed in young (P20) KO compared to WT mice. This suggested a delayed maturation of the PNN in GRIN2A KO mice. In both ages, KO mice had the tendency to display higher immunoreactivity for 8-oxo-dG (an oxidative stress marker) than age-matched WT mice. Our primary findings revealed a susceptibility to redox dysregulation in GRIN2A KO mice. Indeed, an additional oxidative challenge (dopamine uptake inhibitor, GBR12909) applied during early postnatal life (P10-P20) significantly increased oxidative stress in KO but not WT mice. GBR12909-treated KO mice had also a decreased number of parvalbumin-IR neurons and a further weakening of the PNN. These effects were prevented by a treatment with the antioxidant, N-acetylcysteine. The expression of sulfiredoxin 1 (from the peroxiredoxin system) and the modulatory subunit of glutamate-cysteine ligase (from the glutathione system) were significantly reduced in KO compared to WT mice, suggesting that antioxidant capacities were weakened in mice lacking NR2A. Early postnatal oxidative challenge had long term consequences in KO mice as it led to oxidative stress, reduced number of parvalbumin-IR neurons, and abnormal PNN at early adulthood (P60). Moreover, an early-life treatment with GBR12909 reduced the power of fast oscillatory activity in prefrontal slices of adult KO but not WT mice. Altogether, these indicate that functional impairment of NR2A-containing NMDAR confers a susceptibility to redox dysregulation, which can have long term impact on parvalbumin interneuron networks.

Disclosures: **P. Steullet:** None. **R. Cardis:** None. **J. Cabungcal:** None. **K.Q. Do:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

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Topic: A.07. Developmental Disorders

Support: Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO) 917.10.372

Title: Loss of oligophrenin-1 in medial prefrontal cortex leads to age-dependent synaptic dysfunction

Authors: *T. KROON, H. MANSVELDER, R. M. MEREDITH;
VU Univ., Amsterdam, Netherlands

Abstract: Oligophrenin-1 (OPHN1) is a Rho GTPase-activating protein whose dysfunction leads to syndromic intellectual disability. Absence of OPHN1 has been shown to affect synaptic function and morphology of dendrites and spines in cultured neurons, as well as in acute hippocampal slices. However, little is known about the effect of OPHN1 on other regions of the brain. Furthermore, it is not known whether the role of OPHN1 at the synapse depends on the developmental stage of the animal. The medial prefrontal cortex (mPFC) is involved in decision making and social behaviour, making it a promising target for the study of neurodevelopmental disorders. To study the effect of OPHN1 dysfunction throughout development, we assessed synaptic function in mPFC slices from OPHN1 knockout mice at several ages using whole-cell electrophysiology. We find a reduction of both glutamatergic and GABAergic synaptic transmission that is dynamic and dependent on age, indicating that OPHN1 functions in a developmentally regulated manner.

Disclosures: T. Kroon: None. **H. Mansvelder:** None. **R.M. Meredith:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 32.21/D20

Topic: A.07. Developmental Disorders

Support: NIH Shared Instrumentation grant S10RR023706

John and Mary Franklin Foundation

Title: Do neuroanatomical abnormalities in Pax6-deficient mice change as a function of age?

Authors: *M. K. GRANT[1], A. M. BOBILEV[2], K. K. JOHNSON[1], K. HEKMATYAR[3], J. D. LAUDERDALE[1];
[1]Cell. Biol., [2]Neurosci., [3]Bio-Imaging Res. Ctr., Univ. of Georgia, Athens, GA

Abstract: The *PAX6* gene encodes a highly conserved transcription factor required for various aspects of anatomical and functional development of the eye and brain. Heterozygous loss-of-function mutations in *PAX6* cause aniridia in humans and the *Small eye* trait in rodents. Although aniridia is best known as a panocular eye disorder, the condition has a number of structural and functional abnormalities in the brain. Previous studies of patients with aniridia using structural magnetic resonance imaging (MRI) have shown changes in interhemispheric connectivity, grey matter volume, and accelerated age-related cortical thinning. The full scope of neuroanatomical changes in humans, and the conservation of these changes in model organisms remain poorly understood. We predicted two major contributors to the changes in adult brain structure and function: 1) structural changes that occur as a result of altered development, and 2) structural changes in brain regions that require PAX6 for maintenance throughout adulthood. The current study evaluated neuroanatomical consequences of Pax6 haploinsufficiency using the PAX6$^{Sey\ Neu/+}$ mouse model to distinguish between developmental and age related changes across the lifespan. This study employed MRI using a 7T Agilent system to acquire structural brain images using 3D T2 weighted fast spin echo sequences. Thirty-two mice (16 PAX6$^{Sey\ Neu/+}$, 16 wild-type littermates; 50% age 4-5 months, 50% 12-13 months, outbred background) underwent MRI scans under isofluorine (TR/TE 400/44 msec, nt=4). Following in vivo imaging, animals were euthanized following approved protocols, and their brains sectioned for histological comparison. PAX6$^{Sey\ Neu/+}$ mice 4-5 months old show significant volume increases in grey matter in the medial olfactory bulb, cerebral cortex, midbrain, and cerebellum. Increases in white matter volume were seen in the posterior corpus callosum forceps, arbor vitae, dorsal hippocampal commissure, and cerebellum. Comparisons to histological sections show that MRI grey matter changes reflect abnormalities in restricted cell layers in several brain regions, particularly in the medial olfactory bulb and dentate gyrus of the hippocampus. White matter changes reflect overall differences in tract size. In addition, preliminary results comparing age groups suggest changes in specific brain structures are exacerbated in older PAX6$^{Sey\ Neu/+}$ mice, indicating that disruption of Pax6-dependent maintenance functions in the adult brain leads to an accelerated aging phenotype. Overall, this model provides a comparison for human aniridia towards identifying conserved neuroanatomical consequences of PAX6 deficiency.

Disclosures: **M.K. Grant:** None. **A.M. Bobilev:** None. **K.K. Johnson:** None. **K. Hekmatyar:** None. **J.D. Lauderdale:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 32.22/D21

Topic: A.07. Developmental Disorders

Title: Gnas imprinted gene, a new player connecting neurodevelopment, sleep and cognition

Authors: *C. GARCIA-GARCIA, E. BALZANI, C. CHIABRERA, E. ALBANESI, L. CANCEDDA, V. TUCCI;
Inst. Italiano di Tecnologia, Genova, Italy

Abstract: Recent evidence has pointed out that imprinted genes, which exert fundamental roles in embryonic development, are also important in the regulation of sleep and cognitive processes in adulthood. Moreover, sleep abnormalities and cognitive defects are hallmarks of several neurodevelopmental disorders, suggesting that neurodevelopment is a pivotal time window for the formation of sleep and cognition. However, the link between developmental mechanisms and sleep remains largely unknown. Recently, our group has demonstrated that loss of imprinting in the GNAS locus dramatically affects sleep physiology and cognitive functions in mice. GNAS main product, Gαs, codifies for the G protein α-subunit, essential for the generation of intracellular second messenger cAMP. In order to further understand the role of Gαs in neural development and sleep formation, we knocked down its expression with 3-electrode intrauterine electroporation in mice hippocampus and frontal cortex during development. Our results demonstrate that Gαs is involved in pyramidal neuron morphology and migration during neurodevelopment. Specifically, Gαs knocked down neurons are characterized by more and longer branches; while the 20% of them are misplaced in the stratum radiatum of the hippocampus and the layer I of the frontal cortex. Moreover, we discovered that Gαs downregulation during development has also a modulatory effect on sleep and memory consolidation later in life. Specifically, Gαs downregulation in hippocampal neurons leads to a detrimental effect in NREM quality, and dramatically reduces the contextual freezing response of these animals following fear conditioning. Overall, this work provides new insights into the link between genomic imprinting, neurodevelopment, sleep and cognition.

Disclosures: **C. Garcia-Garcia:** None. **E. Balzani:** None. **C. Chiabrera:** None. **E. Albanesi:** None. **L. Cancedda:** None. **V. Tucci:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

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Program#/Poster#: 32.23/D22

Topic: A.07. Developmental Disorders

Support: MR/K004603/1

Title: Effects of Transient P7 DISC1 signalling disruption on LTP in the prefrontal cortex

Authors: *N. R. HARDINGHAM[1], K. FOX[2];
[2]Sch. of Biosci., [1]Cardiff Univ., Cardiff, United Kingdom

Abstract: DISC1 has been identified as a schizophrenia risk factor and has an important role in brain development. We have used a transgenic mouse that expresses a fragment of the DISC1 c-terminal (DISC1-cc) and acts as a dominant negative when activated for 24-48 hours by a single injection of tamoxifen. Transient activation of the mutant protein at P7 results in schizotypic behaviour in the mouse (Li *et al*, 2007) and a loss of experience dependent plasticity and LTP in layer 2/3 of adult barrel cortex (Greenhill et al 2015) Schizophrenia has been strongly linked to dysfunction in the prefrontal cortex with both functional and structural deficits documented. Therefore we set out to investigate whether following P7 disruption of DISC1 signalling, synaptic plasticity is similarly affected in the prefrontal cortex to the barrel cortex. We recorded from layer 3 neurons in medial prefrontal cortex, stimulating the hippocampal input in a modified coronal slice. We used two protocols to induce LTP, a theta burst protocol, consisting of trains of 5 pre-synaptic stimulations followed by 5 post-synaptic spikes at a frequency of 20Hz and an HFS protocol consisting of trains of presynaptic stimulation at 50Hz. We applied dopamine chronically or acutely; either perfusing slices in ACSF containing 3μM dopamine or used a spritzer to apply a 10s puff of 50μM dopamine at the end of the theta burst protocol. Using a low concentration of picrotoxin (2.5 μM or 5μM) in addition to the 3μM dopamine produced significant LTP in wild types (143 ± 78% change in mean amplitude) but not in DISC1-cc mice following P7 DISC1 disruption (15 ± 16% change in mean amplitude, effect of genotype $p<0.001$). Dopamine was necessary for induction of LTP in WTs (change in mean amplitude = 3 ± 24% in absence of dopamine). Our results suggest that development of LTP in adult prefrontal cortex requires normal DISC1 function at P7, in common with barrel cortex. The lack of adult LTP cannot be explained by an alteration in the inhibitory system, as the impairment in LTP is present in DISC1-cc mice in the presence of a GABA antagonist, which in turn implicates the deficit in the function of the excitatory synapses.

Disclosures: **N.R. Hardingham:** None. **K. Fox:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 32.24/D23

Topic: A.07. Developmental Disorders

Support: NJ Governor's Council for Medical Research and Treatment of Autism 10-407-SCH-E-0

Rutgers GSBS Spring 2016 PhD completion and acceleration fellowships

Title: Engrailed-2 is a cell-autonomous regulator of proliferation and survival in cultured hippocampal neural stem cells

Authors: *M. DURENS[1], S. CHUNG[2], E. DICICCO-BLOOM[2];
[1]Rutgers Univ. - Busch Campus, Piscataway, NJ; [2]Rutgers Robert Wood Johnson Med. Sch., Piscataway, NJ

Abstract: En2 is a transcription factor with canonical function in mid-hindbrain development. Its roles in cerebellar patterning are well-studied, and gene deletion (En2-knockout, En2-KO) produces diverse cerebellar defects. However, almost nothing is known about En2 function outside the mid-hindbrain, despite several studies finding low-level expression in forebrain areas including hippocampus. Significantly, En2-KO mice display deficits in forebrain-related behaviors including spatial memory, fear conditioning, and social interaction. En2-KO hippocampus exhibits structural deficits including reduced weight, size, and neuron number. At P21, En2-KO dentate gyrus contains 17% less granule cells, and exhibits 2-fold increase in neural stem cell (NSC) proliferation and 70% increase in cell death. While we showed decreased monoamine innervation contributes to these defects, we hypothesize that En2 expression in hippocampus also contributes to dysregulated neurogenesis. This study examines possible cellular roles for En2 in hippocampal NSC development by using neurosphere cultures. To define cell-autonomous effects, dissociated cells from P7 En2 wild-type (WT) and KO mice were incubated in defined media with EGF and FGF until neurospheres formed. We assessed neurosphere size and number, and markers of proliferation (BrdU) and apoptosis (cleaved-caspase3, pyknosis). We detected expression of En2 mRNA in (WT) but not KO-derived neurospheres. Expression was also detected through immunostaining of an En2-LacZ reporter. Consistent with the heterogeneous composition of neurospheres, the LacZ reporter was only detected in a subset of cells, whose identity is being defined by double labeling with neural markers Sox2, Tbr2, and Tuj1. Compared to WT spheres, KO spheres exhibit a 22% increase in diameter ($p<0.01$), as well as 2-fold increases in proliferation ($P<0.005$) and apoptosis ($p<0.05$), consistent with the phenotype observed in dentate gyrus in vivo. The majority of proliferating cells (~90%) in both genotypes were Sox2+ suggesting that En2 may have a role in maintaining

quiescence of NSCs or promoting differentiation. Overall, these studies suggest En2 expression plays a cell-autonomous role in regulating proliferation and survival in hippocampal NSCs. In turn, these findings in culture prompt further investigation in vivo, where there is absence of En2-LacZ signal, and no apparent evidence of mRNA by in situ hybridization. More broadly these observations suggest that En2 may influence hippocampal development and function through both cell autonomous and non-cell autonomous mechanisms.

Disclosures: M. Durens: None. **S. Chung:** None. **E. DiCicco-Bloom:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 32.25/D24

Topic: A.07. Developmental Disorders

Title: Cerebellar corticogenesis in the lysosomal acid phosphatase (acp2) mutant mice; purkinje cell neurodevelopmental disorder

Authors: *N. ASHTARI[1], X. JIAO[2], M. RAHIMI BALAEI[2], K. BAILEY[2], S. GHAVAMI[2], H. MARZBAN[2];
[1]Dept. of Human Anat. and Cell Sci., [2]Univ. of Manitoba, Winnipeg, MB, Canada

Abstract: Introduction: A mouse mutant called *nax* (naked-ataxia); resulting from a spontaneous mutation in Lysosomal Acid Phosphatase (*Acp2*) gene shows severe cerebellar defects and neuronal degeneration in its cerebellum. Cerebellum is a brain region important for motor control and cognition. In the *Acp2* mutant mouse, three layer cortex (Granule cells (gcs), Purkinje cells (Pcs) and Molecular layer) were found to decrease significantly and monolayer Pcs turn to multi-layered Pcs that ectopically invade the molecular layer. Reelin is an important large extra-cellular signaling protein important in Pcs monolayer formation in cerebellar cortex. It is expressed by gcs and is required for Pcs distribution from the clusteric stage to establish a monolayer of Pcs between the molecular and granular layers of the cerebellar cortex. We hypothesize that the establishment of mono layered Pcs is independent to the Reelin pathway, however rely on Reelin through MAPK signaling pathway.
Methods: *Acp2* mutant mice were used for this study and molecular expression and distribution were assessed by immunohistochemistry and Western blotting.
Results: The cerebellar cortex of the *Acp2* mutant mice reveals the presence of Pcs in a randomized, dispersed manner spanning the entire molecular layer rather than a monolayer in the cerebellar cortex. The pattern of Reelin expression shows a down-regulation in both wild type and *nax* mouse, while lower amount of protein is detected in *nax* mutant at P4 (around Pc layer

formation) compared to wild type. Pcs differentiation is severely delayed in the *Acp2* mutant cerebellar cortex while the presence of Reelin is comparable with wild type during early postnatal development. It is indicative of Reelin effect during clustric stages however failed to form mono layer Pcs.
Conclusion: It is concluded that multilayer Pcs may be due to the failure of appropriate cross-talk between *Acp2* and the Reelin signaling pathway during early postnatal cerebellar development.

Disclosures: **N. Ashtari:** None. **X. Jiao:** None. **M. Rahimi Balaei:** None. **K. Bailey:** None. **S. Ghavami:** None. **H. Marzban:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 32.26/D25

Topic: A.07. Developmental Disorders

Title: Neurofibromin loss of function leads to increased spontaneous grooming in *Drosophila*

Authors: *L. B. KING[1], M. KOCH[1,1], K. R. MURPHY[2,4], Y. VELAZQUEZ[1,3], W. W. JA[2], S. TOMCHIK[1];
[1]Dept. of Neurosci., [2]Dept. of Metabolism and Aging, [3]SURF Program, The Scripps Res. Inst., Jupiter, FL; [4]Integrative Biol. Grad. Program, Florida Atlantic Univ., Jupiter, FL

Abstract: Neurofibromatosis I is a common genetic disorder that results in tumor formation and predisposes individuals to a range of cognitive and behavioral symptoms, including deficits in attention, visuospatial skills, learning, language development, sleep, and autism spectrum disorder-like traits. The *nf1*-encoded neurofibromin protein (Nf1) exhibits high conservation, from the common fruit fly, *Drosophila melanogaster*, to humans. *Drosophila* is a powerful model with which to investigate the signaling cascades upstream and downstream of Nf1, and exhibits similar behavioral phenotypes to mammalian models. In order to understand how loss of Nf1 affects motor behavior in flies, we combined traditional infrared beam based activity monitoring with video analysis of grooming behavior. In *nf1* mutants, spontaneous grooming was increased up to 7x. This increase in activity was distinct from previously-described dopamine-dependent hyperactivity, as dopamine transporter mutants exhibited slightly decreased grooming. In addition, we verify the utility of a published set of conditional driver lines to produce reliable and precise grooming behavior targeted towards specific body parts. Here, we use optogenetic stimulation of a red-shifted channel rhodopsin, CsChrimson, to produce grooming in an open field arena. Overall, these data suggest that loss of *nf1* results in increased

activity that is at least in part manifested as increased grooming, providing a platform to dissect the molecular genetics of neurofibromin signaling across neuronal circuits.

Disclosures: L.B. King: None. **M. Koch:** None. **K.R. Murphy:** None. **Y. Velazquez:** None. **W.W. Ja:** None. **S. Tomchik:** None.

Poster

032. Genetic Animal Models of Neurodevelopmental Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 32.27/D26

Topic: A.07. Developmental Disorders

Support: Start-up funds Lewis & Clark College

Title: Determining the locomotor effects and expression of thrombospondin in *Drosophila melanogaster* larva

Authors: E. LOWENSTEIN[1], *N. A. VELAZQUEZ ULLOA[2];
[1]Biochem. and Mol. Biol. Program and Dept. of Mathematical Sci., [2]Lewis & Clark Col., Portland, OR

Abstract: Thrombospondin (TSP), a gliotransmitter, has been implicated in promotion of synapse formation through binding to the $\alpha_2\delta$-1 subunit of neuronal voltage-gated Ca^{2+} channels in rat retinal ganglion cell cultures, as well as through neuroligin-1 in rat hippocampal neurons. In *Drosophila melanogaster*, $\alpha_2\delta$-3 and neuroligin-2, with functional homology to $\alpha_2\delta$-1 and neuroligin-1, promote formation of boutons at the neuromuscular junction and neuroligin-2 mutants display abnormal locomotion. Additionally, TSP has been shown to be involved in tendon adhesion in *D. melanogaster* (D-TSP). Given TSP's role in the nervous system, this study aims to utilize *D. melanogaster* to study the effects of TSP at multiple levels of action: developmental expression and associated behavioral phenotypes. Characterization of the expression of TSP in the larvae brain through whole-mount immunohistochemistry is underway, and affinity chromatography and qPCR will be used to validate the microscopy results. Preliminary locomotion analysis of D-TSP knockout larvae in open crawling chamber suggests reduced reverse crawling and turns. Future studies hope to probe the role of TSP in the *D. melanogaster* embryo and adult to understand its function within the framework of development.

Disclosures: E. Lowenstein: None. **N.A. Velazquez Ulloa:** None.

Poster

033. Dopamine

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 33.01/D27

Topic: H.01. Animal Cognition and Behavior

Support: NCTR E6979

Title: The effects of dopaminergic receptor ligands on the performance of nonhuman primates in a comprehensive, translational cognitive operant test battery (OTB)

Authors: *J. L. WALTERS, J. J. CHELONIS, M. P. GILLAM, J. C. TALPOS, M. G. PAULE; Natl. Ctr. for Toxicological Res. (NCTR), Jefferson, AR

Abstract: The effects of dopaminergic receptor ligands on cognitive function have been widely studied in rodents, yet limited research has assessed the effects of these compounds on cognition in nonhuman primates. Thus, the purpose of this experiment was to assess the sensitivity of the National Center for Toxicological Research (NCTR) operant test battery (OTB) to detect the effects of acute administration of dopamine (DA) receptor agonists and antagonists in rhesus monkeys. Here, we sought to determine the differential sensitivity of tasks in the NCTR OTB to manipulations of the DA system and characterize the effects of these compounds in a preclinical model with greater relevance to humans. The test battery is thought to measure aspects of motivation, learning, color and position discrimination, time estimation, and short-term memory and has been proven to be sensitive to an array of psychotropic compounds. Quinpirole (0.001-0.75 mg/kg), sulpiride (0.3-10.0 mg/kg), SCH 23390 (0.03-0.3 mg/kg), SKF38393 (0.03-3.0 mg/kg), and corresponding vehicles were administered to young adult males trained to perform the NCTR OTB, which consisted of progressive ratio, incremental repeated acquisition, temporal response differentiation, conditioned position responding, and delayed matching to sample tasks. Results revealed all four dopaminergic compounds differentially affected OTB performance. The D2/D3 agonist, quinpirole, produced the most disruptive effects, decreasing accuracies in all of the tasks with some impairments occurring at doses that did not affect response rates. The D1/D5 partial agonist, SKF38393, did not impair accuracy in any of the tasks and produced minimal effects on response rates while leaving cognitive abilities relatively intact. The D1 antagonist, SCH 23390, and the D2/D3 antagonist, sulpiride, were both found to decrease response rates while having limited to no effects on accuracies, suggesting that these compounds, at the doses given here, primarily disrupt motor behaviors while having modest or no effects on cognition. In summary, these results demonstrate that the NCTR OTB is sensitive to detecting differential effects of compounds that act at D2/D3 receptors and D1/D5 receptors, indicating that this battery is a valid tool for detecting undesirable effects associated with pharmacological manipulations of the DA system. Further, the data suggest this battery can be used to assess the

effects of current and future drugs designed to treat aspects of disorders/diseases related to DA system disruption, such as schizophrenia, Parkinson's disease, and attention deficit hyperactivity disorder. Supported by NCTR/FDA E6979

Disclosures: **J.L. Walters:** None. **J.J. Chelonis:** None. **M.P. Gillam:** None. **J.C. Talpos:** None. **M.G. Paule:** None.

Poster

033. Dopamine

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 33.02/D28

Topic: B.07. Synaptic Transmission

Support: FONDECYT N° 110392

FONDECYT N° 1150244

Title: Lateral septum stimulation induces and increase in GABA and dopamine extracellular levels in the rat ventral tegmental area

Authors: I. M. VEGA[1], H. E. YARUR[1], *K. GYSLING[2];
[1]Dept. of Cell. and Mol. Biol., [2]Pontificia Univ. Catolica De Chile, Santiago, Chile

Abstract: The lateral septum (LS) is involved in positive reinforcement as shown by James Olds and Peter Milner in 1954. The LS belongs to a complex limbic circuit in which the ventral tegmental area (VTA), prefrontal cortex (PFC), and the nucleus accumbens (Nac) connections play a critical role in the motivated behavior. The dysfunction of this circuit is crucial in diseases such as addiction. Dopamine (DA) is the key neurotransmitter of this circuit, and is mainly released from VTA neurons that connect the VTA with the rest of the limbic circuit, inducing and regulating motivated behavior. In this sense, the regulation of dopamine release in the VTA is critical for the activity of VTA dopamine neurons and for the functional integration of the rest of limbic system areas. In this study we performed in vivo dual-probe microdialysis to reveal the neurochemical relationship between LS and VTA, when we stimulate the LS such as Olds and Milner.We observed a significant increase in the extracellular levels of GABA and DA in the VTA after a depolarizing stimulus in the LS. We also observed that the infusion of muscimol/baclofen in LS resulted in a significant decrease in VTA GABA levels after LS stimulation but not in VTA DA levels that remain unchanged. We also studied the effect of LS stimulation after VTA infusion of bicuculline (GABA-A receptor antagonist) or CPG-52432 (GABA-B receptor antagonist). These results showed that in the presence of bicuculline in the

VTA, DA VTA levels did not increase after LS stimulation but VTA GABA extracellular levels increased.Thus, our data suggest that there is a gabaergic projection from the LS to VTA gabaergic interneurons and that its activation results in a disinhibition of VTA dopaminergic neurons. Further studies should prove if this proposed connections are involved in the positive reinforcement effects induced by LS stimulation.

Disclosures: I.M. Vega: None. **H.E. Yarur:** None. **K. Gysling:** None.

Poster

033. Dopamine

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 33.03/D29

Topic: H.01. Animal Cognition and Behavior

Support: R01MH048404

T32DA031111

Title: Sex differences in behavior and neurophysiology during reward approach and punishment avoidance

Authors: *T. G. CHOWDHURY[1], N. W. SIMON[1], R. DUTTA[2], B. MOGHADDAM[1]; [1]Dept. of Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; [2]Stanford Univ., Stanford, CA

Abstract: Men and women show different rates of diagnosis of various psychiatric illnesses. Men are more commonly diagnosed with schizophrenia and substance abuse disorders, while major depression and anxiety disorders are more common in women. These epidemiological patterns suggest that biological sex differences may underlie the gender disparities in vulnerability to psychiatric disease. To study the sex differences in the neurobiology relevant to psychiatric illnesses, we have used a novel paradigm for simultaneously testing two different motivated behaviors, namely approach and avoidance, in rats. Furthermore, we tested the effect of an experimental model of anxiety on these two. Rats are first trained on two separate tasks: (1) to perform a nose-poke action to obtain a reward and (2) to perform a lever-press action to avoid an impending foot-shock. Once the rats are trained to a criterion of >70% on these two tasks, they enter the testing phase in which both tasks are presented during the same session. This allows concurrent measurement of reward seeking and punishment avoidance. Male and female rats showed similar rates of successful reward attainment, and anxiety caused a similar reduction in correct reward trials completed in males and females. While females acquired the avoidance behavior faster than males, males showed a greater rate of shock avoidance once the behavior

was stable. In males, anxiety diminished performance on avoidance trials, while the drug had no effect in female avoidance behavior. We are currently exploring the neural correlates of these behaviors by recording from VTA dopamine neurons.

Disclosures: T.G. Chowdhury: None. **N.W. Simon:** None. **R. Dutta:** None. **B. Moghaddam:** None.

Poster

033. Dopamine

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Program#/Poster#: 33.04/D30

Topic: H.01. Animal Cognition and Behavior

Support: NRF-2015R1A2A2A01007838

Title: Agitating dopamine d1 receptor prevents long-term special memory loss.

Authors: *J. ZHANG;
Dept. of Oriental Phamaceutical Sci., Seoul, Korea, Republic of

Abstract: Dopamine D1 receptors 1 (D1 receptors) have a function of memory improvement according to the previous studies, but it is unclear whether agitating D1 receptors can prevent memory loss or extend memory. In our study, we hypothesized whether D1 receptor agonist, SKF 38393 could prevent spatial memory loss using Barnes Maze test (BM test). We found that the acquired spatial memory through three trials per training for four days in BM test has maintained for 21 days, and the memory had begun to fade down from the 14^{th} day after training. So on the 14^{th} Day after training administration of SKF 38393 for 7 days has prevented the natural memory extinction process, and administration of the D1 receptor antagonist, SCH 23390 blocked this process. In the western blotting, administration of SKF 38393 increased the phosphorylation of PKA and CREB, which indicated that SKF 38393 ameliorated memory consolation through cAMP-PKA-CREB signaling pathway. These results suggest that dopamine D1 receptors are associated with the consolation of long-term spatial memory by cAMP-PKA-CREB signaling pathway. Key words: SKF 38393; Long-term memory; Dopamine D1 receptor; Memory persistance

Disclosures: J. Zhang: None.

Poster

033. Dopamine

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Topic: B.07. Synaptic Transmission

Support: NIH F30 DA040996

NIH DA 35821

NIH NS 95809

NIH T32 GM007250

Title: Regional heterogeneity of dopamine D2-receptor signaling in the dorsal striatum and nucleus accumbens

Authors: *P. F. MARCOTT, C. P. FORD;
Physiol. and Biophysics, Case Western Reserve Univ., CLEVELAND, OH

Abstract: The striatum is the input nucleus of the basal ganglia and is involved in goal-directed behaviors, motivation, and reward processing. Subdivided into the dorsal striatum (DStr) and nucleus accumbens (NAc), both regions are made up predominantly of medium spiny neurons (MSNs) and receive dense dopaminergic innervation from the midbrain. Despite these similarities, the DStr and NAc have different afferent and efferent connections as well as unique behavioral functions. Regional differences in dopamine release properties and reuptake have been identified using fast scan cyclic voltammetry, but it is important to understand how these differences correspond to differences in postsynaptic receptor activation in these striatal subregions. To examine how the synaptic release of dopamine activates D2-receptors on MSNs in the DStr and NAc, G-protein activated inwardly rectifying potassium (GIRK2; K_{ir} 3.2) channels were virally overexpressed in the striatum and the resulting outward currents were used as a sensor of D2-receptor activation. Optogenetic stimulation of dopamine terminals evoked robust D2-receptor mediated inhibitory postsynaptic currents (IPSCs) in GIRK2-expressing MSNs. D2-IPSCs recorded in the NAc were significantly slower than those in the DStr. Regional differences in reuptake, spillover, terminal density, and transmitter diffusion distance were examined as potential explanations for the difference in D2-IPSC kinetics. The finding that D2-IPSCs have different kinetics in the dorsal and ventral striatum may have important consequences for how D2-receptors encode dopamine signals in these striatal subregions.

Disclosures: P.F. Marcott: None. **C.P. Ford:** None.

Poster

033. Dopamine

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Topic: B.07. Synaptic Transmission

Support: NIH T32 Predoctoral Training Grant in Cognition and Cognitive Disorders

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NIH T32 Predoctoral Training Grant in Cell and Molecular Biology

Title: Altered dopamine D4 receptor dependent regulation of synaptic transmission and hippocampal circuit function in PGC-1α-/- mice

Authors: *L. BRADY, A. BARTLEY, Q. LI, L. DOBRUNZ;
Neurobio., Univ. of Alabama At Birmingham, Birmingham, AL

Abstract: GABAergic interneurons control neuronal excitability, integration, and plasticity & have been shown to be altered in various neurological diseases that have cognitive impairment as a core symptom. In schizophrenia for example, post mortem studies have consistently shown transcriptional dysregulation in GABAergic interneurons, in particular fast-spiking interneurons containing the calcium binding protein parvalbumin (PV). To model this, our lab uses mice with genetic deletion of PGC-1α, a transcriptional coactivator that has been shown to be altered in a several neurological disorders. 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 in hippocampus, caused by enhanced inhibition and increased GABA release from interneurons. PGC-1α-/- mice also have enhanced gamma oscillation power and impaired nesting behavior, indicating hippocampal circuit dysfunction. GABAergic inhibitory synaptic transmission has been shown to be modulated by dopamine (DA) in hippocampus. In particular, DA D4 receptors have been observed to be located on PV+ GABAergic interneurons in hippocampus, and have been shown to be important for regulating gamma oscillations. 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. We observed that blocking D4 receptors resulted in an increased 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. We also found that specific D4 receptor antagonists rescued the deficit in basal synaptic transmission observed in PGC-1α-/- mice, and normalized the increased power of hippocampal gamma oscillations and the deficit in nesting

behavior. This suggests 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 also provide mechanistic support for atypical antipsychotics that have a higher affinity for D4 receptors, like Clozapine, in the treatment of cognitive symptoms of neurological disorders.

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

Poster

033. Dopamine

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 33.07/D33

Topic: B.07. Synaptic Transmission

Title: Dopaminergic transmission in nucleus accumbens shell neurons are modulated by methylphenidate.

Authors: ***C. REYES-VAZQUEZ**, A. VÁZQUEZ-ALVAREZ, D. PINEDA-VÁZQUEZ, S. RAMOS-MEJIA, B. PRIETO-GÓMEZ;
Depto. De Fisiología, Mexico DF, Mexico

Abstract: Dopaminergic (DA) neurons from ventral tegmental area (VTA) project to both, the core and shell subregions of the Nucleus Accumbens (NAc) in these regions DA release is induced by natural stimuli as well as by drugs of abuse. DA synaptic transmission is critically involved in reward-motivated behaviors, which are altered in addiction. Also, glutamate (GLUT) is packaged and released by a subset of mesolimbic DA neurons, eliciting Excitatory Postsynaptic Currents (EPSCs) onto medium spiny neurons in NAc. There is no precise information about the modulation of GLUT release from DA midbrain terminals by Methylphenidate (MTP). Using electrical stimulation to selectively activate midbrain DA fibers, we compared the properties and modulation of EPSCs measured using whole-cell recordings in rat brain slices. Rats (18 weeks old) that showed sensitization to methylphenidate in an open-field paradigm, and naive control rats, were anesthetized with isoflurane and killed by decapitation. Brains were quickly removed, mounted, and sliced. Slice preparation and whole cell patch clamp recordings were performed using standard techniques. For electrical stimulation, a glass pipette filled with ACSF was placed in the NAc shell and a rectangular pulse (0.2 ms) was applied every 2 min. The amplitude of the current pulse (100–250 μA) was adjusted to use the minimal current needed to generate a maximal and stable response. In both groups of rats, electrically induced EPSCs were inhibited by a DA receptor D2R agonist and showed a marked paired-pulse depression that required 2 min for a full recovery. MTP depressed EPSCs

amplitude by 50% in control rats, and until 80 % in sensitized rats. But, in both groups, MTP enhanced the overall DA transmission from midbrain DA neurons. AMPA and NMDA receptor-mediated EPSCs were equally inhibited by MTP in sensitized rats, suggesting a presynaptic mechanism of action. These findings demonstrate that MTP inhibits DA and glutamate release from midbrain DA neurons via presynaptic D2R but has differential overall effects on their transmissions in the NAc. Suggesting that MTP, by blocking DA reuptake, facilitates the feedback inhibition of DA and glutamate release from these terminals.

Disclosures: **C. Reyes-Vazquez:** None. **A. Vázquez-Alvarez:** None. **D. Pineda-Vázquez:** None. **S. Ramos-Mejia:** None. **B. Prieto-Gómez:** None.

Poster

033. Dopamine

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 33.08/D34

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH NINDS R01 NS084975

The Grainger Foundation

Title: Kinetic characterization of stimulation-evoked striatal dopamine release in a 6-hydroxydopamine-lesioned parkinsonian rodent model

Authors: ***S. PAEK**, J. K. TREVATHAN, A. D. BATTON, C. D. BLAHA, A. J. BIEBER, J. L. LUJAN, K. H. LEE;
Mayo Clin., Rochester, MN

Abstract: **Background** Dopamine (DA) is an important neurotransmitter, exhibiting critical roles in neurologic function during Parkinson's disease (PD). As such, the ability to characterize DA responses to therapeutic interventions such as deep brain stimulation (DBS) has important implications for studying the pathophysiology of disease. The non-linear and time-varying dynamics of stimulation-evoked DA responses have been characterized using mathematical models of DA release in healthy anesthetized animals. However, the complex relationship between electrical stimulation and consequent stimulation-evoked DA release has yet to be characterized in a Parkinsonian animal model. Thus, this study characterizes DA responses in the striatum of 6-hydroxydopamine (6-OHDA) lesioned rats in response to medial forebrain bundle (MFB) DBS. **Methods** The MFB was electrically stimulated in urethane-anesthetized 6-OHDA lesioned male Sprague-Dawley rats using a comprehensive range of stimulation parameters.

Concurrently, striatal DA release was measured using fast scan cyclic voltammetry in combination with carbon fiber microelectrodes. Evoked responses were characterized using a multi-compartment parametric model of DA release. The resulting kinetics were then compared to the kinetic responses from healthy anesthetized rats. **Results** Preliminary data suggest that the kinetics of stimulation-evoked DA release can be described using compartmental models and that these models can describe the forward relationships between electrical stimulation parameters (i.e., stimulus pulse width and amplitude) and stimulation-evoked extracellular dopamine responses. Further analysis will determine whether these relationships are altered following 6-OHDA lesioning. **Conclusions** Characterization of DBS induced DA release in a model of PD is an important step towards understanding neurotransmitter dynamics in the context of neurologic disease and therapeutic interventions. Ultimately, this work will aid in understanding the neurochemical effects of DBS and further the development of novel therapeutic strategies.

Disclosures: S. Paek: None. **J.K. Trevathan:** None. **A.D. Batton:** None. **C.D. Blaha:** None. **A.J. Bieber:** None. **J.L. Lujan:** None. **K.H. Lee:** None.

Poster

033. Dopamine

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 33.09/E1

Topic: B.07. Synaptic Transmission

Support: Lundbeck Foundation

Title: Differential dopamine potency across striatal d1 positive neurons

Authors: S. E. PEDERSEN[1], T. F. ANDREASSEN[1], J. K. DREYER[2], U. GETHER[1], *K. L. MADSEN[2];
[1]Dept. of Neurosci. and Pharmacol., Univ. of Copenhagen, Copenhagen N, Denmark; [2]Univ. of Copenhagen, Copenhagen, Denmark

Abstract: The major target of dopamine (DA) signaling is the medium spiny neurons (MSNs) located in the nucleus accumbens and the dorsal striatum of the brain. The majority of MSNs are classically categorized as belonging to either the direct or indirect pathway, defined by the expression of either D1 or D2 receptors (D1R or D2R), respectively. Sensitivity towards cortical inputs is regulated by, amongst others, DA. Local changes in DA levels have opposite effect on the direct and indirect pathways; In D1R positive neurons DA increases cAMP and protein kinase A (PKA) signaling, whereas DA leads to a decrease in cAMP/PKA signaling in D2R

positive neurons.
In this study we investigate the spatiotemporal regulation of PKA activity in striatal neurons in response to dopamine stimulation in an isolated MSN culture system. This approach allows us to study individual neurons with great temporal and spatial specificity. Using a FRET based biosensor of PKA activity (AKAR3) we are able to distinguish downstream effects of D1R activation reflected by intracellular time-dependent changes in the FRET ratio. Single neurons show significant FRET ratio changes upon stimulation with physiologically relevant concentrations of dopamine (100nM) illustrating the viability of this assay as a tool to monitor discrete signaling events in MSNs. Moreover, our data reveal a high degree of heterogeneity in the concentration-dependent PKA activation downstream of D1R in these neurons. Further, we show that D1R expression levels can modulate this observed variability in potency. Altogether, these findings illustrate the possibility of a hitherto unknown mechanism of action of DA-induced striatal signaling.

Disclosures: **S.E. Pedersen:** None. **T.F. Andreassen:** None. **J.K. Dreyer:** None. **U. Gether:** None. **K.L. Madsen:** None.

Poster

033. Dopamine

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 33.10/E2

Topic: B.07. Synaptic Transmission

Support: DA35821

NS95809

Title: ACh and opioid regulation of dopamine D2 receptor mediated transmission in striatal medium spiny neurons

Authors: *Y. CAI, A. A. MAMALIGAS, C. P. FORD;
Case Western Reserve Univ., Cleveland, OH

Abstract: In the striatum, cholinergic interneurons (ChIs) regulate dopamine (DA) release by activating nicotinic acetylcholine receptors (nAChRs) on DA terminals. However, it is unclear how post-synaptic dopamine D2 receptors (D2Rs) on medium spiny neurons (MSNs) respond to acetylcholine-mediated DA transmission. Here we compared D2R activation through two different pathways: either direct midbrain DA release or ChI evoked release. To examine this, G protein-coupled inwardly rectifying potassium channels (GIRK2) were overexpressed in striatal

MSNs while ChR2 was expressed in DA neurons in DAT-Cre mice or ChIs in ChAT-Cre mice. Optogenetic stimulation of both ChIs and DA terminals evoked D2-receptor inhibitory postsynaptic currents (D2-IPSCs) in GIRK expressing MSNs. D2-IPSCs in both cases were abolished by D2R antagonist sulpiride (400nM), however only D2-IPSCs evoked by optogenetic activation of ChIs were abolished by the nAChR antagonist DHBE (1 uM). D2-IPSCs evoked by a single electrical stimulus were reduced by ~40% by DHBE (1 uM) indicating that electrical stimulation drove the release of DA both directly from DA terminals and also by the disynaptic release from ChIs. Bursts (5 stimuli at 40 Hz) of DA terminal stimulation potentiated the amplitude of D2-IPSCs by about 20% compared with single optogenetic stimulation. Bursts of ChI stimulation (5 stimuli at 40 Hz), however, did not potentiate the amplitude of IPSCs. This was likely due to desensitization of nAChRs as a result of ChI burst firing. In addition, the opioid receptor agonist U69593 (300nM) and the opioid receptor agonist DAMGO (1uM) inhibited D2-IPSCs evoked by optogenetic stimulation of ChIs. Taken together, the data suggests synchronous activation of ChIs regulates D2R activation in the striatum and probably also indicates a differential regulation pattern of opioids on D2R activation mediated by ChIs and DA neurons

Disclosures: **Y. Cai:** None. **A.A. Mamaligas:** None. **C.P. Ford:** None.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.01/E3

Topic: B.02. Ligand-Gated Ion Channels

Support: Swiss National Science Foundation grant 315230_156929/1

Title: Shedding light on the subunit arrangement of extrasynaptic $\alpha_4\beta_2\delta$ $GABA_A$ receptors

Authors: ***N. WONGSAMITKUL**, R. BAUR, E. SIGEL;
Univ. of Bern, Bern, Switzerland

Abstract: Extrasynaptic $\alpha_4\beta_2\delta$ $GABA_A$ receptors are considered to be an important drug target. However, subunit stoichiometry and arrangement of these receptors is difficult to establish. To predefine subunit arrangement, we constructed concatenated receptors. The δ subunit was substituted in any of the five positions in the $\alpha_1\beta_2\gamma_2$ receptor with γ_2 replaced by β_2. α_4, β_2 and δ subunits were concatenated to dimeric and trimeric subunits. Five pentameric $GABA_A$ receptors were built with two different ways from the combination of trimers and dimers, and two representative pentameric constructs were engineered. The resulting receptors were expressed in *Xenopus* oocytes and their electrophysiological and pharmacological properties were

characterized using the two-electrode voltage clamp technique. We determined GABA EC_{50} in the presence of THDOC (3α, 21-dihydroxy-5α-pregnan-20-one), sensitivity to DS2 (the δ-selective 4-chloro-*N*-[2-(2-thienyl)imidazo[1,2-a]pyridine-3-yl benzamide) and ethanol. All functional receptors were sensitive to DS2 and none of them was affected by ethanol. The subunit arrangement of $\alpha_4\beta_2\delta$ $GABA_A$ receptors seems to be flexible as the δ subunit can assume multiple positions in a receptor pentamer.

Disclosures: **N. Wongsamitkul:** None. **R. Baur:** None. **E. Sigel:** None.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.02/E4

Topic: B.02. Ligand-Gated Ion Channels

Support: Swiss National Science Foundation grant 315230_156929/1

Title: Multiple sites of action of CGS 9895 on $GABA_A$ receptors

Authors: *M. C. MALDIFASSI, R. BAUR, E. SIGEL;
Univ. of Bern, Bern, Switzerland

Abstract: $GABA_A$ receptors are the main inhibitory neurotransmitter receptors in the brain and are targets for numerous clinically important drugs such as benzodiazepines, anxiolytics and anesthetics. Previously, CGS 9895 was described as a positive allosteric modulator acting through the α+/β- interface in the extracellular domain of $GABA_A$ receptors. The localization of the binding site was based on a steric hindrance approach, rather than on direct effects of point mutations. In the current study we further characterized modulation by this compound that seems to have six sites of action. We investigated $GABA_A$ receptors expressed in *Xenopus laevis* oocytes using voltage-clamp electrophysiology. We have identified the α_1Y209 residue present at this interface as a key residue for CGS 9895 modulation. Additionally, the interaction between this residue and various structural analogs was characterized, allowing tentative positioning of CGS 9895 versus α_1Y209. Not all compounds were found to be sensitive to mutations at the α_1Y209 residue. In addition, the interaction of CGS 9895 with flurazepam was characterized. Flurazepam is hypothesized to act at the same subunit interface in the extracellular domain. We also provide evidence that the $GABA_A$ receptor harbors additional modulatory sites for CGS 9895 at each of the subunit interfaces in the trans membrane domain.

Disclosures: **M.C. Maldifassi:** None. **R. Baur:** None. **E. Sigel:** None.

Poster

034. GABAA Receptors

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Program#/Poster#: 34.03/E5

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant R01GM085237

Barrow Neurological Foundation

Title: Competitive antagonists facilitate the recovery of α1β2γ2 $GABA_A$ receptors from desensitization

Authors: *Y. CHANG[1], X. XU[1,2], D. ROBERTS[1], G. ZHU[2];
[1]Barrow Neurolog. Inst., St. Joseph's Hosp. & Med. Ctr., Phoenix, AZ; [2]Inst. of Pesticide and Envrn. Toxicology, Zhejiang University, China, Hangzhou, China

Abstract: The continuous presence of an agonist drives its receptor into a refractory state, termed desensitization. However, it is still unknown whether a competitive antagonist can reverse the agonist-induced desensitization and facilitate receptor recovery from desensitization. In this study, we tested the hypothesis that a competitive antagonist, SR95531, can facilitate the recovery from functional desensitization of the α1β2γ2 $GABA_A$ receptor. The α1β2γ2 $GABA_A$ receptor was expressed in *Xenopus* oocytes, and receptor function was evaluated using the two-electrode voltage-clamp technique. Our results demonstrated that after termination of GABA perfusion, the GABA-response recovery was faster in the presence of SR95531. Furthermore, SR95531 co-application with GABA during the steady-state GABA-induced current caused rebound of the GABA-induced current after removal of SR95531. The SR95531 co-application-induced current rebound was much higher than the steady-state desensitization level. This SR95531-induced current rebound was time dependent and concentration dependent. The concentration dependence of the current rebound had a high potency component and a low potency component. Interestingly, these two components were similar to the two potency components of the SR95531 inhibition of the steady-state current. In addition, bicuculline exhibited a similar facilitation of desensitization recovery. Finally, our results are consistent with that SR95531 has low affinity to the desensitized receptor but high affinity to the non-desensitized receptor. In conclusion, competitive antagonists can facilitate recovery of the α1β2γ2 $GABA_A$ receptor from desensitization by two mechanisms: binding to the desensitized receptor and converting it to the non-desensitized state and binding to the resting state receptor and preventing re-desensitization.

Disclosures: **Y. Chang:** None. **X. Xu:** None. **D. Roberts:** None. **G. Zhu:** None.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.04/E6

Topic: B.02. Ligand-Gated Ion Channels

Title: Pharmacology of the human ε subunit from the $GABA_A$ receptors

Authors: *D. C. BERTRAND[1], S. BERTRAND[1], E. NEVEU[1], M. A. ACKLEY[2];
[1]Hiqscreen, Vesenaz - GE, Switzerland; [2]SAGE Therapeut., Boston, MA

Abstract: $GABA_A$ receptors are ligand gated channels members of the family of four transmembrane domain receptors, which also comprise the nicotinic, $5HT_3$ and glycine receptors. This family includes multiple genes encoding for several individual subunits, allowing expression of a vast repertoire of functional proteins with specific physiological and pharmacological properties. In human this is readily illustrated by the observation that 19 genes encoding for distinct $GABA_A$ receptor subunits have been identified and are known to be expressed in specific areas of the brain. Further pharmacological complexity is brought by the incorporation of three or more distinct subunits in a single receptor complex. For example, $GABA_A$ receptors are typically composed of two $\alpha_{(1-6)}$, two $\beta_{(1-3)}$ and one other subunit ($\gamma_{(1-3)}$, δ, ε, π or θ) and the specific combination of subunits that make up a functional receptor can exert distinct biophysical and pharmacological properties to the channel.
In the human, the ε subunit is encoded by a 506 amino acid long sequence expressed in human on the chromosome X at location p22.33-q28 in a cluster with the gene encoding for the α3 subunit. The ε subunit shows distinct distribution in the CNS and can show plasticity in expression during certain CNS disorders. We have explored how incorporation of this subunit into GABA receptors can exert distinct properties to the receptor.
Expression of the $\alpha_1\beta_2$ subunits in *Xenopus* oocytes in a (1:1) ratio yielded functional receptors displaying an average current to GABA of 0.36 μA and an EC_{50} of 3.5 μM. Co-expression in sibling oocytes of the ε resulted in current that was around ten-fold larger and that was accompanied by an increase in sensitivity and differences in time course of the response. Moreover, these receptors were significantly distinct from other subunit combinations such as $\alpha_1\beta_2\gamma_2$. Further evidences about the incorporation of the ε in the receptor complexes are illustrated by the pharmacological signature of these heteromeric $GABA_A$ receptors.
Taking advantage of the automated HiClamp system we review the singularities of $GABA_A$ receptors that include the ε subunit.

Disclosures: D.C. Bertrand: None. **S. Bertrand:** None. **E. Neveu:** None. **M.A. Ackley:** None.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.05/E7

Topic: B.02. Ligand-Gated Ion Channels

Support: NHMRC Project Grant

Title: The challenge in comparing the pharmacology of agonists and modulators at alpha4beta2gamma2 and alpha4beta2delta GABAA receptors

Authors: *N. ABSALOM[1], L. H. BANG[2], M. CHEBIB[1], P. K. AHRING[1];
[1]Univ. of Sydney, Sydney, Australia; [2]Neurosearch A/S, Ballerup, Denmark

Abstract: Extrasynaptically located γ-aminobutyric acid (GABA) receptors type A are often characterized by the presence of a δ subunit in the receptor complex. These δ-containing receptors respond to low ambient concentrations of GABA or respond to spillover of GABA from the synapse to give rise to slow phasic and tonic currents. In certain brain regions such as thalamocortical neurons, tonic inhibition is estimated to represent the majority of total GABA-mediated inhibition, and has thus raised substantial interest in extrasynaptic receptors as drug targets. Unfortunately, the α4β2/3δ receptors have proven difficult to study in recombinant *in vitro* expression systems due to the inherently low current levels elicited in response to GABA. In this study, we sought to characterize the function of a range of agonists and positive allosteric modulators at α4β2δ and α4β2γ2 receptors. All the agonists tested (GABA, THIP, muscimol, and taurine) displayed between 8 and 22 fold increase in potency at the α4β2δ receptor. In contrast, the modulatory potencies of steroids (allopregnanolone, THDOC and alfaxalone), anesthetics (etomidate, pentobarbital) DS1 and DS2 were similar at α4β2δ and α4β2γ2 receptors. When evaluating modulatory efficacies, the neurosteroids and anesthetics displayed highest efficacy at α4β2γ2 receptors whereas DS1 and in particular DS2 had the highest efficacy at α4β2δ receptors. Overall, none of the tested compounds displayed significant selectivity and a great need for identifying new δ-selective compounds remains. Further, α4β2δ and α4β2γ2 receptors have such divergent intrinsic activation properties that valid comparisons of modulatory efficacies are at best challenging.

Disclosures: **N. Absalom:** 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; NHMRC Project Grant, Australia. **L.H. Bang:** None. **M. Chebib:** 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; NHMRC Project Grant, Australia. **P.K. Ahring:** A. Employment/Salary

(full or part-time): Imk Almene Fond, Denmark. 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; Saniona A/S, Denmark.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.06/E8

Topic: B.02. Ligand-Gated Ion Channels

Support: HHMI 52007557

Blakeslee Foundation

Title: Positive modulation of $GABA_A$ receptor currents by isomers of 2,6-Dimethylcyclohexanol

Authors: *A. C. HALL[1], L. COWDHURY[1], C. J. CROFT[1], S. GOEL[1], N. ZAMAN[1], A. C.-S. TAI[1], E. M. WALCH[1], K. M. SHEA[1,2], C. D. HALL[2], D. JISHKARIANI[2,3], G. G. PILLAI[3]; [1]Smith Col., Northampton, MA; [2]Univ. of Florida, Gainesville, FL; [3]Univ. of Tartu, Ravila, Estonia

Abstract: $GABA_A$ receptors meet all the pharmacological requirements necessary to be considered important targets for the action of general anesthetic agents in the mammalian brain. In a patch-clamp study, the relative modulatory effects of 2,6-dimethylcyclohexanol diastereomers were investigated on human γ-aminobutyric acid type A ($GABA_A$, $\alpha_1\beta_3\gamma_{2s}$) receptor currents stably expressed in human embryonic kidney cells (WSS-1 cells). *Cis,cis, trans,trans*; and *cis,trans* isomers were isolated from commercially available 2,6-dimethylcyclohexanol and were tested for positive modulation of sub-maximal GABA responses. Co-applications of 30 μM 2,6-dimethylcyclohexanol isomers produced a range of positive enhancements of control GABA responses with a rank order for positive modulation: *cis,cis* > *trans,trans* ≥ mixture of isomers >> *cis,trans* isomer. For example, the addition of 30 μM *cis,cis* isomer resulted in ~2-3 fold enhancement of the ~EC_{20} GABA current while the *cis,trans* isomer produced a nominal enhancement of the current.
In molecular modeling studies, the three cyclohexanol isomers bound with the highest binding energies to a pocket within the transmembrane helices M1 and M2 of the β_3 subunit through hydrogen-bonding interactions with a glutamine at the 224 position and a tyrosine at the 220 position. The energies for binding and the hydrogen bond lengths within this pocket corresponded with the relative potencies of the agents for positive modulation of $GABA_A$

receptor currents (*cis,cis* > *trans,trans* >> *cis,trans* 2,6 dimethylcyclohexanol).
In conclusion, the stereochemical configuration of the dimethylcyclohexanols is an important molecular feature in conferring positive modulation of $GABA_A$ receptor activity and for binding to the receptor, a consideration that needs to be taken into account when designing novel anesthetics with enhanced therapeutic indices.

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Poster

034. GABAA Receptors

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Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant GM108580

NIH Grant GM108799

NIH Grant GM058448

Title: Multiple non-equivalent interfaces mediate direct activation of α1β3 $GABA_A$ receptors by propofol

Authors: ***A. S. EVERS**[1], M. M. EATON[1], A. GERMANN[1], R. ARORA[1], L. CAO[1], X. GAO[1], D. SHIN[1], A. WU[1], D. C. CHIARA[2], J. B. COHEN[2], J. H. STEINBACH[1], G. AKK[1]; [1]Anesthesiol., Washington Univ. In St. Louis, Saint Louis, MO; [2]Harvard Med. Sch., Boston, MA

Abstract: Propofol is an allosteric activator of the γ-aminobutyric acid type A ($GABA_A$) receptor. Photo-labeling studies with propofol analogues have identified potential interaction sites in the transmembrane domain of the receptor at both the "+" and "-" sides of the β subunit. We have examined the functional contributions of the putative binding sites at the β-α and α-β interfaces in direct gating of the α1β3 $GABA_A$ receptor by propofol. Electrophysiological assays were performed on human α1β3 $GABA_A$ receptors expressed in *Xenopus* oocytes, using standard two-electrode voltage clamp. Individual tryptophan mutations at the "-" side of the β3 subunit at Y143 in loop 7 of the extracellular domain, or F221 or Q224 near the extracellular end of the first transmembrane segment in the β3 subunit (βM1), or the "+" side of the β3 subunit at M286 in βM3 had a modest effect on receptor activation by propofol. Combination of β3(M286W) with

one of β3(Y143W), β3(F221W), or β3(Q224W) strongly reduced gating efficacy of propofol. Control experiments with GABA showed no systematic effect of mutations. Structural modeling revealed that the mutated residues at the "-" surface of β3 contribute to a cavity predicted to bind propofol with favorable energies, while the mutated residue at the "+" surface of β3 contributes to a distinct pocket in the transmembrane domain that binds propofol and other general anesthetics. We propose that α1β3 $GABA_A$ receptors can be activated by propofol interactions with the β-β, α-β or β-α interfaces where distinct regions control channel gating.

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Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.08/E10

Topic: B.02. Ligand-Gated Ion Channels

Title: Diphenylpyraline, a histamine receptor antagonist, acts as a $GABA_A$ receptor agonist

Authors: *D. B. WILLIAMS[1], J. J. HARP[2];
[1]Biol. Sci., Winston Salem State Univ., Winston Salem, NC; [2]Biol. Sci., Winston-Salem State Univ., Winston-Salem, NC

Abstract: Compounds derived from the antihistamine diphenylpyraline (DPP) have been demonstrated to be high affinity $GABA_A$ receptor agonists. As a result, we hypothesized that DPP itself could also be a $GABA_A$ receptor agonist. $GABA_A$ receptors consisting of α1β2 (injected 1:1 ratio) or α1β2γ2s (injected 1:1:5 ratio) subunits were expressed in *Xenopus* oocytes. GABA and DPP induced currents were measured using a two-electrode voltage clamp set at -60 mV. GABA and DPP were applied for 20-30 s (until peak current). Perfusion with a modified Ringer's was set at 5 ml/min, with drugs washed out 3-5min between applications. DPP currents were reported as the % maximum as measured by 1 mM GABA. DPP concentrations ranged from pM to μM. Dose response curves were fit to one or two site models, varying the slope; the best fit is reported. At α1β2 receptors, in the absence of GABA, DPP was a strong $GABA_A$ agonist, able to induce up to 98% of maximal current with an EC_{50} of 0.18 pM. However, higher concentrations of DPP (still no GABA) inhibited its own current, down to 24% of maximal GABA current, with an IC_{50} of 7.8 nM. At α1β2γ2s receptors, DPP alone induced current to about 60% of maximal, with a similar pM EC_{50} as the α1β2 receptors. DPP did not cause any significant antagonism of its own currents in the α1β2γ2s receptors. We conclude that DPP is a

high affinity $GABA_A$ agonist with potential antagonistic actions depending on the receptor subunit composition. These results may explain some of the side effects, such as drowsiness, of many commercially available antihistamines.

Disclosures: D.B. Williams: None. **J.J. Harp:** None.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.09/E11

Topic: B.02. Ligand-Gated Ion Channels

Support: Swiss National Science Foundation

Title: Acute and chronic itch are suppressed by positive allosteric modulators of α2- and α3-$GABA_A$ receptors

Authors: *W. T. RALVENIUS[1], D. BENKE[1], M. PAGANI[1], H. JOHANNSSEN[1], U. RUDOLPH[2], H. U. ZEILHOFER[1,3];
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Abstract: *Aim of investigation:*
Loss of inhibitory dorsal horn neurons leads to chronic pruritus in mice, while acute activation of such neurons inhibits itch. We tested the hypothesis that drugs that enhance GABAergic inhibition in the dorsal horn would reduce scratching behavior in mice.
Methods:
Behavioral tests were done in wild-type mice and in $GABA_A$ receptor triple point mutated mice in which only a single $GABA_A$ receptor subtype was left diazepam (DZP) sensitive. Acute itch was induced by intradermal cheek injection of α-methyl-serotonin, histamine or chloroquine. To assess effects on chronic itch, atopic dermatitis was induced by repeated application of oxazolone. Scratching bouts were quantified as a measure of itch. We tested the anti-pruritic effects of the classical benzodiazepine agonist DZP in triple $GABA_A$ receptor point mutated mice and of the non-sedative α2- and/or α3-$GABA_A$ receptor-preferring agents TPA023B, TP003, and N-desmethyl clobazam (NDMC) in wild-type mice.
Results:
Activation of α2- and α3-$GABA_A$ receptor in the $GABA_A$ receptor point-mutated mice reduced scratching behavior in both acute and chronic itch, while α5 $GABA_A$ receptors had only minor

effects. The α2- and α3-$GABA_A$ receptor-preferring agents TPA023B, NDMC and TP003 also reduced scratching in acute and chronic itch models. Tolerance to the anti-pruritic effects did not develop after a 10-day treatment with TPA023B or NDMC. Electrophysiological recordings in spinal cord slices demonstrated that TPA023B enhances GABAergic inhibition in gastrin releasing peptide (GRP) positive dorsal neurons, which are known to be required for the relay of pruritogenic signals within the spinal cord. Finally, we show that the spinal expression patterns of MrgprA3 and GRP, markers of primary and secondary pruritoceptors, overlap with those of α2-and α3-$GABA_A$ receptors. No such co-expression was seen for α1-and α5-$GABA_A$ receptors.
Conclusion:
Our findings suggest that α2- and α3-$GABA_A$ receptors are promising targets for the development of novel antipruritic agents.

Disclosures: W.T. Ralvenius: None. **D. Benke:** None. **M. Pagani:** None. **H. Johannssen:** None. **U. Rudolph:** None. **H.U. Zeilhofer:** None.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.10/E12

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH RO1 NS 33300

Title: De novo GABRB3 mutations disrupt $GABA_A$ receptor gating and expression and lead to Early Infantile Epileptic Encephalopathy

Authors: *C. C. HERNANDEZ[1,2], Y. ZHANG[3], N. HU[2], D. SHEN[4], W. SHEN[2], X. LIU[3], W. KONG[3], Y. JIANG[3], R. MACDONALD[2];
[1]Neurol., Vanderbilt Univ. Med. Ctr., Nashville, TN; [2]Dept. of Neurology, Vanderbilt Univ. Med. Ctr., Nashville, TN; [3]Dept. of Pediatrics, Peking Univ. First Hospital., Beijing, China; [4]The Grad. Program of Neuroscience, Vanderbilt Univ., Nashville, TN

Abstract: *De novo* mutations in the γ-aminobutyric acid type A ($GABA_A$) receptor β3 subunit gene *GABRB3* were found in three cases, two heterozygous and one mosaic, with early infantile epileptic encephalopathy (EIEE) (Zhang Y, et al. 2015). We determined the impact of *GABRB3* mutations on $GABA_A$ receptor function and biogenesis. $GABA_A$ receptor α1 and γ2L subunits were co-expressed with wild-type and mutant β3 subunits in HEK 293T cells. Currents were measured using whole cell and single channel patch clamp techniques. Surface expression was determined using surface biotinylation and confocal microscopy. Potential structural

perturbations in mutant $GABA_A$ receptors were explored using structural simulations. We found that the EEIE-associated *GABRB3*(L170R), *GABRB3*(T288N), and *GABRB3*(A305V) mutations located along the axis between the N-terminal domain and the channel pore reduced whole cell currents by decreasing the gating of the receptor. $GABA_A$ receptors containing different mutant β3 subunits had reduced cell surface expression at different levels but without total loss of surface receptors. In addition, structural simulations predicted mutation-induced rearrangements of neighboring subunits that may underlie both assembly and channel kinetic defects of $GABA_A$ receptors, which may contribute to the pathogenesis of EEIE. Although it is difficult to predict what effect a mosaic mutation would have, our results suggest that the impact on $GABA_A$ receptors function is comparable to that of heterozygous *GABRB3* mutations. This work was supported by the NIH RO1 NS 33300 grant to RLM.

Disclosures: C.C. Hernandez: None. **Y. Zhang:** None. **N. Hu:** None. **D. Shen:** None. **W. Shen:** None. **X. Liu:** None. **W. Kong:** None. **Y. Jiang:** None. **R. Macdonald:** None.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.11/E13

Topic: B.02. Ligand-Gated Ion Channels

Support: CONACYT: 220224

PAPIIT-DGAPA: IN200913

Title: Functional properties of GABArho receptors from cerebellar astrocytes

Authors: *A. PÉTRIZ[1], D. REYES-HARO[2], M. GONZÁLEZ-GONZÁLEZ[2], R. MILEDI[2], A. MARTÍNEZ-TORRES[2];
[1]Univ. Nacional Autónoma De México-Instituto, Queretaro, Mexico; [2]Cell. and Mol. Neurobio., Univ. Nacional Autónoma De México-INB, Queretaro, Mexico

Abstract: The cerebellum plays a fundamental role in fine motor control, mainly due to GABAergic neurotransmission. Differential distribution of distinct types of GABA receptors at cerebellar inhibitory synapses has been reported and seems to contribute to specific function at each type of synapses (synaptic or extrasynaptic), due to their peculiar characteristics. As neurons, glial cells express a plethora of $GABA_A$ receptor subunits.
In the present work, we assessed the expression of $GABA_A$ receptors which comprised the GABAρ subunits in $GFAP^+$ cells located in the roof of the fourth ventricle and in the granular

layer of cerebellum during early postnatal development. Immunofluorescence revealed the presence of GABAρ in $GFAP^+$ cells during postnatal development, in contrast, expression in the adult was restricted to Purkinje neurons and a subset of ependymal glial cells.
Astrocytes from cerebellum express functional $GABA_A$ which are inhibited by bicuculline (100 μM) and TPMPA (IC50, 5.9 ± 0.6 μM), indicating the presence of a GABAρ component. GABAρ subunits are assembled as homo- or heteropentamers. Co-immunoprecipitation and double immunofluorescence demonstrated protein–protein interactions between GABAρ1 and GABAα1 in the plasma membrane. Three populations of $GABA_A$ receptors in astrocytes were identified: 1) classic $GABA_A$, 2) bicuculline-insensitive GABAρ, and 3) $GABA_A$–GABAρ hybrids. Clusters of $GABA_A$ receptors were distributed in the perinuclear space and along the processes of $GFAP^+$ cells. Time-lapse microscopy showed clusters of GABAρ2-GFP were relatively immobile, with mean displacement of 9.4 ± 0.9 μm (in 30 min) and a net distance traveled of 1–2 μm (in 30 min), due mainly to diffusion directional movement or simple diffusion. Modulation of GABAρ dynamics may be a novel mechanism of extrasynaptic transmission regulating GABAergic control of $GFAP^+$ cells during early postnatal development. The relative low mobility of GABAρ2 in astrocytes together with the distribution of endoplasmic reticulum in the processes suggested the possibility of the presence of ribosomes in this space; thus we labeled the ribosomal binding proteins RpL29 and RpS5 with fluorescent tags and explored their distribution in $GFAP^+$ of cerebellum maintained in organotypic culture. We detected abundant label of both proteins in distal processes of $GFAP^+$, moreover we found all along the processes co-localization of ribosomal proteins with the endoplasmic reticulum, suggesting the possibility that synthesis of proteins may occur in these sites. Therefore, the local protein synthesis may be the mechanism by which glial cells dynamically modulate at the synapsis.

Disclosures: **A. Pétriz:** None. **D. Reyes-Haro:** None. **M. González-González:** None. **R. Miledi:** None. **A. Martínez-Torres:** None.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.12/E14

Topic: B.02. Ligand-Gated Ion Channels

Support: Swedish Research Council, grant to BB

Title: Human peripheral blood mononuclear cells (PBMCs) express receptors for the neurotransmitters GABA and Glutamate

Authors: *A. BHANDAGE[1], C. HELLGREN[2], Z. JIN[1], S. KOROL[1], E. OLAFSSON[1], I. SUNDSTRÖM[2], B. BIRNIR[1];
[1]Dept. of Neuroscience, Uppsala Univ., Uppsala, Sweden; [2]Dept. of Women's and Children's Hlth., Uppsala Univ., Uppsala, Sweden

Abstract: Background: Usually neurotransmitter signalling is associated with the central nervous system (CNS). But the evidence for neurotransmitter signalling outside of the CNS is increasing. It takes place in cells of the immune system where immune cells can secrete neurotransmitters and do often express neurotransmitter receptors (1). Immune cells can also encounter neurotransmitters while travelling throughout the body. Thus, neurotransmitter signalling may have an important role in immune functions.
Aim: We examined if human peripheral blood mononuclear cells (PBMCs) can express receptors activated by GABA or glutamate and if their expression is influence by physiological conditions such as gender, pregnancy and depression.
Materials and Methods: The PBMCs were isolated by density gradient centrifugation of the EDTA blood. The mRNA expression of all subunits of GABA-A (19 subunits), GABA-B (2 subunits), ionotropic glutamate (18 subunits) receptor (iGluR) and 6 chloride transporters in the PBMCs was accessed by real time RT-qPCR and compared in four experimental groups: men, non-pregnant women, healthy pregnant women and depressed pregnant women.
Results: Most of the GABA-A and iGlu receptor subunit mRNAs were expressed in the PBMCs. Out of those, the GABA-A rho2, GluK4, GluK5, GluN2C and GluN2D subunits had higher expression level than other subunits. In pregnant women, the GABA-A delta and rho2 subunits were upregulated while in depressed pregnant women, the beta1 and epsilon subunits expression were altered (2). In depressed pregnant women, the GABA-B1 was up-regulated (2). The cotransporters NKCC1 and KCC4 were down-regulated in all pregnant women (2). Similar to GABA-A and GABA-B receptor subunits, the expression of specific iGlu receptor subunits was changed by pregnancy and depression.
Conclusions: The results are consistent with the immunomodulation of PBMCs by the neurotransmitters GABA and Glutamate and their receptors. The human PBMCs differentially express neurotransmitter receptors subunits and chloride transporters that are modified by gender, pregnancy and depression. The results indicate that cross-talk is taking place between the nervous and the immune system.
1. Barragan A., et al. Acta Physiol. 2015; 213(4):819-827.
2. Bhandage A.K., et al. Acta Physiol. 2015; 213:575-585.

Disclosures: A. Bhandage: None. **C. Hellgren:** None. **Z. Jin:** None. **S. Korol:** None. **E. Olafsson:** None. **I. Sundström:** None. **B. Birnir:** None.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.13/E15

Topic: B.02. Ligand-Gated Ion Channels

Support: Swedish Research Council

Swedish Brain Foundation

Title: Metabolic hormones modulate GABA-A receptors mediated synaptic and tonic currents in rat hippocampal and amygdala neurons.

Authors: *B. BIRNIR, S. V. KOROL, O. BABATEEN, Z. JIN;
Dept. of Neurosci., Uppsala Univ., Uppsala, Sweden

Abstract: INTRODUCTION. The inhibitory neurotransmitter GABA (gamma-aminobutyric acid) activates GABA-A receptors generating phasic and tonic inhibition in neurons and regulates neuronal networks activity. Glucagon-like peptide-1 (GLP-1) is a gut hormone that promotes insulin secretion in a glucose-dependent manner. Interestingly, GLP-1 and insulin receptors are found in many brain regions. We studied how GLP-1 and its analogs plus insulin affected inhibitory synaptic and tonic currents in hippocampal pyramidal neurons and amygdala neurons.
METHODS. Brain slices from Wistar rats and whole-cell patch-clamp were used to record GABA-A receptors mediated currents in the hippocampal and amygdala neurons. Quantitative RT-PCR was run on samples from rat brain samples. Immunohistochemistry was used to identify the receptors in the brain slices.
RESULTS. GLP-1 and its mimetics transiently enhanced amplitudes and frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) and enhanced inhibitory tonic current in CA3 neurons. Insulin enhanced GABA-A mediated tonic conductance in hippocampal CA1 neurons. The GLP-1 and insulin receptors mRNAs and proteins were detected in the hippocampal and amygdala samples. In amygdala neurons, acute application of insulin increased GABA-A mediated synaptic currents and reduced the action potential firing frequency.
CONCLUSIONS. Our results show that GLP-1 and insulin enhance sIPSCs and extrasynaptic GABA-A receptors in hippocampal and amygdala neurons demonstrating that metabolic hormones influence function of these brain structures.
Korol SV et al. (2015) *Diabetes* 64:79.
Jin Z, et al.(2011). *PLoS One* 6: e16188.

Disclosures: B. Birnir: None. **S.V. Korol:** None. **O. Babateen:** None. **Z. Jin:** None.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.14/E16

Topic: B.02. Ligand-Gated Ion Channels

Support: Banco di Sardegna Foundation Grant 2012.0255

Title: Neonatal estradiol exposure to female rats changes $GABA_A$ receptor expression and function during adulthood

Authors: *P. PORCU[1], A. LOCCI[2], G. TALANI[1], E. SANNA[1,2], A. CONCAS[1,2];
[1]Neurosci. Inst., Natl. Res. Council of Italy (CNR), Monserrato, Italy; [2]Dept. of Life and Envrn. Sci., Univ. of Cagliari, Cagliari, Italy

Abstract: Exposure of developing female rats to estradiol during the perinatal period affects brain sexual differentiation and induces a long-lasting dysregulation of the gonadal axis with decreased progesterone secretion, and a persistent reduction in brain concentrations of its neuroactive metabolite allopregnanolone. Given that allopregnanolone is a potent modulator of $GABA_A$ receptor ($GABA_AR$) expression and function, we evaluated whether neonatal estradiol treatment, alters $GABA_AR$ expression and function in the hippocampus of adult female rats.
On the day of birth, pups received a single administration of β-estradiol 3-benzoate (EB; 10 μg in 50 μl of sesame oil, s.c.) or vehicle. Experiments were performed in 90-120 days old rats. Given that $GABA_AR$ subunit expression varies in response to steroid fluctuations that occur during the estrus cycle, vehicle-treated rats in the proestrus and diestrus 1 phases were used as controls. Neonatal EB treatment increased the abundance of extrasynaptic α4 (+47%) and δ (+30%) subunits ($p<0.05$), and decreased the abundance of synaptic α1 (-36%), α4 (-32%), and γ2 (-36%) subunits ($p<0.05$), compared to vehicle-treated rats in proestrus. Plasma allopregnanolone levels in the same rats were higher in diestrus 1 vehicle-treated rats vs. proestrus ones (+30%, $p<0.05$), and were decreased by neonatal EB treatment compared to vehicle-treated rats in proestrus (-69%, $p<0.001$) and diestrus 1 (-76%, $p<0.001$). Modulation of GABAergic tonic currents recorded in DG granule cells was increased in EB-treated rats and vehicle-treated rats in diestrus 1, compared to vehicle-treated rats in proestrus, with a THIP-induced larger shift in holding current and greater increase in the noise variance ($p<0.05$). EB treatment also altered GABAergic phasic currents with a decrease in decay time ($p<0.05$).
The changes in the expression and function of $GABA_ARs$, induced by neonatal EB treatment, may not be related to the fluctuations in allopregnanolone concentrations, given that vehicle-treated rats in diestrus, which have opposite neurosteroid levels than EB-treated rats, show the same functional changes in $GABA_ARs$ as EB-treated rats. Rather, these changes may represent a compensatory mechanism to counteract the persistent long-term reduction in allopregnanolone

concentrations, induced by neonatal estradiol treatment. These enduring changes in $GABA_AR$ plasticity may be relevant for regulation of neuronal excitability in the hippocampus and for the etiology of psychiatric disorders that originate in development and show sex differences.

Disclosures: P. Porcu: None. **A. Locci:** None. **G. Talani:** None. **E. Sanna:** None. **A. Concas:** None.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.15/E17

Topic: B.02. Ligand-Gated Ion Channels

Support: DK "MOLTAG" FWF W 1232-B24

Title: Novel, simplified $GABA_A$ receptor modulators based on the scaffold of Valerenic acid and derived from a ligand-based pharmacophore model

Authors: *M. J. STADLER[1], G. PARISI[2], S. MONTICELLI[2], D. LUGER[1], W. HOLZER[2], T. SEIDEL[2], C. SCHWARZER[3], V. PACE[2], T. LANGER[2], S. HERING[1];
[1]Pharmacol. and Toxicology, Univ. of Vienna, Wien, Austria; [2]Pharmaceut. Chem., Univ. of Vienna, Vienna, Austria; [3]Med. Univ. Innsbruck, Innsbruck, Austria

Abstract: Valerenic acid (VA) is a major constituent of common *Valerian, a prevalently used herbal medicinal plant* that selectively modulates $GABA_A$ receptors comprising β_2 or β_3 subunits. VA's anxiolytic and anticonvulsive properties without concomitant sedation combined with a promising pharmacokinetic profile make this compound an interesting drug candidate [1, 2]. The aims of this study were the synthesis of simplified molecules maintaining subunit-selective properties based on the VA scaffold and their *in vitro* and *in vivo* characterization.
A pharmacophore model based on the known $\beta_{2/3}$ subunit-selective $GABA_A$ modulators VA, loreclezole, etomidate, suggested a series of novel VA analogues. Their effect on GABA-induced chloride currents (I_{GABA}) through $GABA_A$ receptors composed of $\alpha_1\beta_{1\text{-}3}\gamma_{2S}$ subunits expressed in *Xenopus laevis* oocytes was analysed by means of the two-microelectrode voltage clamp technique. Pentylenetetrazole-tests (PTZ) were performed to investigate potentially anticonvulsant activity.
Efficacy of I_{GABA} enhancement by derivatives AR-013, AR-016, SM-226-1 and SM-392-1 was comparable to that of VA, while slightly reduced potency was observed. Compound SM-408-2 diplayed selectivity for $GABA_A$ receptor containing β_2 or β_3 subunits and was significantly more potent and efficient than VA. PTZ-induced seizure threshold was shifted by SM-408-2 at

concentrations of 0.1 mg/kg bodyweight indicating potent anticonvulsant activity. The other studied compounds were either less efficacious or did not display significant potentiation of I_{GABA} at concentrations ≥30 μM.
Our data suggest that pharmacophore based development of $\beta_{2/3}$-selective VA analogs may be a promising approach for development of non-sedating anxiolytics and anticonvulsants. SM-408-2 displayed higher in vitro and in vivo potency than VA and may thus serve as a scaffold for the development of novel, selective $GABA_A$ receptor modulators.
1) Hintersteiner J, Haider M, Luger D, Schwarzer C, Reznicek G, Jäger W, and others. 2014. Esters of valerenic acid as potential prodrugs. Eur. J. Pharmacol. 735:123-131.
2) Khom S, Strommer B, Ramharter J, Schwarz T, Schwarzer C, Erker T, and others. 2010. Valerenic acid derivatives as novel subunit-selective GABAA receptor ligands -in vitro and in vivo characterization. Br. J. Pharmacol. 161:65-78.

Disclosures: **M.J. Stadler:** None. **G. Parisi:** None. **S. Monticelli:** None. **D. Luger:** None. **W. Holzer:** None. **T. Seidel:** None. **C. Schwarzer:** None. **V. Pace:** None. **T. Langer:** None. **S. Hering:** None.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.16/E18

Topic: B.02. Ligand-Gated Ion Channels

Title: Elucidating putative binding site for THIP on α4-containing receptors

Authors: ***L. Y. HARTIADI**, N. ABSALOM, H. J. LEE, P. K. AHRING, M. CHEBIB;
Fac. of Pharm., The Univ. of Sydney, Sydney, Australia

Abstract: 4,5,6,7-tetrahydroisoxazolo (5,4-c)-pyridine-3-(ol) (THIP) is a $GABA_A$ receptor agonist that displays 'superagonism' on δ-containing receptors and partial agonism on γ-containing receptors. Radioligand binding studies have shown that THIP competes with GABA, indicating that THIP binds to the same site as GABA. However, the selectivity of THIP on δ-containing receptors over γ-containing receptors remains elusive. It is believed that the δ subunit replaces the γ subunit to form a stoichiometry of β-α-β-α-δ. We hypothesize that there is an additional binding site for THIP that resides on the interface of α4-δ and that this site confers THIP sensitivity for δ-containing receptors.
In this study, THIP activation on α4β1-3δ and α4β1-3 $GABA_A$ receptors expressed in *Xenopus laevis* oocytes were compared using two-electrode voltage clamp electrophysiology. To further determine where THIP binds, mutations on α4 principal site were generated and their effects

towards GABA and THIP on α4β2δ and α4β2 receptors were studied.
THIP potency at α4β2-3δ and α4β2-3 was similar while THIP was more potent at α4β1δ compare to α4β1. THIP acted as a superagonist on all of the receptors, except for α4β1 where THIP displayed full agonism. Although there were no changes to the GABA concentration-response curves for the mutants, a significant change on the efficacy of THIP was observed for all receptors. The amino acids tested in the experiments contribute in part to the efficacy of THIP at α4-containing receptors.

Disclosures: **L.Y. Hartiadi:** None. **N. Absalom:** None. **H.J. Lee:** None. **P.K. Ahring:** None. **M. Chebib:** None.

Poster

034. GABAA Receptors

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Program#/Poster#: 34.17/E19

Topic: B.02. Ligand-Gated Ion Channels

Support: MOST103-2321-B002-035

MOST104-2745-B002-004

MOST104-2325-B002-010

MOST104-2314-B002-053-MY3

MOST 104-2923-13-002-006-MY3

NHRI-EX104-10251NI

Title: Hispidulin alleviates methamphetamine-induced hyperlocomotion and impairment of prepulse inhibition via cerebellar alpha6-containing GABA-A receptors

Authors: *H.-J. LEE[1,2], Y.-H. LIAO[3], H.-L. CHEN[3], W.-J. HUANG[4], P.-C. FAN[5], L.-C. CHIOU[2];
[1]Natl. Taiwan Univ., Taipei, Taiwan; [2]Dept. of Pharmacology, Col. of Medicine, Natl. Taiwan Univ., Taipei, Taiwan; [3]Grad. Inst. of Pharmacology, Col. of Medicine, Natl. Taiwan Univ., Taipei, Taiwan; [4]Dept. of Pharmacognosy, Taipei Med. Univ., Taipei, Taiwan; [5]Dept. of Pediatrics, Col. of Medicine, Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Previously, we found that a patient with intractable motor tic disorder was responsive to the ground leaf juice of a local herb, *Clerodendrum inerme* (*CI*). Her tics subsided 1 hour after

taking *CI*.[1] Using methamphetamine-induced hyperlocomotion (MIH)[2] and impairment of prepulse inhibition of acoustic startle response (PPI) in mice, we identified an active *CI* constituent, hispidulin. It is a flavonoid reported to be a positive allosteric modulator (PAM) of $GABA_A$ receptors consisting of various α subunits, including the α6 subunit ($\alpha6GABA_AR$) that mainly exists in cerebellar granule cells. In a screening assay for 92 neurotransmitter receptors/degradation enzymes/transporters, we found hispidulin displayed significant (>50% at 10 μM)) binding affinity only at $GABA_ARs$ ($IC_{50:}$ 0.73~1.78 μM) and catecholamine-o-methyl-transferase (COMT) ($IC_{50:}$ 1.32 μM). We further elucidated whether COMT and cerebellar $\alpha6GABA_ARs$ are action targets of hispidulin for its alleviation of MIH and PPI impairment. Hispidulin given by either *i.p* (10-30 mg/kg) or intra-cerebellar (*i.c.b.*, 10 nmol) injection, significantly alleviated MIH and PPI impairment. The *i.c.b.* effect of hispidulin was prevented by *i.c.b.* furosemide, a non-competitive $\alpha6GABA_AR$ antagonist, and was mimicked by *i.c.b.* Ro 15-4513, a $\alpha6GABA_AR$ PAM, but not by *i.c.b.* diazepam, an $\alpha6GABA_AR$-inactive benzodiazepine. Conversely, *i.cb.* diazepam did not affect MIH or PPI impairment, while it reduced MIH when given systemically at doses (4-10 mg/kg, *i.p.*) having significant benzodiazepine adverse effects, including sedation, anxiolysis and motor-impairment. Hispidulin at systemic doses effectively alleviated MIH did not affect apomorphine-induced hypolocomotion and stereotypy behaviors. A typical COMT inhibitor, OR-482 (10 mg/kg. *i.p.*), did not affect MIH. These results suggest that hispidulin alleviates MIH and PPI impairment in mice via acting as a PAM of cerebellar α_6GABA_ARs. This study also suggests the potential for using selective α_6GABA_AR PAMs as a novel treatment for neuropsychiatric disorders with sensorimotor gating deficit.

[1] Fan et al. (2009) *J. Child Neurol.* **24**:887.
[2] Huang et al. (2015) *J Ethnopharmacol* **166**:18.

Disclosures: **H. Lee:** None. **Y. Liao:** None. **H. Chen:** None. **W. Huang:** None. **P. Fan:** None. **L. Chiou:** None.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.18/E20

Topic: B.02. Ligand-Gated Ion Channels

Support: MOST103-2321-B002-035

MOST 104-2923-13-002-006-MY3

MOST104-2314-B002-053-MY3

MOST104-2325-B002-010

MOST104-2745-B002-004,

NHRI-EX104-10251NI

Title: The cerebellar alpha6-containing GABA-A receptor: A novel target for neuropsychiatric disorders

Authors: *L.-C. CHIOU[1,2,3,4], C.-Y. WU[3], H.-J. LEE[2], J.-C. DU[3], C. HOR[3], M. ERNST[5], W. SIEGHART[5], J. M. COOK[6];
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Abstract: Previously, we found that hispidulin, a constituent of a local herb that effectively remitted tic attacks in a patient with intractable motor tic disorders, significantly alleviates methamphetamine-induced hyperlocomotion (MIH) and impairment of prepulse inhibition of acoustic startle response (PPI), via acting as a positive allosteric modulator (PAM) of cerebellar α6 subunit-containing $GABA_A$ receptors (α6$GABA_A$Rs).[1] Since hispidulin is also a PAM of other $GABA_A$Rs, here we used Compound 6, a newly developed PAM selective for α6$GABA_A$Rs[2] as a tool to examine the role of cerebellar α6$GABA_A$Rs in animal models mimicking Tourette syndrome (TS) and other neuropsychiatric disorders with sensorimotor gating deficit. These include MIH and PPI impairment, apomorphine-induced climbing behaviors, and stereotypy behaviors induced by intra-dorsal striatal injection of the siRNA of SlitTrk. The latter a TS model we established[3] based on the findings that SliTrk is only expressed in adult striatal cholinergic interneurons that are reduced in postmortem brains of TS subjects. Compound 6 (10 mg/kg, *i.p.*) significantly alleviated MIH and PPI impairment. These effects were antagonized by intra-cerebellum microinjection of furosemide, a selective α6$GABA_A$R antagonist. Compound 6 did not affect apomorphine-induced stereotypy climbing behaviors, but significantly reduced the stereotypy behaviors in mice induced by siRNA of SlitTrk. At the same dose, Compound 6 has no motor-impairing, sedative or anxiolytic activity. These results suggest that Compound 6 successfully gets into the brain to alleviate MIH and PPI impairment possibly via enhancing cerebellar inhibitory control on the striatal dopaminergic activity through positively modulating α6GABAARs in cerebellar granule cells, but not affect dopamine receptor response directly. Cerebellar α6GABAARs may be a new target for the treatment of TS or neuropsychiatric disorders with sensorimotor gating deficit, and α6$GABA_A$R selective PAMs may be a novel therapy for these neuropsychiatric disorders.

[1]Lee et al. Hispidulin alleviates methamphetamine-induced hyperlocomotion and impairment of prepulse inhibition via cerebellar alpha6-containing GABA-A receptors. *Neuroscience 2016*, San Diego, CA, USA.
[2]Varagic et al. Subtype selectivity of alpha+beta- site ligands of $GABA_A$ receptors: identification of the first highly specific positive modulators at alpha6beta2/3gamma2 receptors. *Br J*

Pharmacol **169**:384.
[3]Du et al. The role of Slitrk-1 gene in striatal cholinergic interneurons in behavioral manifestations mimicking Tourrette syndrome. *Neuroscience 2015*, Prog. No. 686.17, 2015, Washington DC, USA.

Disclosures: L. Chiou: None. **C. Wu:** None. **H. Lee:** None. **J. Du:** None. **C. Hor:** None. **M. Ernst:** None. **W. Sieghart:** None. **J.M. Cook:** None.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.19/E21

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant R01 NS087031

Title: Neurosteroids selectively disinhibit the cortex and facilitate cortical spreading depression.

Authors: *A. PARGA[1,2], S. VENUGOPAL[2], T. ANDERSON[2];
[1]Univ. of Arizona, Phoenix, AZ; [2]Univ. of Arizona - Col. of Med. - Phoenix, Phoenix, AZ

Abstract: Migraine is one of the most common neurological disorders occurring in 20% of women and 6% of men. While the underlying cause of migraine remains unknown, a key finding is that the brains of migraine patients are hyperexcitable. Cortical inhibition mediated by GABAergic interneurons is thought to play an important role in regulating brain excitability and the development of cortical spreading depression (CSD). CSD is a neurological process that underlies migraine aura and can lead to activation of the migraine pain pathway. Neurosteroids are brain-derived hormones which can be directly synthesized de-novo within individual neurons and glia and are potent allosteric modulators of GABA-A receptors. Frequent triggers of migraine including stress, diet, the menstrual cycle and pregnancy are known to increase neurosteroid levels. The aim of this study was to examine the role of neurosteroids, such as allopregnanolone, on migraine using an *in-vitro* model of CSD. Using a combined intrinsic optical imaging (IOS) and patch-clamp electrophysiology approach we determined that application of endogenous and synthetic neurosteroids paradoxically increases the probability of inducing CSD by up to 40%. This was in contrast to the GABA-A delta specific agonist THIP (gaboxadol) which failed to increase the probability of inducing CSD. To investigate the underlying mechanism we performed a detailed analysis of the action of neurosteroids on inhibitory cortical synaptic neurotransmission. We found that in contrast to THIP, neurosteroids preferentially act to increase phasic and tonic inhibition onto inhibitory interneurons while

decreasing inhibition onto excitatory pyramidal neurons. These results suggest neurosteroids facilitate CSD by selectively disinhibiting the cortex which may increase the incidence of attacks of migraine with aura.

Disclosures: A. Parga: None. **S. Venugopal:** None. **T. Anderson:** None.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.20/E22

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH grant MH-096463

NIH grant HL-118561

NIH grant NS-076517

FWF grant DK W1232

Title: Different Benzodiazepines seem to interact differently with $GABA_A$ receptors

Authors: *P. SCHOLZE[1], A. A. ELGARF[1], F. STEUDLE[1], G. LI[2], J. M. COOK[2], M. ERNST[1]; [1]Med. Univ. Vienna, Vienna, Austria; [2]Dept. of Chem. and Biochem., Univ. of Milwaukee, Milwaukee, WI

Abstract: Gamma-Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the central nervous system. $GABA_A$ receptors exist in an enormous diversity of subtypes. Six α, three β, three γ, the δ, ε, θ, π, and three ρ subunits can, and most of them actually do, assemble into a multitude of different homo- or hetero-pentameric receptors, each of them displaying unique pharmacological properties. Selective modulation of $GABA_A$ receptors containing the α5 subunit is believed to have considerable therapeutic potential in several psychiatric and neurological conditions characterized by cognitive dysfunction.
Some α5-preferring (selective) compounds have already been identified, such as the chiral imidazobenzodiazepine SH-053-2'F-R-CH3 (Gallos et al., 2015) and other structurally similar compounds.
Aim of the current study was to compare the binding affinities and electrophysiological efficacies of several different benzodiazepines; all of them structural variations of well-known compounds and predicted to be potentially α5-selective. Efficacies were tested using two-electrode voltage clamp recordings in rat recombinant $GABA_A$-R subtypes expressed in Xenopus

oocytes. Potencies were investigated by inhibiting [3H]flunitrazepam binding to human kidney 293 cells, transiently transfected with expression plasmids coding for different $GABA_A$-R subunits.
Surprisingly, results indicated that benzodiazepine-derivatives with similar chemical modifications showed dissimilar trends for their efficacies, affinities and "α5-selectivity". Specifically, comparing chiral derivatives (R-, S-methyl isomers) showed slight differences for some compounds, while for another compound one enantiomer had a greatly reduced activity and binding affinity compared to the other enantiomer. Accordingly, this suggests that some compounds interact differently with the $GABA_A$-receptor binding pocket than others and stimulates further interest for better understanding the structure binding relationship between various ligands and their binding sites on different $GABA_A$-R subtypes.
Literature:
Gallos G, Yocum GT, Siviski ME, Yim PD, Fu XW, Poe MM, Cook JM, Harrison N, Perez-Zoghbi J, Emala CW, Sr. (2015) Am J Physiol Lung Cell Mol Physiol 308:L931-942.

Disclosures: **P. Scholze:** None. **A.A. Elgarf:** None. **F. Steudle:** None. **G. Li:** None. **J.M. Cook:** None. **M. Ernst:** None.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.21/E23

Topic: B.02. Ligand-Gated Ion Channels

Support: NSF CNIC award ID 1322626

Title: Investigations into the pharmacology and physiology of gabaergic neurotransmission in planaria.

Authors: *L. RAMAKRISHNAN;
Chem., St. Cloud State Univ., Saint Cloud, MN

Abstract: Planaria (flatworms) belonging to the phylum Platyhelminthes, possess a mammalian-like bilaterally symmetric nervous system and all major neurotransmitters. Though the presence of GABA and its metabolic enzymes in the nervous system of planaria are proven, its pharmacological and physiological actions remain poorly understood due to *lack of direct evidences supporting the presence of specific GABA-binding receptor proteins*. Planarians exhibit seizure-like movements (pSLM), defined as asynchronous paroxysms (e.g. C-like movements, twitching behaviors), after being exposed to pro-convulsants such as, glutamatergic

agonists or GABAergic antagonists. We characterized pharmacological alteration of GABAergic neurotransmission in planaria using Picrotoxin (PTX), a classical convulsant that acts exclusively on GABAergic neurotransmission. We also conducted behavioral pSLM testing of four potential anticonvulsant drugs, which have been previously tested in rat and mice epilepsy models. The chosen drugs, which are structurally related in terms of having a lactam functional group, are: 2-phenylbutyramide (2-PBA), N-carbamoyl-2-phenylbutyramide (NC-2PBA), 3-ethyl-3-phenylpyrrolidin-2-one (3-EPPD) and 3-methyl-3-phenylpyrrolidine-2,5-dione (3-MPPD). The results from the screenings demonstrated potential anticonvulsant activity by 2-PBA and NC-2PBA but not by 3-EPPD and 3-MPPD. Further testing of 2-PBA up to a maximal concentration of 1 mM demonstrated 50-60% alleviation of 5 mM picrotoxin-induced pSLM activity. To investigate expression and localization of GABAergic proteins in planarian nervous system, we plan to conduct glutamate decarboxylase and GABAergic protein gene silencing using siRNAs which will be delivered using alanine-based cationic polymer nanoparticles. We have conducted proof of concept studies in HEK293 cells expressing GFP, wherein GFP gene silencing was induced by delivering siRNA targeting GFP mRNA was delivered using cationic polymer nanoparticles.

Disclosures: L. Ramakrishnan: None.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.22/E24

Topic: B.02. Ligand-Gated Ion Channels

Support: NJCBIR Grant CBIR14IRG024

NJCBIR Grant 11-3223-BIR-E-O

Title: Developmental characterization of intrinsic physiology and inhibitory regulation of Dentate Semilunar Granule Cells.

Authors: *A. GUPTA[1,1], B. SWIETEK[1], J. GUEVARRA[1], Y. SHAH[2], V. SANTHAKUMAR[1]; [1]Rutgers, Newark, NJ; [2]The Col. of New Jersey, Ewing, NJ

Abstract: Semilunar granule cells (SGCs) are a class of excitatory dentate projection neurons distinguished from the classical granule cells (GCs) by their somato-dendritic morphology and intrinsic physiology. In young adult 30-40 day rats, SGCs have greater synaptic and extrasynaptic (tonic) GABAergic inhibition than GCs. This study was conducted to identify

whether SGCs are anatomically and physiologically distinct from GCs regardless of animal age and to determine the developmental changes in intrinsic physiology and inhibitory regulation of SGCs during early life. Acute hippocampal slices from 11-150 postnatal day (PD) male Wistar rats particularly at two early developmental time points: late neonatal (PD11-13) and adolescent (PD28-42) were used to obtain whole cell recordings from SGCs in the inner molecular layer and GCs. Current and voltage clamp recordings were used to record intrinsic physiology and spontaneous inhibitory synaptic currents (sIPSCs). Tonic GABA currents were measured as the baseline current is blocked by Gabazine (10 μM). All recorded cells were filled with biocytin and processed to distinguish SGCs by the semilunar shape of the soma in the inner molecular layer and wide dendritic arbors compared to GCs. Morphological reconstructions revealed SGCs with greater dendritic angle than GCs in sections from PD11-150 rats. SGC firing rate (120pA-200pA) was significantly higher in late neonatal than adolescents. Although input resistance was not different between the two groups, the membrane sag in response to hyperpolarizing current injection was significantly higher in late neonatal rats compared to adolescent rats suggesting reduction in SGC H-currents during development. Analysis of synaptic currents revealed that sIPSC frequency in SGCs was consistently higher than in GCs from age-matched rats. Frequency of sIPSCs in SGCs and GCs was significantly higher in adolescent rats. Tonic GABA current amplitude in SGCs was also significantly greater than in GCs in adolescent rats. However, in keeping with developmental expression of tonic GABA currents in other cell types, tonic GABA currents amplitude was low and not different between SGCs and GCs in late neonatal rats. These data demonstrate that SGCs are distinct from GCs in morphology and inhibitory synaptic inputs from early in development. We speculate that complementary changes in H-currents and tonic GABA currents may underlie the relative stability of SGC input resistance during development.

Disclosures: A. Gupta: None. **B. Swietek:** None. **J. Guevarra:** None. **Y. Shah:** None. **V. Santhakumar:** None.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.23/E25

Topic: B.02. Ligand-Gated Ion Channels

Support: Epilepsy foundation

NIH NS044370

Title: The role of neurosteroid-delta-GABAR interaction in epileptogenesis

Authors: *S. JOSHI[1], K. RAJASEKARAN[3], J. WILLIAMSON[2], J. KAPUR[2];
[1]Dept of Neurol., [2]Univ. of Virginia, Charlottesville, VA; [3]Univ. of Texas, Dallas, VA

Abstract: We determined the role of neurosteroid-δ subunit-containing GABAA receptor (δ-GABAR) interaction in epileptogenesis following statue epilepticus (SE).
SE was induced in adult rats by lithium-pilocarpine. The surface expression of δ-GABARs was determined using biotinylation assay and Western blotting. The neurosteroid modulation of tonic current was studied by whole cell patch clamp technique. The GABAR subunit mRNA expression was determined by real-time PCR. The time course of onset of spontaneous seizures following SE was studied by continuous video-EEG monitoring.
We compared the time course of changes in δ, α4 and γ2 subunit expression following SE to onset of epilepsy, marked by the 2nd spontaneous seizure, which ranged between 7-18 days. There was a specific and early down-regulation of δ-GABAR expression before the animals became epileptic. The total and surface expression of δ subunit was reduced in hippocampi of 7 days post-SE (7-SE) animals. In contrast, the expression of α4 and γ2 subunits was unchanged at this time. The expression of mRNAs of these subunits matched with the expression of their proteins. The reduction in δ-GABAR expression as accompanied by diminished neurosteroid modulation of tonic current of DGCs in 7-SE animals.
We tested the effect of inhibition of neurosteroid synthesis on the onset of epilepsy. Animals were treated with finasteride at 4 days post-SE prior to the appearance of spontaneous seizures. Finasteride-treated animals (86%) experienced seizures; however, seizures did not occur in vehicle-treated or untreated animals. Finasteride treatment also accelerated the onset of epilepsy, 2nd spontaneous seizure. The time to 2nd spontaneous seizure ranged from 5-8 days in finasteride-treated animals, which was significantly shorter than that in vehicle-treated or untreated animals (7-18 days).
Next, we prevented the down-regulation of δ-GABAR expression and determined whether it suppressed epileptogenesis. NMDAR blockade with MK801 or ketamine during SE prevented the reduction in reduction in δ subunit mRNA and protein expression, without terminating or shortening the duration of SE. The neurosteroid modulation of tonic current of DGCs was also preserved in MK801- or ketamine-treated animals. Lastly, MK801 treated animals did not develop epilepsy during 30 days post-SE monitoring, whereas only a fraction of ketamine-treated animals became epileptic.
These studies revealed that reducing neurosteroid synthesis prior to seizure onset hastened epileptogenesis and suppression of δ-GABAR downregulation inhibited epileptogenesis.

Disclosures: S. Joshi: None. **K. Rajasekaran:** None. **J. Williamson:** None. **J. Kapur:** None.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.24/E26

Topic: B.02. Ligand-Gated Ion Channels

Support: This research was funded by GW Pharmaceuticals

Title: Modulation of inhibitory receptors by non-psychoactive cannabinoids

Authors: J. ASSIS MANUEL[1], R. A. GRAY[2], T. D. M. HILL[2], A. J. RUIZ[1], *R. J. HARVEY[1]; [1]UCL Sch. of Pharm., London, United Kingdom; [2]GW Res. Ltd, Cambridge, United Kingdom

Abstract: Glycine and type A γ-aminobutyric acid receptors (GlyRs and $GABA_A$Rs) are ligand-gated ion channels that mediate fast inhibitory neurotransmission in the nervous system. Direct modulation of GlyRs and $GABA_A$Rs by a number of cannabinoids has been previously described. However, the direct effect of cannabidiol (CBD) and cannabidivarin (CBDV) on these receptors has not yet been studied in depth. To further interrogate the effect of CBD and CBDV on glycinergic and GABAergic systems, we examined the affinity of CBD and CBDV for $GABA_A$R ligand, benzodiazepine, Cl^- channel and GABA transporter binding sites by radioligand binding and used whole-cell patch-clamp recordings to examine the effect of CBD and CBDV on Cl^- conductance mediated by transiently expressed GlyR and $GABA_A$Rs. Radioligand binding reveals that CBD and CBDV possess no appreciable affinity for the $GABA_A$R ligand or benzodiazepine binding sites (displacement of [^{3}H]muscimol or [^{3}H]diazepam) or the GABA transporter at concentrations up to 10μM. However, both CBD (IC_{50} 2.57 μM) and CBDV (IC_{50} 2.47 μM) displaced specific binding of [^{3}H]picrotoxinin from the $GABA_A$R Cl^- channel. Electrophysiological examinations reveal a direct positive enhancing effect of CBD and CBDV on glycine and GABA mediated Cl^- currents (I_{Gly} and I_{GABA}). Furthermore, the extent of I_{Gly} and I_{GABA} potentiation is dependent on the receptor subunit composition: Cl^- currents mediated by GlyR α3S and $GABA_A$Rs containing α1 and α2 subunits show the greatest potentiation. These findings uncover subtype-specific modulation of inhibitory receptors by CBD and CBDV, offering new opportunities for therapeutic intervention in the nervous system.

Disclosures: **J. Assis Manuel:** 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; This research was funded by a PhD studentship from GW Pharmaceuticals to JAM, AJR and RJH. **R.A. Gray:** A. Employment/Salary (full or part-time): GW Employee. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); **T.D.M. Hill:** A. Employment/Salary (full or part-time): GW

Employee. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); **A.J. Ruiz:** 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; This research was funded by a PhD studentship from GW Pharmaceuticals to JAM, AJR and RJH. **R.J. Harvey:** 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; This research was funded by a PhD studentship from GW Pharmaceuticals to JAM, AJR and RJH.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.25/E27

Topic: B.02. Ligand-Gated Ion Channels

Support: The Swiss national science foundation (SNF)

Title: Electrophysiological and behavioral characterization of a genetic mouse model of diminished synaptic inhibition in the spinal dorsal horn

Authors: *L. TUDEAU[1,2], W. T. RALVENIUS[1], M. POE[3], J. M. COOK[3], H. C. JOHANNSSEN[1], H. U. ZEILHOFER[1,2];
[1]Inst. of Pharmacol. and Toxicology, Univ. of Zurich, Zurich, Switzerland; [2]Inst. of Pharmaceut. Sci., ETH Zurich, Zurich, Switzerland; [3]Dept. of Chem. and Biochem., Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: Diminished synaptic inhibition in the superficial dorsal horn is believed to underlie several chronic pain syndromes. Here, we describe electrophysiological and behavioral changes in a genetic mouse model of diminished spinal synaptic inhibition, i.e. in mice that lack a major $GABA_A$ receptor ($GABA_AR$) α subunit (α2) from the spinal cord and the spinal terminals of peripheral sensory neurons (hoxb8-gabra2$^{-/-}$ mice). We first characterized changes in GABAergic inhibition in the superficial dorsal horn where α2$GABA_ARs$ are abundantly expressed. These experiments were done on a vGAT::ChR2 transgenic background to allow optogenetic activation of inhibitory neurons in spinal cord slices. In hoxb8-gabra2$^{-/-}$ mice, light-evoked GABAergic IPSC amplitudes were reduced by about half ($p = 0.017$) with no significant change in decay kinetics. Despite the reduction in synaptic inhibition, hoxb8-gabra2$^{-/-}$ mice did not show behavioral sensitization to acute painful stimulation, suggesting the presence of a yet-to-be

identified homeostatic plasticity mechanism. Hoxb8-gabra2$^{-/-}$ mice did not only show unchanged sensitivity to acute pain but also developed normal pain sensitization in the CCI model of neuropathic pain, indicating that altered expression and posttranslational modification of α2GABA$_A$Rs are not essential for neuropathic hyperalgesia. However, the antihyperalgesic effects of systemic application of the GABA$_A$R modulator HZ-166 were virtually abolished in hoxb8-gabra2$^{-/-}$ mice, and, in line with this observation, modulation of GABAergic IPSCs by HZ-166 was strongly reduced. These results indicate that adaptive changes can compensate for diminished spinal inhibition to restore normal pain sensitivity and that, although α2GABA$_A$Rs are not required for the induction of neuropathic hyperalgesia, they are essential for the antihyperalgesic actions of the novel benzodiazepine site agonist HZ-166.

Disclosures: L. Tudeau: None. **W.T. Ralvenius:** None. **M. Poe:** None. **J.M. Cook:** None. **H.C. Johannssen:** None. **H.U. Zeilhofer:** None.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.26/E28

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH Grant NS073574

NIGMS Training Grant K12 GM074869

Title: Disinhibition of the HPA axis during the postpartum period induces deficits in maternal behaviors and postpartum depression-like behaviors

Authors: *J. L. MAGUIRE, A. HOOPER, L. C. MELÓN;
Neurosci., Tufts Univ. Sch. of Med., Boston, MA

Abstract: Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis has been implicated in the pathophysiology of postpartum depression, largely based on endocrine changes in patients. However, investigation into the pathophysiological mechanisms underlying postpartum depression has been limited due to the lack of animal models. Our research team has characterized two independent mouse models which exhibit abnormal maternal care and depression-like behaviors during the postpartum period which share a common pathophysiological mechanism, involving the inability to suppress the stress-induced activation of the HPA axis during pregnancy and the postpartum period. The stress-induced activation of the HPA axis is normally suppressed during pregnancy and lactation, which is thought to be a

protective mechanism to prevent adverse effects of stress hormones on the fetus. Our lab recently discovered a critical role for the K^+/Cl^- co-transporter, KCC2, in the stress-induced regulation of the HPA axis at the level of corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus of the hypothalamus (PVN). To determine whether disinhibition of the HPA axis plays a role in mood disorders associated with the postpartum period, we generated mice which lack KCC2 specifically in CRH neurons (KCC2/Crh mice). KCC2/Crh mice exhibit the inability to suppress the HPA axis during pregnancy and the postpartum period, which is associated with an increase in pup mortality, abnormalities in maternal care, and depression-like behaviors during the postpartum period. Silencing CRH neurons governing the activity of the HPA axis using Gi DREADDs stereotaxically injected into the PVN of KCC2/Crh mice, decreases the abnormal maternal care and depression-like behaviors in postpartum KCC2/Crh mice. Conversely, activating CRH neurons in the PVN using Gq DREADDs increases pup mortality, induces abnormalities in maternal care, and depression-like behaviors in postpartum CRH-Cre mice. These data suggest that the inability to suppress the HPA axis during pregnancy and the postpartum period plays a role in postpartum depression-like behaviors and deficits in maternal care in mice.

Disclosures: **J.L. Maguire:** F. Consulting Fees (e.g., advisory boards); SAGE Therapeutics. **A. Hooper:** None. **L.C. Melón:** None.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.27/E29

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH (NINDS) NS089719 (AJ)

NIH T32 GM008602 (OM)

Title: Conventional theory does not adequately explain benzodiazepine-$GABA_A$ receptor interactions

Authors: *O. MOODY[1], A. JENKINS[2];
[2]Anesthesiol., [1]Emory Univ., Atlanta, GA

Abstract: Classical benzodiazepine (BZD)-$GABA_A$ receptor ($GABA_AR$) pharmacology states that positive BZDs allosterically shift the GABA dose-response curve leftwards in a parallel manner. Potentiation (enhanced receptor activation) of $GABA_ARs$ depends on the BZD used and

the subunit composition of receptors, specifically the α and γ subunit isoforms that make up the high-affinity binding site for BZDs on $GABA_A$Rs. Using the clinically-relevant BZD, midazolam, we have shown that the degree of potentiation measured depends on the type of protocol used to expose receptors to midazolam, affecting the time course of action and decay of potentiation. We used whole-cell patch clamp recording of HEK293T cells expressing $\alpha_x\beta_2\gamma_2$ $GABA_A$Rs and exposed cells to submaximal GABA concentrations with and without midazolam (10nM-10μM). First, we found that the degree of potentiation depends on the α subunit isoform. At 1μM midazolam (in presence of EC_{10} GABA), α1-3 and α5-containing receptors showed between 200-350% enhancement of GABA current with α2/3 showing greater enhancement than α1/5 (n=5-12 cells per group). We tried several exposure protocols to get consistent, reversible midazolam potentiation before choosing a protocol that depended on exposing the receptors to GABA after midazolam exposure ended, producing a rapid onset to peak potentiation. Second, a GABA dose-response on $\alpha_1\beta_2\gamma_2$ receptors in the presence of 1μM midazolam showed a significantly smaller ($p<0.05$, t=2.395, df=21) half-maximal (EC_{50}) concentration (35.9 ± 8.3 μM) relative to the EC_{50} in the absence of midazolam (76.8 ± 17.1 μM) (mean ± SEM). The Hill coefficient was also significantly increased ($p<0.05$, t=2.533, df=21) from 1.00 to 1.435 in the presence of 1μM midazolam while the maximum peak current remained unchanged. We found that these findings are consistent with simple allosteric kinetic schemes where BZDs selectively alter the binding of each GABA molecule. Therefore, the assumption of a parallel leftward shift in the GABA dose-response curve in the presence of midazolam does not adequately explain the continued midazolam potentiation seen in the presence of GABA after midazolam exposure has been stopped unless we consider other factors like lipid partitioning, low-affinity BZD sites and the possibility that the Hill equation may misrepresent the types of concentration effects seen in our experimental data.

Disclosures: O. Moody: None. **A. Jenkins:** None.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.28/DP01 (Dynamic Poster)

Topic: B.02. Ligand-Gated Ion Channels

Support: R01-MH100561

Title: Adolescent synaptic pruning in CA3 hippocampus is due to α4βδ gabar expression.

Authors: *J. PARATO, S. SMITH;
SUNY Downstate, Brooklyn, NY

Abstract: During puberty, synaptic pruning occurs in multiple regions of the brain, including the CA1 hippocampus (Yildrim et al., 2008; Afroz, Parato et al., 2016) and prefrontal cortex (Rakic et al. 2011). Dysregulation of pruning is linked to disorders such as schizophrenia (Glantz and Lewis, 2000) and autism (Hutsler and Zhang, 2010). Schizophrenia normally develops after puberty, indicating that adolescent pruning may be a factor. (Rolls et al., 2005; Paus et al., 2008). We have recently shown (Afroz, Parato, et al., 2016).that adolescent synaptic pruning in CA1 hippocampus is due to the emergence of α4βδ GABARs on dendritic spines. These receptors produce a shunting inhibition which impairs activation of NMDA receptors and reduces expression of Kalirin-7 (Kal7), a Rho-GEF (guanine nucleotide exchange factor) which is necessary for spine stability (Ma et al., 2011).
Another region of the brain which undergoes synaptic pruning is CA3, which is a hippocampal region that facilitates the encoding and retrieval of associations, particularly of spatial locations in rodents, and pattern separation in humans (Deuker et al., 2014). Studies have found that scavenging plays a role in pruning in CA3 (Shi et al., 2015), but the possible trigger for this process is not known.
Thus, we examined spine density using Golgi staining and assessed α4 and Kal7 levels across adolescence in the female mouse (puberty, ~PND 35, assessed by vaginal opening; post-puberty, PND 56) using immunohistochemical techniques. We found that GABAR α4 expression increases by 53% at puberty in the CA3 hippocampus ($p<.05$). Knock-out of α4 prevented the post-pubertal decrease in spine density observed in wild-type (pubertal WT, 1.6 ± 0.1 sp/μm; post-pubertal WT, 1.2 ± 0.1 sp/μm, post-pubertal α4-/-,1.6 ± 0.1 sp/μm; $p<.05$). Analysis of spine types, revealed a 48% decrease in mushroom spines ($p<.05$), an effect prevented by α4 knock-out. We also measured a 14% decrease in Kal7 levels from pre-puberty to puberty ($p<.05$), which would decrease spine stability, an outcome not seen in the α4 -/- CA3 hippocampus ($p<.05$), suggesting a mechanism for the high level of spine density in this group of animals. Our data suggest that pubertal expression of α4βδ GABARs triggers synaptic pruning in CA3 hippocampus via decreased expression of Kal7.

Disclosures: **J. Parato:** A. Employment/Salary (full or part-time): SUNY Downstate. **S. Smith:** A. Employment/Salary (full or part-time): SUNY Downstate.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.29/E30

Topic: B.02. Ligand-Gated Ion Channels

Support: NIH/NINDS K08 1K08NS091248

NIH/NINDS 5R01NS40109-14

Title: The developmental decrease in neuronal chloride concentration is independent of slicing trauma in thalamo-cortical brain slices

Authors: *J. C. GLYKYS, K. STALEY;
Dept. of Neurol., Massachusetts Gen. Hosp., Boston, MA

Abstract: The intraneuronal chloride concentration ($[Cl^-]_i$) is critical for determining the polarity of signaling at $GABA_A$ synapses in the central nervous system. Sectioning hippocampal brain slices increases $[Cl^-]_i$ in the superficial layers. It is unknown if this same effect occurs in the neocortex and thalamus. Therefore, we aimed to study whether cutting trauma also increases $[Cl^-]_i$ in the neocortex and thalamus, and whether the effects of trauma change during development. Neuronal $[Cl^-]_i$ (Clomeleon expressing neurons) was imaged by multiphoton microscopy in acute thalamo-cortical brain slices at developmental ages ranging from post-natal day 5 (P5) to P20. We observed: 1) $[Cl^-]_i$ is higher in the most superficial areas in both neocortical and thalamic brain slices at all ages tested and, 2) there is a developmental decrease in $[Cl^-]_i$ that is independent of acute trauma caused by brain slicing. We conclude that $[Cl^-]_i$ has a developmental progression during P5-20 in both the neocortex and thalamus. However, in both brain regions and during development the neurons closest to the slicing trauma have an elevated $[Cl^-]_i$. We hypothesize that this is due to a disruption of the extracellular matrix in addition to the sectioning of neuronal processes.

Disclosures: J.C. Glykys: None. **K. Staley:** None.

Poster

034. GABAA Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 34.30/E31

Topic: B.02. Ligand-Gated Ion Channels

Support: 2R01NS040109-16A1

Title: Characterization of neuronal chloride microdomains

Authors: *N. RAHMATI, K. J. STALEY;
Neurol., Harvard Med. Sch. & Massachusetts Gen. Hos, Boston, MA

Abstract: Chloride is a minor neuronal cytpolasmic anion whose concentration is maintained by secondary cation-chloride cotransporters. Electrophysiological estimates of cytoplasmic neuronal

chloride concentrations are notable for the variance between neurons and also between different compartments of the same neuron. Recently published and preliminary studies from investigators in our group utilizing multiphoton microscopy and transgenic chloride-sensitive fluorophores have confirmed the variance in chloride concentration varies in different brain regions (e.g. in cortical vs. subcortical neurons), different neurons, and different cytoplasmic milieus, including cell bodies, dendrites and even different segments of the same dendrites. If chloride concentrations differ in these microdomains, and this underlies the variance in electrophysiological measures of the reversal potential for $GABA_A$ receptor-mediated chloride currents (EGABA), then the local EGABA should be predictable from the fluorophore data. To test this idea, we are characterizing the chloride microdomains using both microscopic analysis of the high-sensitivity chloride fluorophore Super Clomeleon, and by simultaneous electrophysiological measures of the local EGABA in organotypic slice cultures of murine hippocampus. These studies will shed light on neuronal chloride homeostasis and the existence of functionally significant neuronal chloride microdomains.

Disclosures: **N. Rahmati:** None. **K.J. Staley:** None.

Poster

035. Opioid, Purine, and Peptide Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 35.01/E32

Topic: B.03. G-Protein Coupled Receptors

Support: NSF Grant DGE-1252376

NIH 5R01NS076772

NIH DA027969

NSF CHE7151264

Title: μ-opioid receptor-mediated catecholamine secretion in adrenal tissue

Authors: *L. DUNAWAY, L. SOMBERS;
Chem., North Carolina State Univ., Raleigh, NC

Abstract: The adrenal glands regulate physiological responses to stressors, in part by secretion of the catecholamines, epinephrine and norepinephrine. A variety of peptides, including opioid peptides, are thought to be co-stored with catecholamines in dense core vesicles in adrenal cells. The opioid system is strikingly complex, and the precise interaction between opioid peptides and

catecholamines is not well understood. We have used fast-scan cyclic voltammetry coupled to carbon-fiber microelectrodes to study the kinetic properties of the opioid/catecholamine interaction in a rat adrenal slice preparation. These experiments are complicated by the fact that many peptides, both endogenous and synthetic, foul the electrode surface and interfere with catecholamine detection. To address this issue, a sawhorse waveform was developed to clean the electrode surface, enabling reproducible measurements. This allows simultaneous monitoring of the application and clearance of exogenous opioid peptide (met-enkephalin or its synthetic analog DAMGO), and the effects of this manipulation on local catecholamine dynamics. We found that an acute application of M-ENK or DAMGO, which is specific to the mu opioid receptor, evokes catecholamine release that is sensitive to blockade by naltrexone, a non-selective opioid receptor antagonist. Further, these mu opioid receptor agonists facilitate nicotine-evoked catecholamine release. DPDPE, a delta opioid receptor agonist, does not elicit a physiological response. These results demonstrate that M-ENK evokes catecholamine secretion in the adrenal medulla by binding to mu opioid receptors, providing a chemical mechanism by which opioid peptides can regulate an organism's response to physiological and environmental demands.

Disclosures: L. Dunaway: None. **L. Sombers:** None.

Poster

035. Opioid, Purine, and Peptide Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 35.02/E33

Topic: B.03. G-Protein Coupled Receptors

Support: NIH R01DA027811 (NTZ)

Title: A structure-activity relationship study of the pan-opioid antagonist AT-076 reveals a kappa-selective antagonist with exceptionally high binding affinity

Authors: *E. TUAN, V. B. JOURNIGAN, N. T. ZAVERI;
Astraea Therapeut., Mountain View, CA

Abstract: The kappa opioid receptor is a $G_{i/o}$-coupled G-protein-coupled receptor that binds endogenously to the peptide dynorphin. A mounting body of evidence suggests that the receptor is involved in affective states such as pain, consciousness, stress, and mood. Interestingly, many of the receptor's effects appear to invoke opposing effects to those of the mu-opioid receptor, the receptor that classically mediates the various effects of morphine, heroin, and other addictive opioids. Along these lines, selective kappa-opioid antagonists such as JDTic have shown marked

effects at attenuating depression, anxiety, and drug-dependence in animal models and may have potential as drugs for treating these disorders. In 2015, we reported the discovery of AT-076, the first small-molecule opioid pan antagonist, which had nanomolar affinity at all four opioid receptors: mu-, delta-, kappa-, and nociceptin. AT-076 was discovered from a medicinal chemistry and structure-activity relationship (SAR) study of the common opioid antagonist core scaffold, *trans*-(3R,4R)-dimethyl-4-(3-hydroxyphenyl)piperidine, a pharmacophore that is also present in other opioid antagonists such as the kappa-selective antagonist JDTic, mu-selective antagonist LY246736 (Alvimopan), and the non-selective opioid antagonist LY255582. Unlike JDTic, however, AT-076 has high binding affinity for the nociceptin opioid receptor. We have continued to explore the SAR of this scaffold at the various opioid receptor subtypes and synthesized several AT-076 analogues. We report here the initial in vitro pharmacological characterization of these analogues. Of particular note are AT-496 and AT-501, which possess exceptionally high binding affinity for the kappa opioid receptor. Both compounds also display modest selectivity for the kappa opioid receptor, where they bind with picomolar K_i, over the mu- and nociceptin opioid receptors, where they bind with nanomolar K_i.

Disclosures: **E. Tuan:** None. **V.B. Journigan:** None. **N.T. Zaveri:** None.

Poster

035. Opioid, Purine, and Peptide Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 35.03/E34

Topic: B.03. G-Protein Coupled Receptors

Support: FEDER/ Le Fonds Europeen de Developpment Regional: Region Alsace

Title: A comprehensive brain mapping study of 77 orphan GPCRs in mouse and human brain reveals new drug targets for psychiatric disease

Authors: ***A. EHRLICH**[1,2], G. MAROTEAUX[1,2], M. OSIKOWICZ[1], E. DARCQ[1,2], A. ROBE[3], L. VENTEO[4], J. A. J. BECKER[3], B. KIEFFER[1,3,2];
[1]Douglas Res. Inst. and Hosp., Verdun, QC, Canada; [2]Psychiatry, McGill Univ., Montreal, QC, Canada; [3]Igbmc, Inst. Génétique Biologie Moléculaire Cellulaire, Illkirch, France; [4]Label Histologie, Riems, France

Abstract: G protein-coupled receptors (GPCRs), are seven transmembrane receptors that orchestrate cellular responses required for most physiological functions, making GPCRs obvious pharmacological targets for psychiatric disease. Around 140 GPCRs, are termed “orphan” receptors (oGPCRs), meaning that they have yet to be paired with a putative endogenous ligand

and/or paired with a physiological function and roughly 80 have enriched expression in the central nervous system. Thus, these oGPCRs represent an untapped resource for psychiatric drug discovery yet, a detailed *in vivo* characterization of brain oGPCRs has been lacking. Here, using two distinct methods of in situ hybridization (ISH), indirect DIG-labeled riboprobes and a direct method, RNAscope®, the neuroanatomical expression of 77 oGPCRs are examined in the mouse brain. oGPCR expression patterns were scored across 14 brain regions, and hierarchical clustering compiled the oGPCRs into a heatmap revealing three categories of expression: (1) widespread expressing oGPCRs suggest uniform roles across the brain, (2) moderate expressing oGPCRs suggest refined function in neuronal networks and (3) discrete expressing oGPCRs suggest tightly controlled roles in brain function. Further cross-species analysis of our data of the 77 oGPCRs was done with publicly available mouse and human data-sets, to find our analysis highly correlated. For 26 oGPCRs, the pattern of expression was remarkably found in brain regions with critical roles in reward and aversion pathways, sites of deficit in psychiatric disease. We next obtained human brain tissue from the Douglas Bell Canada Brain Bank for these 26 oGPCRs, total RNA was prepared from 8 control subjects across the 14 brain regions corresponding to those analyzed in the mouse and then submitted to a highly sensitive and direct method of digital gene expression analysis with the Nanostring nCounter system®. This robust analysis correlated highly with our mouse ISH data for the 26 oGPCRs, adding translational value to their unique expression profiles. Taken together, we provide the first cross-species comparison of the expression of 77 oGPCRs in the mouse and human brain, this collective exposes Gpr26, Gpr85, Gpr101, Gpr149 as novel candidates for development of new psychiatric drugs.

Disclosures: **A. Ehrlich:** None. **G. Maroteaux:** None. **M. Osikowicz:** None. **E. Darcq:** None. **A. Robe:** None. **L. Venteo:** None. **J.A.J. Becker:** None. **B. Kieffer:** None.

Poster

035. Opioid, Purine, and Peptide Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 35.04/E35

Topic: B.03. G-Protein Coupled Receptors

Support: NIH-NIAAA F31AA024033

1R01AA023797

Title: Synaptic mechanisms of OPRM1 A118G (MOR N40D) gene variants in human neurons

Authors: *A. HALIKERE[1], J. C. MOORE[2], J. TISCHFIELD[2], R. P. HART[3], Z. P. PANG[4]; [1]Dept. of Neurosci. and Cell Biol., CHINJ, RWJMS, Rutgers Univ., New Brunswick, NJ; [2]Genet., RUCDR/Infinite Biologics, The Human Genet. Inst. of New Jersey, Dept. of Genetics, Rutgers Univ., New Brunswick, NJ; [3]Cell Biol. and Neurosci., The Human Genet. Inst. of New Jersey, Dept. of Cell Biol. and Neuroscience, Rutgers Univ., New Brunswick, NB; [4]Neurosci. and Cell Biol., Child Hlth. Inst. of New Jersey, Robert Wood Johnson Med. School, Rutgers Univ., New Brunswick, NJ

Abstract: Mu opioid receptor (MOR) signaling modulates synaptic transmission and thus plays a pivotal role in regulating reward behaviors relevant to addiction. However, the molecular and synaptic mechanisms of opioid signaling in the context of drug addiction are poorly understood. Interestingly, the single nucleotide polymorphism (SNP) rs1799971 (OPRM1 A118G) in the human MOR has been linked to impaired MOR trafficking and signal transduction and has been implicated in increased predisposition to drug abuse disorders and alcohol use disorders. The A118G SNP produces a non-synonymous amino acid substitution, replacing an asparagine with an aspartate at position 40 (MOR N40D). Although heterologous expression systems and animal studies suggest altered receptor trafficking, ligand binding affinities and altered intracellular signaling, these studies have provided largely inconclusive and even conflicting evidence regarding the cellular and molecular mechanisms of the MOR N40D SNP. To investigate the molecular, cellular, and synaptic consequences of this SNP in a human neuronal context, we derived human neurons, using the induced neuronal (iN) cell technology, from induced pluripotent stem (iPS) cell lines generated from multiple human subjects carrying either homozygous N40 or D40 alleles. Our compelling preliminary data reveal that D40 MOR iNs exhibit more potent inhibition of synaptic release following MOR activation by DAMGO ([D-Ala^2, NMe-Phe^4, Gly-ol^5]-enkephalin) when compared to N40 MOR expressing iNs. Furthermore, pre-exposure of these human neurons to DAMGO for 24 hours diminishes their sensitivity to DAMGO, possibly due to the desensitization and internalization of membrane MORs following prolonged activation of the receptors. Moreover, N40 MOR carrying human neurons regained partial sensitivity to DAMGO during a 7-day pre-exposure paradigm, whereas D40 failed to re-sensitize. We thus hypothesize that human N40 and D40 MORs have differential membrane recycling dynamics. We are currently testing this hypothesis. Our system using iNs carrying human MOR gene variants provides the unique opportunity to study the neurophysiology of MOR regulation on synaptic transmission in a human system and will likely provide novel information about the neurocircuitry of reward behavior.

Disclosures: A. Halikere: None. **J.C. Moore:** None. **J. Tischfield:** None. **R.P. Hart:** None. **Z.P. Pang:** None.

Poster

035. Opioid, Purine, and Peptide Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 35.05/E36

Topic: B.03. G-Protein Coupled Receptors

Title: High content screening of mu-opioid receptor internalizationusing fluorescent rabbit monoclonal antibodies against its N-terminal fragment

Authors: *A. E. KALYUZHNY, J. HAGEN, N. HOPP, M. GRAHEK, J. HUMPHREY, G. DU, P. MURTHA, M. COOPER, C. AUTIN, B. AGGELER;
Bio-Techne, Minneapolis, MN

Abstract: Trafficking of mu-opioid receptors from the cell membrane into cytoplasm is one of the key mechanisms underlying desensitization of these receptors which is implicated in tolerance and dependence to narcotic opiates. Usually, endocytosis of mu-opioid receptors is analyzed qualitatively by utilizing cells transfected with mu-opioid receptor tagged with such haptens as HA and c-*myc* at their N-termini followed by immunocytochemical detection using tag-specific secondary antibodies conjugated to fluorescent dyes. We have developed a more efficient protocol to monitor receptor endocytosis by employing Alexa 594 conjugated rabbit monoclonal antibodies against the N-terminal part of OPRM1. Treatment of HEK293 cells transfected with GFP tag at its N-terminus of rat OPRM1 with agonist of mu-opioid receptors DAMGO produced strong internalization of receptors which was blocked by antagonist Naloxone. The specificity of internalization was confirmed by detecting complete overlap of green (GFP) and red (Alexa 594) fluorescence using confocal microscope. The extent of mu-opioid receptor internalization has been further analyzed using the High Content Screening (HCS) platform, allowing automated examination hundreds of cells treated with drugs. The advantage of this new technique is that it can be applied to native neuronal cells because they do not require transfecting with hapten-containing mu-opioid receptor constructs. By measuring the fluorescence intensity of mu-opioid receptors localized on the cell membrane and in the cytoplasm, it becomes possible to accurately quantify internalization of mu-opioid receptors.

Disclosures: A.E. Kalyuzhny: None. **J. Hagen:** None. **N. Hopp:** None. **M. Grahek:** None. **J. Humphrey:** None. **G. Du:** None. **P. Murtha:** None. **M. Cooper:** None. **C. Autin:** None. **B. Aggeler:** None.

Poster

035. Opioid, Purine, and Peptide Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 35.06/E37

Topic: B.03. G-Protein Coupled Receptors

Support: DFG Grant HI 1288/3-1

Title: $P2Y_1$ receptor-mediated modulation of neuronal activity in the mouse olfactory bulb

Authors: *D. HIRNET, K. SCHULZ;
Div. of Neurophysiol., Univ. of Hamburg, Hamburg, Germany

Abstract: Purine nucleotides such as ATP and ADP modulate the communication between cells throughout the nervous system. In the mouse olfactory bulb (OB), the first olfactory relay station, ATP is released from sensory axons together with glutamate as a neurotransmitter and stimulates calcium signaling in glia cells. It has recently been shown that ATP also evokes neuronal network activity in the OB, though the origin of this effect could not be identified so far. We used spatiotemporal defined photolysis of caged ATP and caged ADP in acute mouse OB slices to investigate purinergic modulation in glomeruli, specific processing units of the OB. Therefore, we released the active molecules locally restricted to glomeruli and recorded the response in mitral cells and external tufted cells conveying to this glomerulus by whole-cell patch clamp. The release of ATP led to a $P2Y_1$ receptor-dependent increase in synaptic activity and a prominent depolarization of mitral/tufted cells (MC/TC). This depolarization was reduced in synaptic isolation (TTX, NBQX, DAP-V, CPCCOEt, ZM241385, DPCPX). However, under these conditions ATP as well as ADP still caused a $P2Y_1$-mediated inward current in MCs. Given the increase in membrane conductivity underlying the induced inward current at negative holding potentials, we conclude that purine nucleotides seem to modulate unspecific cation channels in mitral cells. Our results show, that purinergic signaling is present in one of the processing units in the OB and presumably is able to modulate the processing of odor information.

Disclosures: **D. Hirnet:** None. **K. Schulz:** None.

Poster

035. Opioid, Purine, and Peptide Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 35.07/E38

Topic: B.03. G-Protein Coupled Receptors

Support: FCT

EMBO fellowship

DFG Center for Nanoscale Microscopy and Molecular Physiology of the Brain

LabEx, DISTALZ, DN2M, ANR (ADORATAU) and FUI MEDIALZ

LECMA/Alzheimer Forschung Initiative (AFI)

Investigator FCT (LVL)

Title: The caffeine-binding adenosine A2A receptor induces age-like HPA-axis dysfunction by targeting glucocorticoid receptor function

Authors: *L. V. LOPES[1], V. L. BATALHA[1], D. FERREIRA[1], J. E. COELHO[1], R. GOMES[1], T. SHMIDT[2], Y. BAQI[3], L. BUÉE[4], C. E. MÜLLER[3], M. HAMDANE[4], T. F. OUTEIRO[5], M. BADER[2], S. H. MEIJSING[6], G. SADRI-VAKILI[7], D. BLUM[4];
[1]Inst. de Medicina Molecular, Fac Med. Lisbon, Lisbon, Portugal; [2]MDC, Berlin, Germany; [3]Univ. Bonn, Bonn, Germany; [4]INSERM, Lille, France; [5]Univ. Med. Ctr. Göttingen, Göttingen, Germany; [6]Max Planck Inst. for Mol. Genet., Berlin, Germany; [7]MassGeneral Inst. for Neurodegenerative Dis., Boston, MA

Abstract: Caffeine is associated with procognitive effects in humans by counteracting overactivation of the adenosine A_{2A} receptor ($A_{2A}R$), which is upregulated in the human forebrain of aged and Alzheimer's disease (AD) patients. We have previously shown that an anti-$A_{2A}R$ therapy reverts age-like memory deficits, by reestablishment of the hypothalamic-pituitary-adrenal (HPA) axis feedback and corticosterone circadian levels. These observations suggest that $A_{2A}R$ over-activation and glucocorticoid dysfunction are key events in age-related hippocampal deficits; but their direct connection has never been explored. We now report that inducing $A_{2A}R$ overexpression in an aging-like profile is sufficient to trigger HPA-axis dysfunction, namely loss of plasmatic corticosterone circadian oscillation, and promotes reduction of GR hippocampal levels. The synaptic plasticity deficits triggered by GR in the hippocampus are amplified by $A_{2A}R$ over-activation and were rescued by anti-A_{2A} therapy; finally, we demonstrate that $A_{2A}R$ act on GR nuclear translocation and GR-dependent transcriptional regulation. We provide the first demonstration that $A_{2A}R$ is a major regulator of

GR function and that this functional interconnection may be a trigger to age-related memory deficits. This supports the idea that the procognitive effects of $A_{2A}R$ antagonists, namely caffeine, on Alzheimer's and age-related cognitive impairments may rely on its ability to modulate GR actions.

Disclosures: **L.V. Lopes:** None. **V.L. Batalha:** None. **D. Ferreira:** None. **J.E. Coelho:** None. **R. Gomes:** None. **T. Shmidt:** None. **Y. Baqi:** None. **L. Buée:** None. **C.E. Müller:** None. **M. Hamdane:** None. **T.F. Outeiro:** None. **M. Bader:** None. **S.H. Meijsing:** None. **G. Sadri-Vakili:** None. **D. Blum:** None.

Poster

035. Opioid, Purine, and Peptide Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 35.08/F1

Topic: B.03. G-Protein Coupled Receptors

Support: SAU Start-Up Fund

Title: Exogenous ATP modulates prostaglandin E_2 synthesis during inflammation

Authors: *R. S. AKUNDI, S. AKTER;
South Asian Univ., New Delhi, India

Abstract: Brain inflammation is a common occurrence following responses to varied insults such as bacterial infections, stroke, traumatic brain injury and neurodegenerative disorders. A common mediator for these varied inflammatory responses is prostaglandin E_2 (PGE_2), produced by the enzymatic activity of cyclooxygenases (COX) 1 and 2. Previous attempts to reduce neuronal inflammation through COX inhibition, by use of nonsteroidal anti-inflammatory drugs (NSAIDs), have met with limited success. It is well-known that inflammation and cell injury leads to the release of significant amounts of adenosine triphosphate (ATP). Here, we show that exogenous ATP modulates the inflammatory response of LPS-activated macrophage and neuronal cell-lines by 2-5 fold. We have further identified P2Y6 as one of the purinergic receptors involved in this pathway through the use of specific agonists and antagonists. We also find the involvement of p38 mitogen-activated protein kinase in this pathway. Targeting the P2 receptors, therefore, provides a therapeutic alternative to reduce inflammation in the brain. It paves the way for P2 receptor-based anti-inflammatory drugs (PBAIDs) which will retain the activities of essential COX enzymes, yet will significantly reduce inflammation by decreasing the enhanced production of PGE_2 by extracellular ATP.

Disclosures: **R.S. Akundi:** None. **S. Akter:** None.

Poster

035. Opioid, Purine, and Peptide Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 35.09/F2

Topic: B.03. G-Protein Coupled Receptors

Support: The National Basic Research Program of China 973 Program (2010CB912003)

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Swedish Research Council Grants (2011-3465, 2015-02998)

Title: Trafficking of the neuropeptide galanin and its receptors in live cells

Authors: ***T. LI**[1,2], V. VUKOJEVIć[2], Y. BAI[1], Y. YANG[1], L. TERENIUS[2], T. HÖKFELT[3], P. SVENNINGSSON[2], Z.-Q. XU[1,3];
[1]Dept. of Neurobio., Capital Med. Univ., Beijing, China; [2]Dept. of Clin. Neurosci., [3]Dept. of Neurosci., Karolinska Institutet, Stockholm, Sweden

Abstract: The neuropeptide galanin was tagged at its N terminus with TAMRA or FAM to study trafficking and binding in live PC12 and HEK293 cells. We explored some of these important processes with the help of two quantitative methods: Fluorescence Correlation Spectroscopy (FCS) and its dual-color, two channel variant, Fluorescence Cross-Correlation Spectroscopy (FCCS) as well as Fluorescence recovery after photo bleaching (FRAP). Using these approaches we characterized the cellular dynamics and lateral diffusion at both the nano- and microscale levels. Our results show that in both cell lines functional GalR1-EGFP and GalR2-EGFP were expressed on the plasma membrane and internalized into cytoplasmic vesicles after stimulation with tagged galanin. Moreover, galanin receptors co-localize, co-migrate and form complexes with galanin peptide in live cells, and the complexes dissociate before being delivered to the lysosome. Inhibition of cholesterol-dependent GalR1 internalization by exposure to methyl-β-cyclodextrin or cholesterol oxidase reduced formation of the GalR1-galanin complex, and induced faster lateral diffusion of GalR1 on the membrane. In contrast, cholesterol depletion did not visibly alter lateral mobility of GalR2 and formation of the GalR2-galanin complex. Our study indicates that galanin receptor subtypes exhibit subtle differences with regard to lateral diffusion and that they are differentially dependent on cholesterol.

Disclosures: **T. Li:** None. **V. Vukojević:** None. **Y. Bai:** None. **Y. Yang:** None. **L. Terenius:** None. **T. Hökfelt:** None. **P. Svenningsson:** None. **Z. Xu:** None.

Poster

035. Opioid, Purine, and Peptide Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 35.10/F3

Topic: B.03. G-Protein Coupled Receptors

Support: NIMH MH093412

Brain and Behavior Research Foundation 19417

Title: Oxytocin alters excitatory synaptic transmission in rat insular cortex

Authors: ***J. A. VARELA**, V. KRISHNA, J. P. CHRISTIANSON;
Psychology, Boston Col., Chestnut Hill, MA

Abstract: The neuropeptide oxytocin (OT) is a highly conserved neural modulator and is known to contribute to social and fear behaviors. OT positive axons and OT receptors (OTR) are found in the insula of rat but little is known regarding the physiological consequences of OT acting in this region. We sought to describe the effects of OT on synaptic transmission in layer 5/6 pyramidal neurons of the insular cortex. Studies were done using visualized whole cell patch clamp recordings and extracellular field potentials from 300μm slices taken from 6 to 9 week old male Sprague Dawely rats; data were included for analysis if they were found in the deep layers of Agranular or Granular insular cortex 2.8mm (+/- .5mm) caudal to bregma and appeared to have pyramidal morphology (confirmed by biocytin staining with patch-clamp experiments or by visual inspection on field potential experiments). Bath application of OT (500 nM) decreased of the mEPSC amplitude and increased the inter-event intervals (IEI), ps < 0.05. However, preliminary data shows that in 3 out 4 cells spontaneous excitatory post-synaptic currents (sEPSCs) the IEI was decreased with no clear change in amplitude and sIPSCs amplitude and IEI were unchanged. OT did not influence evoked excitatory post-synaptic potentials (fEPSPs) per se (N=8), but OT applied during low frequency stimulation (LFS, 900 stimulations at 1Hz) appeared to promote long term depression (N=3) when compared to control (N=3); data collection is ongoing. Together these data suggest that OT may alter both presynaptic glutamate release probability and postsynanptic AMPA receptor kinetics to promote LTD-like neuroplasticity.

Disclosures: **J.A. Varela:** None. **V. Krishna:** None. **J.P. Christianson:** None.

Poster

035. Opioid, Purine, and Peptide Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 35.11/F4

Topic: B.03. G-Protein Coupled Receptors

Title: Expression of functional ox2 receptor in the human cervix cell line c33a

Authors: E. PERDONA', F. FAGGIONI, *M. A. CORSI;
Aptuit Ctr. For Drug Discovery & Develop., Verona, Italy

Abstract: Orexin-A and orexin-B, are neuropeptides produced by perifornical and lateral hypothalamic neurons that project throughout the brain and bind two different G protein-coupled receptors, orexin-1 receptor (OX1R) and orexin-2 receptor (OX2R). OX1R signals through Gq coupling whereas OX2R has been reported to be coupled to Gq or Gi/Go. The modulation of the OXRs has the potential to impact various pathophysiological conditions such as stress, anxiety, pain, disturbances of the sleep-wake cycle, addiction and feeding disorders. Biological experiments are most often performed with immortalized cell lines because they are readily available and can be expanded without limitation. However, cell lines may differ from the *in vivo* situation in important aspects such for instance the presence of accessory proteins needed for the signal transmission and the presence of the native receptor coupling machinery. It becomes essential to confirm in primary cells the pharmacology of ligands originally identified on recombinant systems. The aim of this study was to identify and characterize the functional activity of endogenously expressed OXRs in a physiological cell line. Human cervical carcinoma cells, C33A and human neuroepithelioma cells, MCIXC were studied for this scope. The gene expression was studied by RT-PCR whereas functional expression and antagonism profile were characterized by calcium mobilization measurement and D-myo-inositol-1-monophosphate (IP1) accumulation, respectively. Both cell lines expressed OX2R mRNA, whereas OX1R mRNA only in C33A cells. Increasing concentrations of orexin-A- and orexin-B-induced a calcium response only in C33A cells with a potency of 8.06±0.02 and 8.65±0.01, respectively (n=3). No calcium response was observed in MCIXC. To determine the relative effect of OX1R or OX2R in the [Ca^{2+}]$_i$ mobilization induced by orexin-A, C33A cells were pre-incubated with either the OX1R selective antagonist SB-334867 or OX2R selective antagonist JNJ-10397049 or with the dual OX1R/OX2R antagonists ACT-078573 or MK-6096. Increasing concentrations of all five compounds fully inhibited the calcium response induced by orexin-A with fpKi value of 6.06 ± 0.04 for SB-334867, 9.00 ± 0.05 for JNJ-10397049, 9.66 ± 0.06 for ACT-078573 and 9.94 ± 0.05 for MK-6096. These potency values were confirmed with the IP-1 accumulation assay. These results indicate the lack of a Gq/PLC-coupled OX2R in MCIXC cells. Moreover, the calcium mobilization induced by orexin-A in C33A cells is exclusively mediated by OX2R

indicating that C33A cell line could represent a useful tool for the pharmacological characterization of new chemical entities.

Disclosures: E. Perdona': A. Employment/Salary (full or part-time): Aptuit Center for Drug Discovery & Development, Verona, Italy. **F. Faggioni:** A. Employment/Salary (full or part-time): Aptuit Center for Drug Discovery & Development, Verona, Italy. **M.A. Corsi:** A. Employment/Salary (full or part-time): Aptuit Center for Drug Discovery & Development, Verona, Italy.

Poster

035. Opioid, Purine, and Peptide Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 35.12/F5

Topic: B.03. G-Protein Coupled Receptors

Support: FONDECYT No. 1120156

Basal Center of Excellence in Science and Technology CONICYT-PFB12/ 2007

Fondecyt Postdoctorado N°3140355

Title: Wnt-5a/Frizzled-9 regulates dendritic spine formation through Gαo and Gβγ

Authors: *V. T. RAMIREZ, E. RAMOS-FERNÁNDEZ, N. C. INESTROSA;
Pontifical Catholic Univ., Santiago, Chile

Abstract: Wnt ligands play crucial roles in the development and regulation of synapse structure and function. Specifically, Wnt-5a acts as a secreted growth factor that regulates dendritic spine formation in rodent hippocampal neurons, resulting in postsynaptic development that promotes the clustering of the postsynaptic density protein-95 (PSD-95). Here, we focused on the early events occurring after the interaction between Wnt-5a and its Frizzled receptor at the neuronal cell surface. Additionally, we studied the role of heterotrimeric G proteins in Wnt-5a- dependent synaptic development. We report that Frizzled9 (FZD9), a Wnt receptor related to Williams' syndrome, is localized in the postsynaptic region, where it interacts with Wnt- 5a. Functionally, FZD9 is required for the Wnt- 5a-mediated increase in dendritic spine density. FZD9 forms a pre-coupled complex with Gαo and Gβγ under basal conditions that dissociates after Wnt-5a stimulation. Accordingly, we found that G-protein inhibition abrogates Wnt-5a- dependent pathway in hippocampal neurons. In particular, the activation of Gαo appears to be a key factor controlling the Wnt-5a-induced dendritic spine density. In addition, we found that Gβγ is required for the Wnt-5a-mediated increase in cytosolic calcium levels and spinogenesis. Our

findings reveal that FZD9 and heterotrimeric G proteins form a crucial protein complex that regulates Wnt-5a signaling and dendritic spines in cultured hippocampal neurons.

Disclosures: V.T. Ramirez: None. **E. Ramos-Fernández:** None. **N.C. Inestrosa:** None.

Poster

035. Opioid, Purine, and Peptide Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 35.13/F6

Topic: B.03. G-Protein Coupled Receptors

Title: Comparison of Leu^8- and Pro^8-oxytocin potency, efficacy and functional selectivity at the human and marmoset receptors.

Authors: *M. PIERCE[1], S. MEHROTRA[1], M. L. TOEWS[2], J. A. FRENCH[3], T. F. MURRAY[1]; [1]Dept. of Pharmacol., Creighton Univ., Omaha, NE; [2]Pharmacol. and Exptl. Neurosci., Univ. of Nebraska Med. Ctr., Omaha, NE; [3]Psychology, Univ. of Nebraska Omaha, Omaha, NE

Abstract: The nonapeptide oxytocin (OT) is an important modulator of social behavior. OT binds to the oxytocin receptor (OTR) and activates multiple G proteins that exert diverse effects on cell function, including stimulation of cAMP (Gs), inhibition of adenylyl cyclase (Gi/o), stimulation of potassium channel currents (Gi), and activation of phospholipase C (Gq). In mammals, the nonapeptide OT sequence is highly conserved (Cys-Tyr-Ile-Gln-Asn-Cys-Pro-**Leu**-Gly; Leu^8-OT). Five novel OT ligand variants were recently discovered in New World Monkeys. In marmosets (*Callithrix*), an amino acid change to proline occurs in the 8^{th} position of the peptide (Pro^8-OT). Here we used stably transfected CHO cells expressing marmoset (mOTR) or human (hOTR) oxytocin receptors to assess OT-OTR signaling. To assess OTR activation of Gq, functional assays were performed using fluo-3 to measure OT-induced intracellular Ca^{2+} mobilization. To assess OTR coupling to Gi, a FLIPR Membrane Potential (FMP) assay was performed to measure OT-induced changes in membrane potential. The FMP assay reports hyperpolarization occurring as a consequence of stimulation of K^+ channel currents. OT-stimulated [^{35}S]GTPγS binding assays were used to directly assess OTR activation of Gi/o. Pro^8-OT was more efficacious than Leu^8-OT in mOTR CHO cells in Gq-mediated Ca^{2+} mobilization assays, with both peptides displaying similar subnanomolar potencies. In contrast, the potency and efficacy of Pro^8-OT and Leu^8-OT as stimulators of intracellular Ca^{2+} in hOTR cells did not differ. Membrane potential assays demonstrated Leu^8-OT to be substantially more potent and modestly more efficacious than Pro^8-OT in both mOTR- and hOTR-expressing cells. Surprisingly, in both hOTR and mOTR cells, Leu^8-OT-induced hyperpolarization was largely insensitive to treatment with PTX, suggesting a minor role for Gi/o-activation in OT-induced

membrane hyperpolarization. To explore the role of Ca^{2+}-activated potassium channels in OT-induced membrane hyperpolarization we tested the effects of pretreatment with charybdotoxin, paxilline and Tram-34. Only Tram-34, a selective blocker of the intermediate conductance (IK) channel $K_{Ca}3.1$, attenuated the hyperpolarizing response to OT ligands. A dependence of the hyperpolarization on intracellular Ca^{2+} was confirmed by its elimination with BAPTA-AM. Analysis of OT-stimulated [^{35}S]GTPγS binding to hOTR membranes revealed that Pro^8-OT may function as an inverse agonist at the hOTR. A more thorough understanding of these distinct pharmacologic signatures for Leu^8-OT and Pro^8-OT interaction with OTRs may provide insight into therapeutic targeting of OTRs.

Disclosures: M. Pierce: None. **S. Mehrotra:** None. **M.L. Toews:** None. **J.A. French:** None. **T.F. Murray:** None.

Poster

035. Opioid, Purine, and Peptide Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 35.14/F7

Topic: B.03. G-Protein Coupled Receptors

Title: Immunohistochemical localization of a functional PAC1 antagonist antibody in the rodent trigeminovascular system

Authors: *S. MILLER[1], H. LIU[2], J. K. PRETORIUS[3], H. SUN[1], G. HILL DELLA PUPPA[1], D. W. SMITH[3], C. XU[2];
[1]Neurosci., Amgen Inc., Cambridge, MA; [2]Neurosci., [3]Comparative Biol. and Safety Sci., Amgen Inc., Thousand Oaks, CA

Abstract: Pituitary adenylate cyclase-activating polypeptide (PACAP) has been implicated in the pathophysiology of migraine. PACAP binds to the vasointestinal peptide receptors and PACAP receptors (PAC1 receptors). To further understand the mechanism of PAC1 mediated signaling in migraine, we generated mouse monoclonal antibodies against PAC1 receptors. We show that the antibody 1.8.1 blocks PACAP-induced cAMP production in cells overexpressing rat or human PAC1 receptors with nanomolar potency. To explore whether these functional antibodies could be used to map PAC1 receptor localization in tissues, we further tested 1.8.1 binding on fixed cells overexpressing rat, mouse or human PAC1 receptors using immunocytochemistry. Immunofluorescence was detected in cells overexpressing the rodent or human PAC1 receptors, but not in parental cells. The specificity of 1.8.1 for immunohistochemical applications was confirmed by comparing staining in fixed tissues from PAC1 knock out mice versus wild-type littermates. In trigeminal and sphenopalatine ganglion of

wild-type mice, 1.8.1 labelled a fraction of the neurons and in the spinal trigeminal nucleus, 1.8.1 labelled the neuropil. In the same tissues PAC1 knock out mice showed only faint fluorescent labeling, which was considered non-specific. Fluorescent staining of 1.8.1 in rat tissues was compared to in situ hybridization (ISH) with specific PAC1 receptor probes. Neurons in the trigeminal ganglion were less intensely stained than in the sphenopalatine ganglion. The degree of the fluorescent signal corresponded to a lower ISH signal in the trigeminal ganglion compared to the sphenopalatine ganglion. Likewise, staining of the spinal trigeminal nucleus matched the ISH signal in this area. The only discrepancy was found in the dura blood vessels, where staining was detected, but no ISH signal. Together these data contribute to our understanding of PAC1 receptor localization in the trigeminovascular system and as it may relate to migraine pathophysiology.

Disclosures: **S. Miller:** A. Employment/Salary (full or part-time): Amgen Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amgen Inc. **H. Liu:** A. Employment/Salary (full or part-time): Amgen Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amgen Inc. **J.K. Pretorius:** A. Employment/Salary (full or part-time): Amgen Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amgen Inc. **H. Sun:** A. Employment/Salary (full or part-time): Amgen Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amgen Inc. **G. Hill della Puppa:** A. Employment/Salary (full or part-time): Amgen Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amgen Inc. **D.W. Smith:** A. Employment/Salary (full or part-time): Amgen Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amgen Inc. **C. Xu:** A. Employment/Salary (full or part-time): Amgen Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Amgen Inc..

Poster

035. Opioid, Purine, and Peptide Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 35.15/F8

Topic: B.03. G-Protein Coupled Receptors

Support: KAKENHI 25113522

KAKENHI 15K06775

Title: Melanin-concentrating hormone-mediated signaling induces cilium shortening via Gi/o-dependent Akt phosphorylation

Authors: *Y. SAITO[1], A. HAMAMOTO[2], S. YAMATO[1], Y. KOBAYASHI[1];
[1]Hiroshima Univ., Hiroshima 739-8521, Japan; [2]Kurume Univ., Kurume, Japan

Abstract: Primary cilia are microtubule-based organelles present on nearly every cell in the mammalian body. The cilium has an important chemosensory function in many types of cells and ciliary dysfunction is associated with ciliopathies such as polycystic kidney disease and obesity. Although the ciliary membrane is contiguous with the plasma membrane, ciliary localization of protein is tightly regulated and only certain molecules are permitted to traffic there. Melanin-concentrating hormone (MCH) is the natural peptide ligand for two G-protein-coupled receptors (GPCR), MCHR1 and MCHR2. The MCH-MCHR1 system has been implicated in the regulation of feeding, sleep and emotional processing in rodents. Recently, MCHR1 expression was detected in primary cilia of the central nervous system. However, the underlying function and signaling pathway via MCHR1 located in primary cilia is unclear. Here we show that treatment of MCH significantly reduces cilia length in hTERT-RPE1 epithelial cells (hRPE1) transfected with MCHR1. Quantitative analysis indicated that the rate of MCH-induced cilia shorting progressed in time-dependent manner during the first 3 h with an EC50 value of 0.49 nM. MCH elicits receptor internalization, calcium mobilization, ERK activation, and inhibition of cAMP accumulation in MCHR1-expressing hRPE1 cells. However, these fundamental signaling pathways seem to be independent of the pathway that causes cilia shortening. Notably, we found that MCH causes an increase in Gi/o-dependent Akt activity, and that this pathway would be the principal step in the initial stage of MCH-induced ciliary shortening. These data suggests that MCH-MCHR1 governs the sensitivity by controlling the length of the cell's sensory organelle. Further characterization of MCHR1 as a ciliary GPCR provides a potential molecular mechanism to link defects in cilia with obesity. Further studies will be required to determine whether the MCH-MCHR1 axis is directly involved in controlling the cilia length in vivo, and in turn its physiological roles in the brain.

Disclosures: **Y. Saito:** None. **A. Hamamoto:** None. **S. Yamato:** None. **Y. Kobayashi:** None.

Poster

035. Opioid, Purine, and Peptide Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 35.16/F9

Topic: B.03. G-Protein Coupled Receptors

Support: NIH Grant 1R01 HL122921-01

Title: Characterization of leptin receptor expressing neurons in the mouse NTS

Authors: J. DO[1], *T. KOWAL[2], L. FLOREANI[3], D. MCCRIMMON[1], M. MARTINA[1,3]; [1]Physiol., Northwestern University, Feinberg Sch. of Med., Chicago, IL; [2]Physiol., Northwestern Univ. Dept. of Physiol., Chicago, IL; [3]Lab. of Neurogenomics, Intl. Sch. for Advanced Studies, Trieste, Italy

Abstract: Leptin is an anorexigenic hormone acting through activation of specific leptin receptors, which belong to the G-protein-coupled receptors (GPCRs) family. Leptin causes a slow long-lasting depolarization in neurons of the nucleus of the solitary tract (NTS). However, the precise identity of the cells expressing the leptin receptor and the mechanism underlying the leptin-induced depolarization are still unknown. Our study has two main goals: 1- to uncover the expression of individual genes that identify neuronal subpopulations of cells expressing the leptin receptor; and 2- to identify the identity of the ion channel(s) mediating the action of leptin in these different cellular populations. To pursue these goals we combine genetic tagging of leptin receptor expressing neurons, electrophysiological recordings and single cell RT-PCR to test the expression of a list of channels and neuropeptides. We found that NTS leptin receptor expressing neurons appear to belong to two at least distinct populations, one expressing galanin and one expressing CCK. Additionally, in virtually all of the cells harvested we detected the transcript for the leakage channel NALCN. Together our data suggest that leptin receptor expressing NTS neurons form different subpopulations and that in all of them NALCN channels could in part mediate the depolarizing action of leptin.

Disclosures: J. Do: None. **T. Kowal:** None. **L. Floreani:** None. **D. McCrimmon:** None. **M. Martina:** None.

Poster

035. Opioid, Purine, and Peptide Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 35.17/F10

Topic: B.03. G-Protein Coupled Receptors

Support: NS24067

MH64070

Title: Oxytocin increases excitability and burst firing of CA2 pyramidal neurons and tunes their synaptic output

Authors: *N. N. TIRKO[1], M. MITRE[2], R. C. FROEMKE[2], M. V. CHAO[2], R. W. TSIEN[1]; [1]NYU Neurosci. Inst., New York Univ. Sch. of Med., New York, NY; [2]Skirball Inst., NYU Sch. of Med., New York, NY

Abstract: Oxytocin neuromodulation is important in many regions of the mammalian brain responsible for social behaviors, and acts to increase information salience and circuit plasticity. We find that CA2, a distinct hippocampal area that is critical for social memory in mice, robustly expresses oxytocin receptors (OTR) and contains a high density of oxytocinergic axons originating from the paraventricular nucleus.

Oxytocin has been shown to employ various mechanisms to alter circuit function including disinhibition, increased inhibitory tone, and potentiation of excitatory inputs. In CA2 we find that OTR activation strongly modulates both excitatory neurons and fast-spiking PV-positive interneurons, raising two lines of questions: what mechanisms underlie OTR modulation of cell behavior, and how does OTR modulation of excitation and inhibition within CA2 ultimately alter circuit output?

To study the mechanisms downstream of OTR activation, we examined CA2 pyramidal cells. Application of the oxytocin receptor agonist (TGOT) depolarizes the cells (~5 mV; 100% of cells recorded) and increases total membrane resistance and excitability. Additionally, action potential (AP) amplitude and afterhyperpolarization magnitude both decrease, leading to repetitive spiking and high frequency burst activity (~30 Hz). The mechanisms underlying these effects are downstream of G-protein coupled receptor activation of phospholipase C (PLC). We find that PLC activation leads to both reduction of M-current conductance, which depolarizes the cell and increases membrane resistance, and PKC-mediated modulation of voltage-gated Na^+ channels (VGSC), which reduces their maximal conductance and voltage-dependent availability. The effects of TGOT can be mimicked by a combination of M-current inhibition and PKC activation.

Reduction in M-current conductance and phosphorylation of VGSC both shape the firing activity of CA2 pyramidal cells, but so does inhibitory transmission. Pharmacologically blocking inhibition reduced frequency and increased duration of AP bursts. What is the relevance of the burst characteristics? Using optogenetic stimulation, we show that AP burst pattern and frequency are important for controlling the output onto CA1 pyramidal cells, the primary target of CA2 axons. High frequency bursts more efficiently transfer excitatory charge to CA1 cells. We hypothesize that OTR modulation of both excitation and inhibition in CA2 tunes its output onto CA1, potentially sculpting hippocampal information transfer during periods of oxytocin release *in vivo*.

Disclosures: **N.N. Tirko:** None. **M. Mitre:** None. **R.C. Froemke:** None. **M.V. Chao:** None. **R.W. Tsien:** None.

Poster

035. Opioid, Purine, and Peptide Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 35.18/F11

Topic: B.03. G-Protein Coupled Receptors

Support: FONDECYT N° 110392

FONDECYT N° 1150244

CONICYT graduate fellowship to HEY

Title: Functional consequences of the heteromerization between dopamine D1 and type-2 corticotropin releasing factor receptors

Authors: *H. E. YARUR[1], C. A. LOPEZ[2], K. GYSLING[2];
[1]Pontificia Univ. Catolica De Chile, Santiago, Chile; [2]Dept. of Cell. and Mol. Biol., Pontificia Univ. Católica de Chile, Santiago, Chile

Abstract: G-protein coupled receptors (GPCR) are capable of form homomers and heteromers providing new properties in the signaling and/or regulation of the respective GPCRs. It has been shown that D1 dopamine receptors (DAD1) are capable of form a heteromer with type-2α corticotropin releasing factor receptors (CRFR2α) when both receptors are heterologously co-expressed in HEK293t cells. The evidence suggests that their heteromerization changes the subcellular localization of both receptors and could modulate their signaling in the cell system.In order to further study the consequences of DAD1/CRFR2α heteromerization, we have determined the impact of their heteromerization in the recycling of DAD1. To this end, we have analyzed the behaving of a mutated form of DAD1 lacking its recycling domain using protein immunoprecipitation and immunofluorescence microscopy. The results showed that activation of DAD1 does not modify the level of DAD1/CRFR2α heteromerization and that the recycling of DAD1 is not necessary for DAD1/CRFR2α heteromerization. However, data showed that the endocytosis of DAD1 increases its heteromerization with CRFR2α. Interestingly, we observed an increase of CRFR2α in the early endosome compartment when is co-expressed with DAD1 and the mutated form of DAD1. We are evaluating the effect of the agonist in the signaling of DAD1, CRFR2α and the heteromer DAD1/CRFR2α by cAMP and ERK signaling.Thus, our data suggest that heteromerization of CRFR2α with DAD1 increases the availability of functional CRFR2α.

Disclosures: H.E. Yarur: None. **C.A. Lopez:** None. **K. Gysling:** None.

Poster

035. Opioid, Purine, and Peptide Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 35.19/F12

Topic: B.03. G-Protein Coupled Receptors

Support: NSF IOS 1352944

Title: Functional authentication of two isoforms of a gastropod gonadotropin-releasing hormone receptor.

Authors: *S. I. KAVANAUGH, P.-S. TSAI;
Integrative Physiol., Univ. of Colorado, Boulder, CO

Abstract: Gonadotropin-releasing hormone (GnRH) is the most upstream neuroendocrine activator of reproduction in vertebrates. A GnRH-like molecule was previously identified in the mollusk, *Aplysia californica,* interestingly, this GnRH (ap-GnRH) does not appear to have a reproductive role. In our earlier study, we cloned the full-length cDNA of a Type II putative ap-GnRH receptor (ap-GnRHR). This receptor contains two potential translation start sites, each accompanied by a Kozak sequence, suggesting the translation of both a long and a short form of the receptor is possible. The putative ap-GnRHR maintains the conserved structural features and motifs of other Type II GnRH receptors and shares high sequence identity with the octopus GnRHR. The expression of the putative ap-GnRHR short form is ubiquitous in all tissues examined, whereas the expression of the long form is confined to the central nervous system, hermaphroditic duct, ovotestis and osphradium. The goal of this study is to examine, through a series of functional characterizations, if these two receptor isoforms are authentic ap-GnRHR. The cDNA encoding the long or the short receptor was subcloned into the pAWG vector and transfected into Drosophila S2 cells, a protostomian cell line. Transfected cells were subject to a radioreceptor assay using ^{125}I-labeled ap-GnRH as a radioligand. Further, they were treated with various concentrations of ap-GnRH or a related peptide, *Aplysia* adipokinetic hormone (ap-AKH), and measured for the accumulation of cAMP and inositol phosphate (IP). Radioreceptor assay revealed that only the long form of the receptor selectively bound to the radioligand, with cold ap-GnRH displacing the bound radioligand at EC_{50} of 3.54×10^{-8} M. Cells transfected with either form of the receptor did not respond to ap-GnRH or ap-AKH treatment with cAMP accumulation. However, cells transfected with the long receptor increased intracellular IP in a dose-dependent manner when treated with ap-GnRH, leading to a 3- to 6-fold increase in IP accumulation over controls. The results from our studies show that despite the more prevalent expression of the short receptor, only the long receptor is a bona fide ap-GnRHR. Our study also cautions against the naming of a receptor based solely on sequence homology before a thorough functional characterization.

Disclosures: **S.I. Kavanaugh:** None. **P. Tsai:** None.

Poster

035. Opioid, Purine, and Peptide Receptors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 35.20/F13

Topic: B.03. G-Protein Coupled Receptors

Support: NIH Grant ZIA NS002824-26

Title: Tanning the GnRH neurons-Melanocortin receptor 1 in GnRH neurons

Authors: C. HAERING, *S. S. CONSTANTIN, S. WRAY;
NIH, Bethesda, MD

Abstract: The accurate migration, precise signaling and pulsatile activity of Gonadotropin-releasing hormone-1 (GnRH) neurons are crucial for the onset of puberty and fertility in both male and female mammals. Our lab generated transcriptome data of primary GnRH neurons that showed high levels of melanocortin receptor 1 (Mc1R) mRNA. The McR subfamily, known to play a role in pigmentation, inflammation, exocrine secretion and energy homeostasis, consists of 5 members (Mc1-5R) that are G-protein coupled receptors. The broad agonist for McRs, α-Melanocyte-stimulating hormone (α-MSH), derived from proopiomelanocortin (POMC), had been shown to increase calcium oscillations in murine GnRH neurons in acute adult brain slices via Mc3R and Mc4R. We sought to determine whether Mc1R modulates GnRH neuronal activity and the signal transduction pathway utilized. Experiments performed include 1) RT-PCR of single GnRH neurons and 2) calcium imaging to pharmacologically address the function of McRs in the GnRH neuronal population. RT-PCR verified the presence of Mc1R, Mc3R and Mc5R transcript in a subset of neurons. Calcium imaging demonstrated that a subset of GnRH neurons was activated by α-MSH application. Notably, this activation occurred using a low concentration, 5 nM, implicating activation via Mc1r. After blockade of GnRH neuronal inputs using an amino acid blocker cocktail, α-MSH still activated GnRH neurons, indicating direct postsynaptic activation. Future experiments will examine whether expression and function of McRs in GnRH neurons change during development.

Disclosures: **C. Haering:** None. **S.S. Constantin:** None. **S. Wray:** None.

Poster

036. Homeostatic Plasticity

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 36.01/F14

Topic: B.08. Synaptic Plasticity

Support: Ellison Medical Foundation

W.M. Keck Foundation

Title: Exercise-induced neurotransmitter switching in the adult mouse midbrain

Authors: *H. LI, K. B. JACKSON, N. C. SPITZER;
Neurobio. Section, Div. of Biol. Sciences, Kavli Inst. for Brain, UCSD, La Jolla, CA

Abstract: Neurotransmitter (NT) switching is a newly appreciated form of plasticity in the mature brain (Spitzer, 2015). However the environmental cues that trigger it, the extent to which it occurs throughout the CNS and its functions are largely unknown. We have searched for activity-dependent NT switching in motor circuitry where the mechanism underlying plasticity remains obscure.

We began by comparing the number of neurons expressing c-Fos in motor nuclei of mice with free access to running wheels to the number in sedentary control mice. We found that mice that exercised by running for 1 week showed a 5-fold increase in the number of c-Fos+ cells in the midbrain pedunculopontine nucleus (PPN) accompanied by a 33% decrease in the number of choline acetyltransferase (ChAT+) neurons and a 51% increase in the number of glutamate decarboxylase 1 (GAD1+) neurons. Stereological counts showed that the decrease in number of ChAT+ neurons in the PPN occurred exclusively in the caudal region that projects to the brain stem, STN, VTA, and the thalamus, but not in the rostral PPN or in the adjacent LDT nucleus. The number of neurons losing ChAT (n=605±19) matched the number of neurons gaining GAD1 (n=575±156). TUNEL staining revealed the absence of apoptosis and Ki67/DCX staining demonstrated the absence of neurogenesis. Moreover, the number of neurons expressing nitric oxide synthase 1 (NOS1) that is highly co-localized with ChAT in the caudal PPN did not change.

To determine functional correlates of NT switching in the caudal PPN, we performed behavioral analyses and found that runners progressively increased the running episode duration and acquired better motor control during self-training on running wheels. They also performed better than controls on the rotarod motor learning/coordination test; no significant difference was seen in the locomotor activity test or analysis of metabolism. To test for causal relationships, we are determining whether overexpressing ChAT via stereotaxic injection of ChAT-Cre mice with AAV-DIO-ChAT prevents the acquisition of motor learning by running.

To find out whether ChAT loss and GAD1 gain occur in the same cell population, we are

combining sensitive FISH with IHC to triple-stain ChAT mRNA, GAD1 mRNA and NOS1 protein to see whether levels of ChAT and GAD1 change in the same population of neurons. This study is expected to be pertinent to understanding the mechanism underlying motor learning as well as neurological disorders of the motor system.
Supported by the Ellison Medical Foundation and the W.M. Keck Foundation to NCS.

Disclosures: **H. Li:** None. **K.B. Jackson:** None. **N.C. Spitzer:** None.

Poster

036. Homeostatic Plasticity

Location: Halls B-H

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Program#/Poster#: 36.02/F15

Topic: B.08. Synaptic Plasticity

Support: NIH R21 MH105706 to NCS.

Title: Neurotransmitter switching in the adult mammalian hippocampus

Authors: *S. ZAMBETTI, J. O. CONNORS, N. C. SPITZER;
Biol. Sci., UC San Diego, LA Jolla, CA

Abstract: Neuroplasticity is the ability of the brain to adapt in response to internal and external stimuli. Although much progress has been made in the characterization of classical forms of synaptic plasticity (Feldman et al., 2009), the mechanisms underlying changes in brain structure and function are not completely understood. The discovery of a form of plasticity called neurotransmitter (NT) respecification or switching, in which a neuron acquires a new function by losing, gaining or replacing a neurotransmitter (Spitzer, 2015), adds complexity to the nervous system but casts new light on these mechanisms. We have investigated whether NT switching contributes to the plasticity of the hippocampus, focusing our attention on adult neurogenesis. Environmental enrichment and voluntary running increase neurogenesis in the subgranular zone (SGZ) of the dentate gyrus (DG) in adult mice (Vivar et al., 2013). Here we demonstrate that running not only increases neurogenesis and improves learning performance but also changes the NTs expressed in a neuronal population in the hilus of the DG. Adult mice that have engaged in voluntary running for 1 week display a robust decrease in the number of neurons expressing neuropeptide Y (NPY) and a parallel increase in the number of neurons expressing the glutamatergic marker, VGluT1. However, the number of somatostatin (SST) neurons in the hilus and the number of NPY neurons in the CA1 (stratum oriens) remain constant. No cell death or neurogenesis is involved: experimental animals do not exhibit changes in apoptosis (TUNEL) or in the number of BrdU-DCX positive cells in the hilus after 1 week of voluntary running.

Together our data suggest that hilar NPY neurons acquire a glutamatergic phenotype when losing NPY. NPY knockout mice show reduced neurogenesis (Decressac et al., 2011) and hilar NPY neurons project to the outer molecular layer (Deller & Leranth 1990), the target zone of the input from the entorhinal cortex (EC; Förster et al., 2006). We hypothesize that the changes in the number of NPY and glutamatergic neurons act as a regulatory mechanism for neurogenesis and change the response of granule cells to the EC. Viral manipulation of the expression level of NPY and VGluT1 will allow us to understand the function of NT switching in the hilus with respect to neurogenesis. Our strategy is to prevent the loss of NPY and gain of glutamatergic phenotype during running by stereotaxically injecting Cre-dependent viruses encoding NPY and small interference (si) VGluT1 into the DG of NPY-Cre mice (Shi et al., 2013) before the onset of the running period.

Disclosures: S. Zambetti: None. **J.O. Connors:** None. **N.C. Spitzer:** None.

Poster

036. Homeostatic Plasticity

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 36.03/F16

Topic: B.08. Synaptic Plasticity

Support: HHMI International Predoctoral Fellowship

Ellison Medical Foundation

W.M. Keck Foundation

Title: Neuronal activity regulates neurotransmitter switching in the adult brain

Authors: *D. MENG[1,2], S. LEUTGEB[1,2], K. DEISSEROTH[3,4], N. C. SPITZER[1,2];
[1]Div. of Biol. Sci., [2]Kavli Inst. for Brain and Mind, UC San Diego, La Jolla, CA; [3]Dept. of Bioengineering and Howard Hughes Med. Inst., [4]Dept. of Psychiatry & Behavioral Sci., Stanford Univ., Stanford, CA

Abstract: The nervous system responds to changes in endogenous activity and the external environment by modulating its function through various forms of neuroplasticity, many of which occur at the synapse. It has been believed that neurotransmitters are fixed and invariant in the brain. However, recent work has demonstrated neurotransmitter switching, a newly recognized form of neuroplasticity, both in the developing and adult nervous system. Neuronal activity has been shown to play an essential role in neurotransmitter switching in the developing nervous system, acting by a non-cell-autonomous mechanism through the release of BDNF (Guëmez-

Gamboa et al., 2014; Marek et al., 2010).
Here we have investigated the role of neuronal activity in neurotransmitter switching in the adult nervous system. Previous work from our lab demonstrated that exposing adult rats to a long-day photoperiod (19L:5D) decreases the number of dopaminergic neurons in the paraventricular nucleus of the hypothalamus (PaVN) compared to the number in a balanced-day photoperiod (12L:12D). We have now suppressed the activity of different neuronal populations in the PaVN with viral and genetic tools to determine the effects on transmitter switching in dopaminergic neurons.
To suppress overall PaVN neuronal activity, a Cre-dependent AAV virus expressing inwardly rectifying potassium channels (AAVdj-DIO-Kir) was stereotaxically injected into the PaVN along with a Cre-expressing AAV virus under the Synapsin promoter (AAV1-Syn-Cre). To suppress the activity of PaVN excitatory neurons, AAVdj-DIO-Kir was injected with a Cre-expressing AAV virus under the CaMKII promoter (AAV9-CaMKII-Cre). To suppress the activity of dopaminergic neurons, TH (tyrosine hydroxylase)-Cre transgenic rats were injected with AAVdj-DIO-Kir.
Preliminary results show that 1) Suppressing overall neuronal activity does not affect the number of dopaminergic neurons in the PaVN after 12L:12D; 2) Suppressing activity of excitatory neurons decreases the number of dopaminergic neurons in the PaVN after 12L:12D without affecting the number of neurons expressing nitric oxide synthase, suggesting that the effect is specific; 3) Specifically suppressing activity of PaVN inhibitory dopaminergic neurons blocks transmitter switching after 19L:5D without changing TH expression after 12L:12D, suggesting a cell-autonomous activity-dependent mechanism for neurotransmitter switching in the adult brain.

Disclosures: **D. Meng:** None. **S. Leutgeb:** None. **K. Deisseroth:** None. **N.C. Spitzer:** None.

Poster

036. Homeostatic Plasticity

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 36.04/F17

Topic: B.08. Synaptic Plasticity

Support: WM Keck Foundation

Title: Neurotransmitter switching in mouse prefrontal cortex

Authors: *S. K. GODAVARTHI, N. C. SPITZER;
Neurosci., Univ. of California San Diego, San Diego, CA

Abstract: Expression of appropriate neurotransmitters (NTs) is essential for development and function of neural circuits (Misgeld et al., 2002; Root et al., 2008). However, the NT expressed by a neuron is not immutable and fixed, but capable of change in response to alteration in electrical activity – both during development and in adult stages (Borodinsky et al., 2004; Demarque and Spitzer, 2010; Dulcis et al., 2013; Meng et al., SfN 2016). This NT switching is proposed to play a key role in normal development and maintenance of homeostatic plasticity (Demarque and Spitzer, 2012), but imbalances in excitation-inhibition are frequently reported in neurodevelopmental disorders. Accordingly, we are investigating if and how inadvertent and unwanted NT switching contributes to these disorders, specifically autism and autism spectrum disorders. We have focused on prefrontal cortex (PFC), a key player in the emergence of the autism phenotype both anatomically and behaviorally (Amaral et al., 2008.
Our strategy is to compare NT expression between the PFC of control mice and environmental models of autism (EMA) obtained by injecting valproate or poly inosine:cytosine in pregnant dams at E12.5 (Shi et al,. 2009; Sui et al., 2012). EMA mice at P10 showed a ~50% decrease in GAD67+ neurons (inhibitory neuron marker), specifically in the medial PFC when compared to controls. No such difference was detected in the lateral PFC. We found a parallel increase in the number of VGluT1+ cells (excitatory neuron marker) in the medial PFC of EMA mice. TUNEL staining revealed no change in apoptosis and NeuN counts were comparable between EMA and control groups. Moreover, there was no change in number of nitric oxide synthase (NOS1)+ cells, suggesting that the observed NT switching is specific. These data indicate that there may be respecification of GAD67+ neurons in the medial PFC in EMA, with VGluT1 as the switching partner.
We are currently seeking a marker for the subpopulation of neurons that loses GAD67, using antibodies to calcium-binding proteins and peptide transmitters, which will enable prevention of NT switching and determination of the consequences for behavior. In order to learn if the changes persist into adulthood, we will examine EMA mice at P90 for the expression of these NTs.
This study is expected to be pertinent to understanding the behavioural alterations resulting from unintended and unanticipated NT switching. The results will provide insight into the way multiple causative factors give rise to the common set of behavioural symptoms seen in autism spectrum disorders.

Disclosures: S.K. Godavarthi: None. **N.C. Spitzer:** None.

Poster

036. Homeostatic Plasticity

Location: Halls B-H

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Program#/Poster#: 36.05/F18

Topic: B.08. Synaptic Plasticity

Support: NSF318

Title: Investigating mechanisms by which the WDR proteins regulate the deubiquitinating enzyme USP-46 *In vivo* to control *C. elegans* AMPA receptor GLR-1

Authors: *M. HODUL[1,2], C. L. DAHLBERG[3,2], P. JUO[1,2];
[1]Neurosci., [2]Developmental, Mol. and Chem. Biol., Tufts Univ., Boston, MA; [3]Dept. of Biol., Western Washington Univ., Bellingham, WA

Abstract: Ubiquitination is a key regulator of synapse development and function. Addition and removal of ubiquitin is an important mechanism that regulates the abundance of glutamate receptors at synapses. We previously identified Ubiquitin Specific Protease-46 (USP-46) as the first deubiquitinating enzyme (DUB) to regulate glutamate receptors. We showed that *usp-46* loss-of-function mutants have decreased levels of the AMPA-like glutamate receptor GLR-1 in the ventral nerve cord (VNC) of *C. elegans*. USP-46 deubiquitinates GLR-1 and promotes its degradation in the lysosome. USP-46 has low enzymatic activity and overexpression of the DUB by itself does not affect GLR-1 levels, suggesting that USP-46 activity is tightly controlled in neurons. We and others have shown that the WD40 repeat proteins, WDR-48 and WDR-20, interact with USP-46 and increase its catalytic activity in vitro. We previously found that co-expression of both WDR proteins increases GLR-1 abundance in the VNC in a USP-46-dependent manner. Here we show that neuronal expression of either WDR-48 or WDR-20 alone results in a small increase in USP-46 protein abundance *in vivo*, whereas co-expression of the WDR proteins results in a large 3-4 fold increase in USP-46 protein levels. These data indicate that the WDR proteins promote the abundance of USP-46 *in vivo*. We tested the hypothesis that the WDR proteins promote USP-46 protein levels by stimulating USP-46 to deubiquitinate itself *in trans* by measuring the effects of WDR protein co-expression on the abundance of a catalytically-inactive version of USP-46(C>A). We found that the WDR proteins were still able to increase the levels of USP-46(C>A) by 3-4 fold, suggesting that USP-46 does not regulate its own stability. Since USP-46 levels and activity are sensitive to the expression levels of the WDR proteins and synaptic activity can regulate GLR-1 levels, we are currently investigating whether the expression and/or localization of the WDR proteins are altered in various synaptic activity mutants.

Disclosures: **M. Hodul:** None. **C.L. Dahlberg:** None. **P. Juo:** None.

Poster

036. Homeostatic Plasticity

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant RO1 NS083402

UIUC ICR start-up fund

UIUC James Scholar Preble Research Award

Title: Dynamic changes in Kv7/KCNQ channel complex at the axonal initial segment of hippocampal neurons during homeostatic plasticity

Authors: *Z. HUANG, S. LEE, J. CAVARETTA, G. LEE, H. CHUNG;
Univ. of Illinois At Urbana-Champaign, Urbana, IL

Abstract: Homeostatic intrinsic plasticity maintains neuronal firing within an optimal operating range; its dysfunction is hypothesized to cause multiple neuronal hyperexcitability disorders including epilepsy and chronic pain. However, the molecular players mediating homeostatic intrinsic plasticity and the sites of their actions remain largely unknown. We have recently reported that chronic activity blockade by voltage-gated sodium (Na_v) channel antagonist tetrodotoxin (TTX) leads to homeostatic regulation of action potential firing rates in hippocampal culture neurons, and identified K_v7/KCNQ potassium channel genes and current as possible key players of this regulation. Kv7/KCNQ channels composed of Kv7.2/KCNQ2 and Kv7.3/KCNQ3 subunits are voltage-gated potassium channels that potently limits repetitive and burst firing of action potentials in neurons. Consistent with their ability to inhibit excitability, they are preferentially enriched at the plasma membrane of the axon with the highest concentration at the axon initial segment (AIS). Here, we investigated the extent to which chronic activity blockade alters expression of K_v7 channels, and their interacting proteins including calmodulin (CaM), Ankyrin-G, AKAP79/150, and syntaxin-1A. Immunoblot analysis showed a significant reduction in the expression of $K_v7.3$, CaM, and AKAP79/150 in hippocampal neurons after 48 h TTX treatment. In contrast, syntaxin 1A level was enhanced by TTX treatment. Immunocytochemistry revealed a noticeable decrease in $K_v7.2$ expression specifically at the axonal initial segments (AIS) of hippocampal neurons upon 48 h TTX treatment, whereas the same treatment increased the level of AIS marker Ankyrin-G and shortened the distance from soma to the start of the AIS. The investigation of chronic inactivity-induced changes of CaM, AKAP79/15, and syntaxin 1A in the axon is ongoing. These results suggest that alterations of Kv7 channel protein complex at the AIS may in part contribute to the expression of homeostatic intrinsic plasticity.

Disclosures: **Z. Huang:** None. **S. Lee:** None. **J. Cavaretta:** None. **G. Lee:** None. **H. Chung:** None.

Poster

036. Homeostatic Plasticity

Location: Halls B-H

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Program#/Poster#: 36.07/F20

Topic: B.08. Synaptic Plasticity

Support: German Research Foundation

Title: Homeostatic plasticity induces rapid temperature adaptation of the life-saving C-start mediated by NMDA receptors and gap junctions at Mauthner cell level in larval zebrafish

Authors: ***A. HECKER**, W. SCHULZE, S. SCHUSTER;
Dept. of Animal Physiol., Bayreuth, Germany

Abstract: Homeostatic plasticity describes the ability of neurons to regulate their own synaptic input and excitability. These regulations usually occur after ex- or intrinsic perturbations, which affect those neuronal properties. However, the majority of studies regarding homeostatic plasticity were done using cell cultures, slices or electrophysiology. In this study we tried to link homeostatic plasticity of distinct brainstem neurons directly to its elicited behavior in larval zebrafish by making use of a combination of non-invasive imaging techniques, behavioral observations and pharmacology.
We extrinsically altered properties of the life-saving escape response (C-start) and its command neurons (Mauthner (M)-cells) by significantly decreasing the water temperature. We used simultaneous live-cell calcium imaging of both M-cells to indirectly measure their neuronal activity and monitored the linked C-start escape latency over 10 h (100 min at 28.5°C and 500 min at 19.5°C) in semi-fixed larvae. The latency of an acoustically elicited C-start shows a rapid adaptation after an initial cooling-induced slowdown. Throughout the experiments the M-cell $[Ca^{2+}]$-signal correlates significantly with the latency: it is lower in the beginning of the cold phase ($\Delta F/F$: 22 %) and is fully compensated at the end of the experiments ($\Delta F/F$: 40 %). The surprising correlation of the M-cell $[Ca^{2+}]$-signal and C-start latency suggests a common mechanism of adaptation.
To identify this mechanism, we repeated the experiments using antidromic (AD) stimulation. Hence, instead of activating the sensory input, we evoked an action potential directly in both M-cells, still causing calcium influxes in both M-cell somata. Again we found an adaptation after an initial decrease of the $[Ca^{2+}]$-signal, suggesting that the adaptation cannot solely be due to mechanisms at the input level. However, both the NMDA receptor antagonist MK-801 as well as

the gap junction blocker carbenoxolone blocked adaptation in AD-stimulated larvae. Homeostatic plasticity is linked to NMDA receptors by altering the number of NMDA receptors at synapses or changing their NR2 subunit composition. Gap junctions as part of club endings in the M-cells lateral dendrite are responsible for electrical transmission and therefore involved in regulating synaptic strength. In this study, we not only demonstrated a concurrent and remarkable rapid adaptation of a behavior and its underlying neural network to an environmental perturbation, but also suggest that this adaptation depends on homeostatic plasticity affecting NMDA receptors as well as synaptic strength through gap junctions.

Disclosures: **A. Hecker:** None. **W. Schulze:** None. **S. Schuster:** None.

Poster

036. Homeostatic Plasticity

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Topic: B.08. Synaptic Plasticity

Support: PAPIIT IN215816

CONACYT 417634

Title: Homeostatic modulation of neocortical plasticity: extinction of aversive taste memory prevents the maintenance of *In vivo* insular cortex LTP

Authors: *A. RIVERA-OLVERA[1], M. L. ESCOBAR[2];
[1]Facultad De Psicologia, UNAM, Ciudad DE Mexico, Mexico; [2]Facultad De Psicologia, UNAM, Ciudad de Mexico, Mexico

Abstract: Accumulating evidence indicates that extinction process does not reflect loss of the original memory, but rather reflects the emergence of a new learning, which in turn requires consolidation in order to be stored in the long term. On the other hand, it is considered that synaptic strength is homeostatically regulated in order to maintain the circuit stability that is crucial for memory storage. Thus, homeostatic plasticity dynamically adjusts the capacity of synapses to express plastic changes depending on previous experience. In particular, training in several behavioral tasks modifies the possibility to induce long-term potentiation (LTP). Our previous studies in the insular cortex (IC) have shown that induction of LTP in the basolateral amygdaloid nucleus (BLA)-IC projection previous to conditioned taste aversion (CTA) training modifies the extinction process of this task. In this regard, we have also reported that prior training in CTA prevents the subsequent induction of LTP generated by high frequency

stimulation (HFS) in the same pathway. Here, we evaluate the effect of CTA extinction training on the ability to induce subsequent LTP in the BLA-IC projection in vivo. Thus, 48h after CTA extinction animals received HFS in BLA in order to induce IC-LTP. Our results show that extinction training allows the induction but not the maintenance of IC-LTP. These findings reveal that extinction training is able to modify the permanence of subsequent synaptic plasticity on IC, suggesting a homeostatic regulation on this pathway. Supported by: PAPIIT IN215816 and CONACYT 417634

Disclosures: A. Rivera-olvera: None. **M.L. Escobar:** None.

Poster

036. Homeostatic Plasticity

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Topic: B.08. Synaptic Plasticity

Support: NIH grant 2R56NS036853-17A1

Simons Foundation

Title: Pharmacological rescue of synaptic scaling.

Authors: *V. TATAVARTY, H. LIN, G. G. TURRIGIANO;
Brandeis Univ., Waltham, MA

Abstract: Multiple genes have been discovered that may contribute to Autism Spectrum Disorders (ASDs). It is unclear at this point if there is an underlying neurophysiological deficit that is common to the Autism caused by the various linked genes. Our previous results demonstrated that lack of MeCP2, an ASD associated gene leads to a deficit in Synaptic Scaling (SS) (Blackman et. al.2012.). SS is a form of synaptic plasticity that controls synaptic strength and firing rate homeostasis both *in vivo and in vitro.* To determine if aberrant SS is a common underlying feature of ASDs, we investigated if the loss of another ASD associated gene SHANK3 causes deficits in SS. SHANK3 is known to be an important regulator of synaptic plasticity and mutations in this gene have been reported in human subjects suffering from ASD. Our results show that cultured cortical neurons expressing a short hairpin directed against SHANK3 are unable to scale up synaptic strength. In addition, SS leads to accumulation of Shank3 at synapses. Interestingly, our preliminary data also shows that treatment of SHANK3-deficient neurons with a therapeutic dose of Lithium can rescue scaling *in vitro*. These results are consistent with the hypothesis that failure to regulate synaptic strength via scaling may

contribute to autism pathogenesis in different genetic backgrounds. Finally, since scaling can be measured quickly and accurately using electrophysiology and fluorescence microscopy in vitro, pharmacological rescue of scaling in general can serve as an ideal assay for high throughput screening of drugs to treat ASDs.

Disclosures: V. Tatavarty: None. **H. Lin:** None. **G.G. Turrigiano:** None.

Poster

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The Physiological Society of Great Britain

The International Brain Research Organisation (IBRO)

Title: Impaired astrocytic calcium signalling interferes with experience-dependent plasticity (EDP) in layers 2/3 of the murine barrel cortex.

Authors: *J. BUTCHER[1], R. E. SIMS[2], H. R. PARRI[2], S. GLAZEWSKI[1];
[1]Sch. of Life Sci., Keele Univ., Newcastle, United Kingdom; [2]Sch. of Life and Hlth. Sci., Aston Univ., Birmingham, United Kingdom

Abstract: In response to changes in whisker experience of adolescent mice, cortical neurons in layers 2/3 of the murine barrel cortex can exhibit two general forms of EDP: input-specific coding plasticity (CP) and homeostatic plasticity (HP). CP refers to changes in neuronal transmission and connectivity of individual synapses and is thought to enable information storage within a neuronal network. HP is most often a global phenomenon that acts as a negative feedback mechanism to keep the activity of a neuronal network within a set operating range. To determine possible astrocyte roles in both CP and HP we utilised IP3-R2 knock out (KO) mice, in which astrocytes exhibit diminished [Ca2+] responses. To evoke CP, all but one whisker (single-whisker experience - SWE) were removed unilaterally for 18 days, followed by regrowth for 5-9 days. To evoke HP, all whiskers were trimmed unilaterally for 1, 3, 7, 14, 25 and 32 days and re-attached on the day of recording. We found no significant differences in the magnitude of principal and surround responses in undeprived WT and KO mice and in the amount of plasticity

induced in SWE animals (all pairs $p>0.05$, U-test, N=20). In all-whisker-deprived WT animals the principal whisker responses showed rapid depression 1 day after deprivation (t-ratio 7.3, $p<0.0001$, N=15), started to recover at 3 days, were above control levels at 7-14 days (t-ratio 3.7, $p<0.003$, N=15), indicating HP, and back to control levels at 25-32 days. However, IP3-R2 KO mice exhibited a linear decay in magnitude of responses with deprivation time which was significant after 14 days ($p<0.05$, N=6) with an impaired HP rebound at 25 and 32 days which was not significant ($p>0.05$, N=10). In acute slice experiments, the LTD/LTP induction frequency curve was shifted towards LTP in the KO mice and mimicked in WT recordings by patch electrode filling of astrocytes with the calcium chelator BAPTA. These data implicate astrocytes as potent regulators of experience-dependent coding depression and homeostatic up-regulation of whisker-evoked responses.

Disclosures: **J. Butcher:** None. **R.E. Sims:** None. **H.R. Parri:** None. **S. Glazewski:** None.

Poster

036. Homeostatic Plasticity

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Program#/Poster#: 36.11/F24

Topic: B.08. Synaptic Plasticity

Support: MRC Grant MR/N003896/1

Title: Evidence for homeostatic and Hebbian plasticity components in cortical layer 2/3 neurons

Authors: ***K. D. FOX**[1], S. D. GREENHILL[2], S. GLAZEWSKI[3];
[1]Cardiff Univ., Cardiff, United Kingdom; [2]Neurosci., Aston Univ., Birmingham, United Kingdom; [3]Biosci., Keele university, Keele, United Kingdom

Abstract: Previous studies have shown that cortical layer 2/3 neurones show Hebbian forms of synaptic plasticity. The layer 4 to layer 2/3 pathway shows both LTP and LTD. LTP is absent in this pathway in mutants lacking autophosphorylation of CaMKII, as is experience dependent potentiation induced by single whisker experience. LTD occurs in this pathway in the deprived whisker's barrel-column, but can be occluded by whisker deprivation. In this study, we wished to determine whether homeostatic plasticity could also be detected in layer 2/3 neurones. We deprived C57BL/6J mice of all whiskers unilaterally for 1, 3, 7 or 14 days and measured principal whisker responses to a standard whisker deflection. Responses were depressed after a single day of whisker deprivation but returned toward baseline after 3 days in a homeostatic fashion despite continued whisker input deprivation. Principal whisker responses slightly overshot baseline values after 7 and 14 days deprivation. The homeostatic response was

prevented in mice lacking synaptic scaling (C57BL/6OlaHsd, Ranson et al 2012, PNAS 109, 1311) and by administration of XPro1595 (Xencor, CA), a selective inhibitor of soluble TNF. TNF-alpha has itself been implicated in synaptic scaling (Stellwagen and Malenka 2006, Nature 440, 1054). We conclude that layer 2/3 neurones exhibit both Hebbian and homeostatic components of synaptic plasticity in the barrel cortex.

Disclosures: K.D. Fox: None. **S.D. Greenhill:** None. **S. Glazewski:** None.

Poster

036. Homeostatic Plasticity

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 36.12/F25

Topic: B.08. Synaptic Plasticity

Support: NSERC

CIHR

Title: Glial sources of TNF during homeostatic synaptic plasticity

Authors: *R. HEIR, H. ALTIMIMI, D. STELLWAGEN;
McGill Univ. Ctr. For Res. In Neurosci., Montreal, QC, Canada

Abstract: In order for neural circuits to function well, overall activity levels must be kept within an optimal range for neurotransmission by homeostatic plasticity mechanisms. The process of homeostatic strengthening of excitatory synapses in response to chronic activity deprivation is mediated by the glial release of tumour necrosis factor alpha (TNF), which modulates synaptic receptor trafficking. While it has become clear that glia play a critical role in some forms of homeostatic plasticity, the glial source—whether from astrocytes or microglia—has been a matter of some debate.
Here we show that both astrocytes and microglia can supply TNF depending on the context. In dissociated neuron-glial cultures we show that astrocytes are capable of supplying TNF during homeostatic plasticity, and that this occurs at least in part by the modulation of mRNA levels. We show that 48 hour activity deprivation of cultures with tetrodotoxin (TTX) results in an increase in TNF mRNA levels as well as an increase in surface GluA1 levels. Depletion of microglia from these cultures does not prevent the production of TNF in response to activity deprivation, nor does it prevent the increase in surface GluA1, suggesting that astrocytes are capable of producing TNF that results in AMPA receptor trafficking in this context. In addition, we use organotypic hippocampal slice cultures in order to investigate glial release of TNF in a

situation more closely representing *in vivo* conditions. We find that in this system, microglia are capable of supplying TNF in response to activity deprivation. Together, these experiments suggest that both microglia and astrocytes can serve as a source of TNF during homeostatic plasticity, and that the glial subtype responsible for TNF release is context-dependent. These experiments will allow a deeper understanding of the mechanisms by which different glial subtypes can contribute to the functioning of synapses.

Disclosures: R. Heir: None. **H. Altimimi:** None. **D. Stellwagen:** None.

Poster

036. Homeostatic Plasticity

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant NS062738

Whitehall Foundation Grant 2014-08-03

Title: How spontaneous activity drives homeostatic synaptic plasticity

Authors: *C. J. NEFF[1], C. A. FRANK[2];
[1]Interdisciplinary Grad. Program in Neurosci., [2]Anat. and Cell Biol., Univ. of Iowa, Iowa City, IA

Abstract: Forms of homeostatic plasticity have been shown to help maintain neuronal output within a physiologically appropriate range. Failure of homeostatic regulation is thought to be a component of diseases such as migraine, ataxia, and epilepsy. Our lab uses the Drosophila neuromuscular junction (NMJ) as a model synapse. The best-characterized model of homeostatic plasticity at the fly NMJ is the potentiation of vesicle release following glutamate receptor perturbations. Perturbing the postsynaptic glutamate receptors causes a decrease in the amplitude of spontaneous release events and triggers a retrograde signal, leading to a compensatory increase in the number of vesicles released per evoked potential. The number of vesicles released per evoked potential is called quantal content, and this increase in quantal content is termed homeostatic potentiation.
From this paradigm, we assume a relationship exists between the amplitude of spontaneous release events and quantal content. If the system regulating these phenomena is truly homeostatic, we expect that the quantal content should change in response to all changes in spontaneous activity amplitude. One way we have tested this is through studying the reversibility

of homeostatic potentiation. We have found that upon removal of the glutamate receptor perturbation midway through development, quantal content decreases to control levels. We are currently studying reversibility in both sustained and acute time scales, as well as studying which molecular factors are necessary for reversibility.
A second experimental paradigm can be used to induce a homeostatic decrease in quantal content. Overexpression of the vesicular glutamate transporter, or VGlut, causes an increase in the amplitude of spontaneous release events, which is offset by the homeostatic depression of quantal content. Through an ongoing genetic screen, we have identified factors that have a deficit in evoked release only in the context of homeostatic depression, which in turn exacerbates the decrease in quantal content. We are interested in determining whether the same factors required for homeostatic depression are also required for the reversal of homeostatic potentiation. Finally, we are studying the reversibility of homeostatic depression.

Disclosures: C.J. Neff: None. **C.A. Frank:** None.

Poster

036. Homeostatic Plasticity

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Support: Provost PhD fellowship

Klingenstein

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Women in Science and Engineering Travel Support

Title: Presynaptic scaling of active zone size and number homeostatically tunes global synaptic strength

Authors: *P. GOEL[1,2], J. PALUCH[1], J. WONDOLOWSKI[1], L. NUNNELLY[1], D. DICKMAN[1,2];
[1]Dept. of Neurobio., [2]USC Grad. Program in Mol. and Computat. Biol., USC, Los Angeles, CA

Abstract: Synaptic structure can change in subtle and dramatic ways throughout the development, growth, maturation, and aging of the brain, yet synaptic strength is typically maintained within remarkably stable physiological ranges. We have investigated mutations and manipulations that lead to extreme changes in synaptic growth and structure at

the *Drosophila* neuromuscular junction. Although these conditions lead to enlarged synaptic vesicle size and inhibited or exuberant synaptic growth, remarkably, synaptic strength is maintained at wild type levels. These synapses have increased quantal size due to excess neurotransmitter release per synaptic vesicle, but express a compensatory, homeostatic decrease in presynaptic release, termed presynaptic homeostatic depression (PHD). Using quantitative imaging and analysis of synaptic architecture, we find that scaling of active zone size compensates for reciprocal changes in active zone number to achieve stable levels of synaptic strength. We further show that when actives zones are not tuned in this way, basal synaptic strength deviates from wild type. Finally, synapses simultaneously expressing PHD and active zone scaling can also homeostatically potentiate synaptic strength when postsynaptic receptors are acutely blocked. This demonstrates the extraordinary plasticity and stability of this synapse, where three homeostatic signaling systems can dynamically respond to diverse challenges and still maintain stable levels of synaptic strength. Together, this suggests that when this synapse is confronted with extreme changes in active zone number, homeostatic tuning of active zone size achieves stable synaptic strength.

Disclosures: P. Goel: None. **J. Paluch:** None. **J. Wondolowski:** None. **L. Nunnelly:** None. **D. Dickman:** None.

Poster

036. Homeostatic Plasticity

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 36.15/F28

Topic: B.08. Synaptic Plasticity

Support: Neuroscience Graduate Program

NIH Grant

Title: Retrograde potentiation of presynaptic release is suppressed when three homeostatic perturbations are chronically combined at an individual synapse

Authors: *X. LI[1,2,3], P. GOEL[1,4,2], J. WONDOLOWSKI[1,2], J. PALUCH[1,2], D. DICKMAN[1,2,4,3]; [2]Dept. of Neurobio., [3]Neurosci. Grad. Program, [4]Grad. Program in Mol. and Computat. Biol., [1]USC, Los Angeles, CA

Abstract: Synapses have the remarkable ability to adaptively modulate synaptic strength in response to perturbations that would otherwise destabilize neurotransmission. However, little is known about how synapses respond when confronted with conflicting homeostatic challenges.

We have interrogated the trans-cellular dialogue orchestrating the homeostatic control of synaptic strength in response to three perturbations to structure and function at the glutamatergic *Drosophila* neuromuscular junction. First, manipulations that increase synaptic vesicle size lead to excessive glutamate release, increased quantal size, but normal synaptic strength due to a homeostatic decrease in quantal content, termed presynaptic homeostatic depression (PHD). Second, acute pharmacological or chronic genetic perturbations to postsynaptic glutamate receptors leads to a decrease in quantal size but normal synaptic strength due to a retrograde homeostatic increase in presynaptic release, termed presynaptic homeostatic potentiation (PHP). Third, we have found that presynaptic active zone size inversely scales in mutants with extreme changes in active zone number, maintaining stable transmission. We have combined these three manipulations at a single synapse over acute and chronic times to elucidate the interface between these homeostatic signaling systems.
We find that synapses expressing active zone scaling and PHD can acutely express PHP to maintain stable synaptic strength, demonstrating a remarkable ability to coordinate diverse homeostatic expression mechanisms. Further, genetic mutations that lead to PHP can be expressed together with active zone scaling or PHD through development to stabilize synaptic strength. However, when a synapse confronts all three challenges over chronic time scales, active zone scaling and PHD are fully expressed, but PHP signaling is completely silenced. Indeed, we find no evidence that PHP signaling is ever permitted to advance beyond the postsynaptic cell. As a result, this silencing leads to a maladaptive, non-homeostatic reduction in neurotransmission, where the homeostat governing synaptic strength ceases to operate. Surprisingly, we find that expression of active zone scaling and PHD interferes with the Tor-dependent modulation of postsynaptic translation required for chronic forms of PHP. Thus, synapses expressing PHD and active zone scaling retain the adaptive ability to acutely potentiate synaptic strength. However, when these challenges are integrated over chronic time scales, potentiation is silenced due to an anterograde disruption of postsynaptic translational signaling.

Disclosures: **X. Li:** None. **P. Goel:** None. **J. Wondolowski:** None. **J. Paluch:** None. **D. Dickman:** None.

Poster

036. Homeostatic Plasticity

Location: Halls B-H

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Program#/Poster#: 36.16/F29

Topic: B.08. Synaptic Plasticity

Support: BBSRC Grant RA0592

Title: Differential expression of JAK2-STAT3 during homeostatic plasticity

Authors: *S. CHOI, G. COLLINGRIDGE;
Ctr. For Synaptic Plasticity, Bristol, United Kingdom

Abstract: Many studies have been published about Hebbian synaptic plasticity which is synapse specific. However, not enough mechanisms were found about homeostatic plasticity which is activity-dependent but not synapse-specific. In this study, we found differential expression of JAK2-STAT3 proteins after 48 hr after bicuculline (Bic) treatment, but not after tetrodotoxin (TTX) treatment NMDA receptor dependent manner. Interestingly, mRNA level of all known JAKs and STATs did not altered after Bic treatment in the same condition. Moreover, we also could find time-dependent increment of STAT3 mRNA which have long poly A tail after Bic treatment. And it is well known that STAT3 mRNA contains consensus CPE site. These data suggest that JAK2-STAT3 pathway may have important role in homeostatic plasticity. And they also suggest that regulation of CPE-mediated local translation might be crucial for regulating homeostatic plasticity.

Disclosures: S. Choi: None. **G. Collingridge:** None.

Poster

036. Homeostatic Plasticity

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Topic: B.08. Synaptic Plasticity

Support: Edward Mallinckrodt, Jr. Foundation

NINDS Grant NS091546

Title: Distinct requirements for pallidin and the schizophrenia susceptibility gene dysbindin in promoting synaptic vesicle recycling and homeostatic plasticity

Authors: *X. CHEN[1,2], W. MA[1], S. ZHANG[1], J. PALUCH[1], W. GUO[1,2], D. K. DICKMAN[1];
[1]Dept. of Neurobio., [2]Neurosci. Grad. Program, USC, Los Angeles, CA

Abstract: Membrane trafficking pathways must be exquisitely coordinated at synaptic terminals to maintain proper functionality, particularly during adaptive responses to high activity and to perturbations of neurotransmission. We have generated null mutations in *Drosophila* in the central Biogenesis of Lysosome-Related Organelles Complex-1 subunit *pallidin* to test whether this complex may be a fundamental mediator of adaptive synaptic responses to stress. We find

that *pallidin* is not required for synapse morphology or structure at the neuromuscular junction. However, *pallidin* is necessary to sustain the synaptic vesicle pool under high frequency stimulation, where we observe a loss of early endosomes with a concomitant increase of tubular endosomal structures. The schizophrenia susceptibility factor *dysbindin* binds to *pallidin* and is necessary for presynaptic homeostatic potentiation, an adaptive increase in presynaptic release in response to perturbation of postsynaptic glutamate receptor function. We find that *dysbindin*, like *pallidin*, is also necessary to sustain neurotransmission during high activity. Surprisingly, *dysbindin* achieves homeostatic potentiation through a modulation of the readily releasable synaptic vesicle pool, while *pallidin* is not necessary for this process and potentiation is fully expressed in *pallidin* mutants. We provide additional evidence that the majority of *dysbindin* is localized to synaptic vesicle pools, while *pallidin* localizes primarily to the presynaptic cytoskeleton. Together, our data reveals shared and distinct functions of *pallidin* and *dysbindin* in activity- and plasticity-dependent trafficking of synaptic vesicles.

Disclosures: X. Chen: None. **W. Ma:** None. **S. Zhang:** None. **J. Paluch:** None. **W. Guo:** None. **D.K. Dickman:** None.

Poster

036. Homeostatic Plasticity

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Topic: B.08. Synaptic Plasticity

Support: Kaken-hi 15H05569

Kaken-hi 15H01417

Title: Hypoperfusion-induced changes in neuronal network oscillations in the mouse forebrain

Authors: *Y. NISHIMURA, R. ABE, T. SASAKI, Y. IKEGAYA;
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Abstract: Neuronal activity is highly sensitive to changes in oxygen tension. Numerous studies have revealed signaling pathways and molecular mechanisms underlying the neuronal degeneration observed with hypoxia/ischemia. However, there is still a lack of information on the pathophysiological basis of ischemia. In this study, we examined the impact of hypoxic/ischemic conditions on neuronal ensemble activity patterns in the mouse brain using *in vivo* extracellular electrophysiological recordings from up to 8 sites in the thalamus, dorsal

hippocampus, and neocortex, while cerebral hypoperfusion was induced by unilateral common carotid artery occlusion (CCAO), a technique that reduces the blood supply to the forebrain. After a few minutes, the occlusion triggered a rapid change in the power of the local field oscillations. In the hippocampus, but not in the neocortex, corpus callosum and thalamus, the absolute power changes at all frequency ranges (relative to the baseline) became less pronounced with time, and no significant changes were observed 30 min after the occlusion-induced hypoperfusion. Next we focused on the hippocampal neuronal activity and tested how more severe ischemic damage alters its network activity by photothrombosis, a model of ischemia that significantly reduces blood supply, which induces neuronal degeneration in a small area of the brain. Similar to CCAO, hippocampal LFP power showed an immediate change in response to photothrombosis and kept decreasing significantly 60 min after the onset of photostimulation. These results suggest that LFP power within the hippocampus never returned to the pre-photostimulation baseline when significant ischemia related to neuronal degeneration was induced. We also tested whether continuous ischemic challenge induced by the occlusion for up to 1 week alters neuronal activity. In the hippocampus, corpus callosum and the thalamus, the chronic occlusion did not lead to a reduction in the power of the local field oscillations. However, in the neocortex, there was a tendency that its neuronal activity decreased. These results indicate that certain neuronal populations have the ability to maintain internal neurophysiological homeostasis against ischemic challenge.

Disclosures: **Y. Nishimura:** None. **R. Abe:** None. **T. Sasaki:** None. **Y. Ikegaya:** None.

Poster

036. Homeostatic Plasticity

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Topic: B.08. Synaptic Plasticity

Support: NIH5R01EY014882

Title: Homer1a mediated experience-dependent synaptic plasticity in mouse primary visual cortex

Authors: *V. B. CHOKSHI[1,2], M. GAO[4], P. WORLEY[3], H.-K. LEE[3,2];
[1]Biol., [2]The Mind Brain Inst., [3]The Solomon H. Synder Dept. of Neurosci., Johns Hopkins Univ., Baltimore, MD; [4]Dept. of Neurol., Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Sensory systems are developed to be able to sense the environment around us. It is well accepted that the information of past experience is stored as changes in the strength of

synapses. Homeostatic regulation of these synapses is required to maintain stability in the neural circuits in response to prolong changes in sensory experience. Experience-dependent homeostatic synaptic plasticity (EHP) has been shown in various model systems including mouse primary visual cortex. Short dark exposure of an animal induces homeostatic scaling up of excitatory synaptic strength, measured in terms of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) currents. In contrast, two hours of brief light exposure following dark exposure, scales down the synaptic strength to normal levels. We found that metabotropic glutamate receptor 5 (mGluR5) acts as an activity sensor to scale down the synapses with light exposure. These receptors are coupled to synaptic proteins by a scaffolding protein, Homer1. Constitutively Homer1 exists as dimers and can couple mGluR5 to its other synaptic targets and downstream signaling molecules. But upon high neuronal activity, neurons express its splice variant called Homer1a (H1a), which acts as monomers and competitively binds mGluR5. Hence, H1a dissociates mGluR5 from the other targets. We found using H1a knockout mice that mGluR5 and H1a interaction is required for scaling down synapses in visual cortex with visual experience. To rule out potential developmental effects of knocking out H1a as well as cell autonomous effects, we are currently using a genetic strategy to acutely knockout H1a in a subset of neurons. So far our results suggest that H1a acts as a sensor of acute increase in neural activity and switches the mode of mGluR5 signaling in order to maintain homeostasis in the visual cortex.

Disclosures: V.B. Chokshi: None. **M. Gao:** None. **P. Worley:** None. **H. Lee:** None.

Poster

036. Homeostatic Plasticity

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Program#/Poster#: 36.20/F33

Topic: B.08. Synaptic Plasticity

Support: NIH MH091220

Title: A mechanism of synaptic homeostasis in ca3 critical for maintaining hippocampal circuit balance

Authors: *F.-J. WENG[1], K. ALVINA[4], Y. ZHANG[1], M. DUSHKO[1], S. LUTZU[4], A. SORENSEN[1], M. HUNG[2], D. GARCIA-DOMINQUEZ[1], D. RICH[3], P. CASTILLO[4], Y. LIN[1]; [1]Dept. of Brain and Cognitive Sci., [2]Dept. of Biol. Engin., [3]Dept. of Chem. Engin., MIT, Cambridge, MA; [4]Dominic P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: For normal brain functions such as learning and memory to take place, the operational dynamic range of neural circuits must be maintained homeostatically, but the underlying molecular and synaptic mechanisms are largely unknown. Here we show that the activity-dependent transcription factor Npas4 plays a key role in setting the homeostatic balance of the hippocampus microcircuit and that it does so by selectively modulating the mossy fiber inputs to CA3 pyramidal neurons. Genetic manipulation of Npas4 selectively altered excitatory synapses on pyramidal neurons in CA3, but not in CA1. Both global knockout and selective deletion of Npas4 in CA3 led to a dramatic increase in the frequency of miniature excitatory synaptic currents (mEPSCs) in CA3 pyramidal neurons, while re-expression of Npas4 in CA3 in the global knockout mouse was sufficient to rapidly restore this synaptic deficit to the wild type level. Npas4 modulates excitatory inputs to CA3 pyramidal neurons through selectively altering synaptic inputs from dentate gyrus granule cells through the mossy fiber pathway, which is one of the many inputs converged onto CA3 pyramidal neurons. We found that mossy fiber synapses were homeostatically modulated to maintain the balance of the hippocampal circuit. Npas4 expressed in CA3 pyramidal neurons acutely modulates the size of thorny excrescences (TE), the distinct postsynaptic structure of mossy fiber synapses formed on CA3 pyramidal neurons. In the absence of Npas4, kainic acid-induced homeostatic down-sizing of TE is blocked. Furthermore, we have identified a previously unknown transcriptional target of Npas4 that mediates homeostatic modulation of TE. Our study therefore identified a molecular and synaptic mechanism controlled by Npas4 that is required to maintain the homeostatic balance of the hippocampus microcircuit. Consistent with this idea, Npas4 total knockout mice are significantly more susceptible to kainic acid-induced seizure. Given our previous finding that Npas4 is selectively required in CA3 for contextual memory formation (Ramamoorthi *et al.* Science, 2011), these new results indicate a critical role for circuit balance in learning and memory.

Disclosures: **F. Weng:** None. **K. Alvina:** None. **Y. Zhang:** None. **M. Dushko:** None. **S. Lutzu:** None. **A. Sorensen:** None. **M. Hung:** None. **D. Garcia-Dominquez:** None. **D. Rich:** None. **P. Castillo:** None. **Y. Lin:** None.

Poster

036. Homeostatic Plasticity

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant NS019546

Title: A presynaptic glutamate receptor confers robustness to neurotransmission and homeostatic potentiation

Authors: *B. KIRAGASI[1,2], J. WONDOLOWSKI[2], Y. LI[3], G. W. DAVIS[4], D. K. DICKMAN[1,2];
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Abstract: Homeostatic signaling systems are thought to interface with other forms of plasticity to ensure flexible yet stable levels of neurotransmission. The role of neurotransmitter receptors in this process, beyond mediating neurotransmission itself, is not known. Through a forward genetic screen using electrophysiology in *Drosophila*, we have identified the non-NMDA ionotropic glutamate receptor subunit *DKaiRID* to be required presynaptically for the homeostatic potentiation of synaptic strength. We find that *DKaiRID* is necessary in motor neurons to enable both acute and long term expression of homeostatic plasticity without altering synapse morphology or ultrastructure. *DKaiRID* confers robustness to the calcium sensitivity of baseline synaptic transmission, with significantly reduced release at lowered extracellular calcium, while high extracellular calcium restores both neurotransmission and homeostatic potentiation. Importantly, acute pharmacological blockade of *DKaiRID* disrupts homeostatic plasticity, indicating that this receptor operates at or near presynaptic release sites to potentiate both baseline and homeostatic release. We also demonstrate that calcium permeability through *DKaiRID* is necessary for the calcium sensitivity of baseline transmission, but is not required for homeostatic signaling. Finally, we have identified an additional receptor and auxiliary subunit that may work with *DKaiRID* to facilitate presynaptic homeostatic potentiation. We propose that *DKaiRID* is a glutamate autoreceptor that confers robustness to promote synaptic strength with active zone specificity.

Disclosures: **B. Kiragasi:** None. **J. Wondolowski:** None. **Y. Li:** None. **G.W. Davis:** None. **D.K. Dickman:** None.

Poster

036. Homeostatic Plasticity

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Topic: B.08. Synaptic Plasticity

Support: K99NS089800-01

R01EY014439

R37NS092635

Title: Homeostatic regulation of cortical firing rates is inhibited by sleep.

Authors: *K. B. HENGEN, A. TORRADO PACHECO, G. G. TURRIGIANO;
Biol., Brandeis Univ., Waltham, MA

Abstract: By continuously recording extracellular signals from ensembles of single neurons in primary visual cortex of freely behaving rats during the critical period for ocular dominance plasticity, we recently demonstrated that 1) individual neurons in freely behaving animals have a firing rate set-point, and 2) when firing rates are perturbed by monocular deprivation (MD), the homeostatic return to the set-point is expressed during waking and not sleep. This observation raises the important question of whether sleep inhibits an otherwise ongoing homeostatic process, or whether normal sleep/wake cycles are essential for the expression of firing rate homeostasis.

To answer this question, we recorded activity from ensembles of cortical single units in juvenile rats (postnatal days 24-34) continuously for 10 days during a monocular deprivation (MD) paradigm. As expected, amongst neurons that were "online" for the entire recording, firing rates dropped during early MD. To directly assess the role of wake and sleep during 24h at the height of the homeostatic (late) phase of MD, we enhanced every other naturally occurring waking state using non-stressful techniques such as novel object introduction and tactile stimulation. Enhanced waking states did not affect firing rates of neurons in the non-deprived (control) hemisphere, while the same behavioral epochs resulted in substantial increases in homeostatic recovery of firing rates.

Our control data indicate that extended waking in a familiar environment is insufficient to drive changes in neuronal firing rates in the primary visual cortex. In contrast, data from neurons in the deprived hemisphere strongly support the conclusion that sleep inhibits homeostatic plasticity during late MD, and that enhanced waking expands the time window for the expression of homeostasis. These results raise the possibility that various plasticity mechanisms may require temporal segregation, or that brain-state related changes in chemical composition may preclude homeostatic plasticity during sleep.

Disclosures: K.B. Hengen: None. **A. Torrado Pacheco:** None. **G.G. Turrigiano:** None.

Poster

036. Homeostatic Plasticity

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant NS083402

University of Illinois at Urbana-Champaign, ICR start-up fund

Title: Homeostatic scaling of intrinsic excitability involves upstream signaling pathways that are distinct from homeostatic scaling of excitatory synapses

Authors: A. WEISS[1], S.-S. JANG[2], E. KIM[1], G. LEE[1], *H. CHUNG[1];
[1]Dept Mol. and Integrative Physiol., [2]Neurosci. Program, Univ. of Illinois At Urbana Champaign, Urbana, IL

Abstract: Homeostatic plasticity maintains neuronal firing and synaptic strength within physiologic limits. Prolonged blockade of neuronal activity for 2 days in culture and in vivo causes a compensatory increase in firing rates as well as synaptic strength. Interestingly, activity blockade for 2-4 weeks in vivo causes temporal lobe epilepsy, implicating homeostatic plasticity in epileptogenesis. However, the molecular mechanism underlying homeostatic plasticity is not fully understood. We have recently reported that prolonged activity blockade by the voltage-gated sodium channel antagonist tetrodotoxin (TTX) leads to homeostatic increases in action potential firing rates, and decreases in multiple K+ channel transcripts in cultured hippocampal neurons (Lee and Royston et al., 2015). This TTX-induced scaling of intrinsic excitability and down-regulation of K+ channel genes is mimicked by inhibition of NMDA receptors (NMDAR) but not L-type voltage-gated Ca2+ channels (VGCC) (Lee and Royston et al., 2015). Here, we show that the same prolonged activity blockade leads to homeostatic increases in miniature excitatory postsynaptic potentials, and such synaptic scaling is mimicked by inhibition of L-type VGCCs but not NMDARs. These results together indicate that homeostatic scaling of intrinsic excitability involves upstream activity sensors and their downstream signaling pathways that are distinct from those underlying homeostatic scaling of synaptic strength. We further found that prolonged inhibition of neuronal activity or NMDARs but not L-type VGCCs markedly decreased BDNF transcripts and TrkB activity. Based on these preliminary results, we are currently testing the hypothesis that down-regulation of BDNF-TrkB signaling pathways upon reduced NMDAR activity leads to transcriptional down-regulation of K+ channel genes and the expression of homeostatic scaling of intrinsic excitability.

Disclosures: **A. Weiss:** None. **S. Jang:** None. **E. Kim:** None. **G. Lee:** None. **H. Chung:** None.

Poster

036. Homeostatic Plasticity

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Topic: B.08. Synaptic Plasticity

Support: Barrett Foundation Research Scholarship

Title: Calcium signaling mediates retrograde homeostatic compensation at the *Drosophila* neuromuscular junction

Authors: *L. GRAY[1], R. BALL[2], G. KAUWE[1], M. MORI[1], E. ISACOFF[2], P. HAGHIGHI[1]; [1]Buck Inst. For Res. On Aging, Novato, CA; [2]Mol. and Cell Biol., Univ. of California, Berkeley, Berkeley, CA

Abstract: Alterations in synaptic activity trigger homeostatic responses that modulate synaptic strength; these responses manifest as changes in postsynaptic receptor expression and retrograde regulation of transmitter release. The *Drosophila* larval NMJ provides an excellent experimental paradigm for studying synaptic homeostasis: removal of the GluRIIA glutamate receptor subunit decreases single channel mean open time, thereby reducing miniature synaptic currents; however, evoked synaptic currents remain comparable to wild type, implying a retrograde compensatory signal to enhance neurotransmitter release. We have hypothesized that a change in synaptic calcium influx, as a result of the genetic removal of GluRIIA, plays a role in retrograde signaling. To address this hypothesis, first we tested whether synaptic calcium influx is altered by genetic manipulation of GluR subunits. To achieve this, we combined two-electrode voltage clamp and calcium imaging using a synaptically targeted fluorescent calcium indicator, SynapGCamp3. Genetic removal of the GluRIIA subunit or overexpression of a GluRIIA mutant transgene ($GluRIIA^{M/R}$), in which a methionine near the opening of the pore was mutated to an arginine, both strongly reduced synaptic calcium influx. Both of these genetic manipulations strongly enhanced presynaptic release. These findings partially support our hypothesis by highlighting a link between reduced synaptic calcium influx and retrograde enhancement of presynaptic release, but raise the question of whether increasing calcium influx in these combinations would block retrograde signaling. To address this, we turned to an obligatory GluR subunit at the NMJ, GluRIIC. GluRIIC does not contain the signature pair of glutamine (Q) residues near the pore of the channel that are shared by most glutamate receptor subunits; this glutamine pair is thought to confer calcium permeability to the channel. We reasoned that engineering a pair of glutamines in this position in GluRIIC ($GluRIIC^{QQ}$) could increase synaptic calcium permeability at the NMJ. We could then test whether restoring calcium permeability to GluRIIA mutants would suppress homeostatic compensation in these animals. Our imaging and electrophysiological examination supports this prediction. Overexpression of $GluRIIC^{QQ}$ restored synaptic calcium influx and suppressed the enhancement in presynaptic release observed in GluRIIA mutant larvae. These findings provide strong evidence in support of our hypothesis that alteration in synaptic calcium influx plays a critical role in triggering the retrograde compensatory signal at the NMJ.

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Poster

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Support: RO1 NS083402 from NIH, NINDS

ICR strat-up fund from the University of Illinois at Urbana-Champaign

Title: Chronic enhancement of neuronal activity induces homeostatic down-scaling in $STEP_{61}$-dependent manners

Authors: *S.-S. JANG[1,2], H. JEONG[1], H. OH[1], S. ROYSTON[1,2], M. VEST[1], J. XU[3], P. LOMBROSO[3,4], H. CHUNG[1,2];
[1]Dept. of Mol. Integrative Physiology, Neurosci., [2]Neurosci. Program, Univ. of Illinois at Urbana-Champaign, Urbana, IL; [3]Chile Study Ctr., [4]Dept. of Neurobio. and Psychiatry, Yale Univ. Sch. of Med., New Haven, CT

Abstract: Prolonged changes in network activity induce homeostatic plasticity at excitatory synapse by regulating postsynaptic α-amino-3-hydroyle-5-methyl-4isoxazolepropionic acid receptors (AMPAR) and N-methyl-D-aspartate receptors (NMDAR), both of which are primary mediators of excitatory synaptic transmission. Previously, we reported that chronic activity blockade of hippocampal neurons induces synaptic scaling by decreasing the expression and activity of Striatal-Enriched protein tyrosine Phosphatase ($STEP_{61}$), which plays a critical role in the dephosphorylation of NMDAR subunit GluN2B and AMPAR subunit GluA2. However, very little is known about the role of $STEP_{61}$ in homeostatic downscaling of excitatory synaptic strength following chronic elevation of neuronal activity, and the signaling pathway involved. In this study, we induced chronic elevation of hippocampal neuronal activity by inhibiting GABAa receptors through the treatment of bicuculine (BC) or picrotoxin (PTX) for 24-48 h. Such prolonged activity elevation enhanced $STEP_{61}$ protein expression and decreased Tyr-phosphorylation of GluA2 and GluN2B from 24-48 h. Consistent with the ability of $STEP_{61}$ to induce internalization of AMPAR and NMDAR through dephosphorylation, we found that prolonged activity elevation for 24-48 h decreases surface expression of GluA2 and GluN2B expression. We are currently investigating how chronic activity elevation increases $STEP_{61}$ expression by examining the signaling pathways downstream of NMDAR and L-type voltage-gated calcium channels. Further insights into the functional implication of $STEP_{61}$ in synaptic down-scaling would be provided by experimental results using pharmacologic and genetic inhibition of STEP.

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Poster

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Topic: B.08. Synaptic Plasticity

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CIHR

Title: Homeostatic scaling-down of excitatory synapses during sleep driven by Homer1a

Authors: ***G. H. DIERING**[1], R. NIRUJOGI[2], R. H. ROTH[1], P. F. WORLEY[1], A. PANDEY[2], R. L. HUGANIR[1];
[1]Neurosci., [2]Inst. of Genet. Med., Johns Hopkins Univ., Baltimore, MD

Abstract: Homeostatic scaling is believed to regulate neuronal firing through global, non-Hebbian, adjustments to synaptic weights while maintaining the information stored through Hebbian plasticity mechanisms. While scaling has clearly been demonstrated in neurons in culture, true physiological functions of homeostatic scaling in vivo are not known. Sleep plays an essential role in normal cognitive functions. Evidence suggests that the benefits of sleep may occur via synaptic mechanisms, including a global weakening of synapses. Using sub-cellular fractionation, biochemistry and quantitative proteomics, we characterized the changes that occur in forebrain excitatory post-synaptic densities (PSD) through the dark/light cycle in mice. During the light phase when mice spend more time asleep we observed reduced levels of synaptic AMPA receptors and reduced AMPA receptor phosphorylation consistent with global synaptic weakening. These changes are driven by the immediate early gene Homer1a which is kept at low levels in the PSD during wake by the neuromodulator noradrenaline. During sleep, Homer1a remodels the mGluR1/5 signaling complex which is important for the consolidation of contextual memory. These findings reveal part of the molecular mechanisms at play during sleep and suggest that the physiological function of homeostatic scaling-down may be to renormalize synaptic strength during sleep.

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Topic: B.08. Synaptic Plasticity

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Title: Neuregulin1 (NRG1)/ErbB4 signaling regulates visual critical period cortical plasticity

Authors: *T. IKRAR[1], Y. SUN[1], N. GONG[1], M. F. DAVIS[2], S. P. GANDHI[2], X. XU[1];
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Abstract: Experience dependent critical period plasticity has been extensively studied in the visual cortex. Adjustments of excitatory and inhibitory synaptic strength are believed to be a major mechanism by which cortical networks adapt to sensory input over a range of timescales from seconds to days. During the developmental critical period, the most dramatic consequence of occluding vision through one eye (monocular deprivation) is a rapid loss of excitatory synaptic inputs to parvalbumin expressing (PV) inhibitory neurons in visual cortex. Subsequent cortical disinhibition by reduced PV cell activity allows for excitatory ocular dominance plasticity. However, the molecular mechanisms underlying critical period synaptic plasticity are unclear. Neuregulin1 (NRG1) is essential for the normal development of the nervous system, and signaling through its tyrosine kinase receptor ErbB4 has been implicated in synaptic plasticity and GABAergic circuit development. We test whether NRG1/ErbB4 signaling regulates functional circuit connections of PV interneurons and excitatory neurons during the critical period of visual development. Here we show that brief monocular deprivation during the critical period down-regulates neuregulin1(NRG1)/ErbB4 signaling in PV neurons (but not excitatory neurons), causing retraction of excitatory inputs to PV neurons. Exogenous NRG1 rapidly restores excitatory inputs onto deprived PV cells through downstream PKCdependent activation and AMPA receptor exocytosis, thus enhancing PV neuronal inhibition to excitatory neurons. NRG1 treatment prevents the loss of deprived eye visual cortical responsiveness *in vivo*. Our findings reveal molecular, cellular and circuit mechanisms of NRG1/ErbB4 in regulating the initiation of critical period visual cortical plasticity.

Disclosures: **T. Ikrar:** None. **Y. Sun:** None. **N. Gong:** None. **M.F. Davis:** None. **S.P. Gandhi:** None. **X. Xu:** None.

Poster

036. Homeostatic Plasticity

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 36.28/F41

Topic: B.08. Synaptic Plasticity

Support: NINDS NS#091546

Klingenstein-Simons Foundation

USC Provost fellowship

USC Woman in Science and Engineering travel grant

Title: A forward genetic screen identifies a link between the homeostatic control of sleep behavior and synaptic plasticity

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[1]Neurosci. Grad. Program, [2]Dept. of Neurobio., USC, Los Angeles, CA

Abstract: Homeostasis is a fundamental form of feedback regulation that precisely maintains the functionality of a system at set point levels of activity. Sleep is an ancient behavior under homeostatic control, yet despite intensive study, the biological function of sleep remains enigmatic. Synaptic plasticity is likely to change during sleep, but the relationship between these processes is unclear and controversial. The *Drosophila* neuromuscular junction has been established as a model system to study the homeostatic control of synaptic plasticity. At this glutamatergic synapse, acute pharmacological perturbation of postsynaptic glutamate receptors triggers a retrograde signal that results in an increase in presynaptic release, restoring proper levels of synaptic strength, a process termed presynaptic homeostatic potentiation (PHP). Using an electrophysiology-based forward genetic screen to isolate genes necessary for PHP, we have identified a sleep gene, *insomniac*. Interestingly, *insomniac* encodes an adaptor that targets substrates for protein ubiquitylation and degradation. Intriguingly, the sleep homeostat has been reported to be impaired in this mutant. Together, these findings provide amongst the first genetic and molecular links between the homeostatic control of sleep and synaptic plasticity. We find that *insomniac* is expressed in both presynaptic motor neurons and postsynaptic muscles, but is needed in the postsynaptic cell to drive the acute induction and expression of PHP. While we find no major defects in synaptic growth or architecture in *insomniac* mutants, Insomniac is localized to Golgi and ER structures, suggesting that modulation of the postsynaptic secretory pathway may be a target of the retrograde homeostatic signaling system. Finally, we have identified a putative substrate that colocalizes with Insomniac in Golgi and ER structures and implicates unconventional protein secretion in the induction of homeostatic plasticity. An

attractive hypothesis is that Insomniac controls postsynaptic secretory pathways to homeostatically modulate synaptic strength, and, perhaps, sleep behavior.

Disclosures: **K. Kikuma:** None. **M. Amar:** None. **H. Yang:** None. **D. Dickman:** None.

Poster

036. Homeostatic Plasticity

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Topic: B.08. Synaptic Plasticity

Support: NIH Grant NS062738

Whitehall Foundation Grant 2014-08-03

NIH Grant T32GM008629

Title: Tyrosine kinase-based synaptic signals drive the long-term maintenance of homeostatic neuroplasticity

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Abstract: Forms of homeostatic neuroplasticity endow neurons and circuits with an ability to maintain physiologically appropriate levels of synaptic output. Broken control of neuronal circuit function is hypothesized to contribute to conditions such as epilepsy and ataxia, as well as complex neurodevelopmental disorders like autism. Yet the molecular signals that direct the homeostatic control of synaptic outputs are poorly defined. We address this gap genetically by utilizing a model synapse, the glutamatergic Drosophila neuromuscular junction (NMJ). At the NMJ, genetic and pharmacological manipulations can decrease the sensitivity of postsynaptic receptors to single vesicles of neurotransmitter (quantal size). Decreased quantal size triggers retrograde, muscle-to-nerve signaling that drives increased transmitter release. In recent years, the NMJ has been key in characterizing conserved presynaptic processes that trigger a rapid homeostatic potentiation of neurotransmitter release. By contrast, almost nothing is known about how postsynaptic cells control the homeostatic potentiation of presynaptic neurotransmitter release or how the NMJ monitors its output over long periods of time. Through genetic screening and electrophysiological analyses, we have characterized how two classical tyrosine kinases in the postsynaptic muscle work to maintain the homeostatic signaling capacity of the NMJ throughout development. Our data demonstrate that the first one of these tyrosine kinases, C-terminal Src kinase (Csk), limits expression of the Neural Cell Adhesion Molecule (NCAM)

homolog Fasciclin II (FasII). Limiting FasII expression is necessary in order to propagate muscle-to-nerve homeostatic signaling. In the absence of Csk function, excess FasII/NCAM protein at the NMJ appears to inhibit the activity of a second tyrosine kinase in the muscle, the Drosophila Heartless (Htl) FGF Receptor. Htl and its signaling effectors are required for homeostatic potentiation and activation of the protein translation factor, Target of Rapamycin (TOR). TOR is an instructive factor previously shown to drive the long-term maintenance of synaptic homeostasis. We hypothesize that postsynaptic Htl/FGFR tunes synaptic strength throughout development by relaying multiple anterograde (nerve-to-muscle) and retrograde (muscle-to-nerve) signals.

Disclosures: **C. Frank:** None. **D.J. Brusich:** None. **A.M. Spring:** None.

Poster

037. Microglia I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 37.01/F43

Topic: B.12. Glial Mechanisms

Support: NIAAA Center Grant P60-AA011605

Title: Dynamic effects of ethanol on microglia

Authors: ***T. J. WALTER**, F. CREWS;
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Abstract: Alcoholism causes significant suffering, and further insight into the causes and consequences of this disease is necessary to provide more effective treatment. Recent research suggests not only that alcohol impacts microglia - the resident macrophages of the brain - but that microglia may contribute to the detrimental effects of alcoholism. Therefore, further investigation of the effects of alcohol on microglia may provide new insight into the causes of alcoholism. To more thoroughly define the effects of alcohol on microglia, BV2 microglial cells were treated with ethanol. The ethanol was allowed to evaporate away, thereby mimicking acute intoxication and withdrawal. Expression of various immune genes was examined at different time points. At early time points, while ethanol was still present, decreased expression of inflammatory genes TNF and Ccl2 was observed. However, at later time points, after the ethanol had evaporated, expression of TNF and Ccl2 was increased. These results suggest a dynamic, biphasic effect of ethanol on microglia. To further examine this dynamic effect, BV2 microglial cells were treated with lipopolysaccharide (LPS), a potent inflammogen, either during or after ethanol exposure. The presence of ethanol blunted LPS-induced expression of TNF and Ccl2.

However, treatment with LPS following ethanol treatment and evaporation increased expression of TNF and Ccl2. To determine whether alcohol induced similar effects in vivo, C57BL/6 mice were gavaged with an acute dose of ethanol. Expression of various immune genes was examined at different time points. Similar to the results obtained in vitro, TNF expression was decreased during intoxication but increased during withdrawal. Other immune genes such as Ccl2, IL-1B and IL-6 only increased during withdrawal. Microglial-specific genes such as CD68 also increased during withdrawal. Overall, these results suggest that alcohol has dynamic, biphasic effects on microglia. During acute intoxication, ethanol exhibits an anti-inflammatory effect, while withdrawal from ethanol has a pro-inflammatory effect. These results may lead to a greater understanding of the causes and consequences of alcoholism and potentially provide new insights for treatment.

Disclosures: **T.J. Walter:** None. **F. Crews:** None.

Poster

037. Microglia I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 37.02/F44

Topic: B.12. Glial Mechanisms

Support: (INIA) – Neuroimmune

Title: The role of microglial tlr-myd88 function in the cns after chronic intermittent ethanol.

Authors: *P. D. RIVERA[1], K. M. MILLER[2], H. S. SWARTZWELDER[2], S. D. BILBO[1]; [1]Dept. of Psychology and Neurosci., Duke Univ., Durham, NC; [2]Dept. of Psychiatry and Behavioral Sci., Duke Univ. Med. Ctr., Durham, NC

Abstract: It is now appreciated that drugs of abuse - including alcohol - promote a neuroimmune response from glia, primarily microglia, which may play an important role in mediating addictive-like behaviors. For example, alcohol consumption is linked to an increase in innate immune receptors, Toll-Like-Receptors [TLRs], within the CNS in humans and rodents. However, because central immune signaling pathways are pleiotropic and redundant, the elucidation of specific molecular pathways underlying excessive alcohol-induced pathophysiology, and therefore effective interventions, has been difficult. One major difficulty is that TLRs may be broadly expressed in CNS tissue. For example, TLR4 is expressed in neurons, astrocytes, and endothelial cells, in addition to microglia. Thus, the specific contribution of microglial TLR responses after excessive alcohol consumption has yet to be deciphered. Therefore, to better understand the pathogenesis of alcohol addiction, the innate immune

response specifically from microglia vs. other cell types after binge chronic intermittent ethanol (CIE) consumption was examined. To do so we used a novel transgenic mouse line in which TLR function is specifically ablated only in microglia within the CNS (CX3CR1-Cre x MyD88$^{f/f}$). Male CX3CR1-Cre$^{+/-}$ x MyD88$^{f/f}$ mice (8 weeks old) were exposed to either CIE (20% w/v) or chronic intermittent water (CIW, controls) for 16 days (10 doses). Blood was collected in a parallel group after ethanol dosing to measure blood alcohol levels (BALs). Mice were sacrificed 24 hrs after the last ethanol dose and the brain was extracted and flash frozen. Brains were sectioned, punches of the central amygdala and prefrontal cortex were taken, and a Toll-like receptor-signaling pathway PCR array was examined. Both CIE and CIW groups showed normal weight gain and reached BALs of ~100 mg/dL. Any changes in the gene expression profiles between the CIE and CIW groups would prove beneficial to understanding the role of microglial TLR-MyD88 signaling. Future directions include examining the gene expression profiles of CIE adolescent mice versus adult mice.

Disclosures: P.D. Rivera: None. **K.M. Miller:** None. **H.S. Swartzwelder:** None. **S.D. Bilbo:** None.

Poster

037. Microglia I

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Program#/Poster#: 37.03/F45

Topic: B.12. Glial Mechanisms

Support: NIH Grant AA013498

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Title: Chronic ethanol exposure provokes diverse microglial activation patterns in region-specific manner in the mouse brain.

Authors: *S. MONTGOMERY[1], M. BAJO[1], S. HUITRON-RESENDIZ[2], T. NADAV[2], L. N. CATES[2], E. F. CRAWFORD[1], K. CHENG[3], H. YIN[3], A. J. ROBERTS[2], M. ROBERTO[1]; [1]Committee on the Neurobio. of Addictive Disorders, The Scripps Res. Inst., LA Jolla, CA; [2]Mol. and Cell. Neurosci., The Scripps Res. Inst., La Jolla, CA; [3]Chem. and Biochemistry, BioFrontiers Inst., Univ. of Colorado Boulder, Boulder, CO

Abstract: Neuroinflammation and aberrant neuroimmune responses are common characteristics of a variety of neurodegenerative diseases and psychiatric disorders, including alcohol use disorder (AUD). Alcohol exposure has been shown to induce diverse neuroimmune responses between brain regions in both animal models and humans. In this study, we focused on microglia, the resident immune cells of the brain and critical regulators of the brain's innate immune response. We used a 2BC-CIE (two-bottle choice - chronic ethanol intermittent vapor exposure) paradigm to induce ethanol dependence in C57BL/6J mice and examined expression of microglial markers (Iba-1 and CD11b) via immunohistochemistry in the central amygdala (CeA) and prefrontal cortex (PFC) - brain regions that play an integral role in the development of alcohol dependence. During early ethanol withdrawal (4-8 h), we found an increase in Iba-1 expression and total optical density (OD) in the PFC but not in the CeA. However, chronic ethanol increased ramification of CD11b+ microglia in the CeA. Further morphological analyses of CD11b+ microglia in the PFC are currently under investigation. In a separate study, we systemically administered a TLR4-inhibitor, T5342126 (57 mg/kg, injected i.p for 14 days) and found that it reduced the OD of Iba-1 in the CeA of both ethanol-dependent and non-dependent mice. Overall, our data indicate that the development of alcohol dependence involves brain region-specific dysregulation of microglial responses, which may contribute to ethanol-induced neuroadaptive changes in the neural networks of those regions. Thus, understanding brain-region specific mechanisms behind chronic ethanol-induced neuroimmune responses and how these responses change throughout different stages of addiction is critical for further development of novel immune-based therapies, specifically for the treatment of AUD.

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Poster

037. Microglia I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 37.04/F46

Topic: B.12. Glial Mechanisms

Title: Microglia morphology in the medial prefrontal cortex following binge alcohol consumption and exercise

Authors: *E. A. BARTON[1], Y. LU[2], M. MEGJHANI[2], M. E. MAYNARD[1], B. ROYSAM[2], J. L. LEASURE[1];
[1]Dept. of Psychology, [2]Dept. of Electrical and Computer Engin., Univ. of Houston, Houston, TX

Abstract: Binge drinking, the most common pattern of alcohol intake in the United States, is characterized by discrete episodes of high blood alcohol content. Binge drinking damages brain structures such as the hippocampus and medial prefrontal cortex (mPFC), causing cognitive impairments. Using a rodent model of binge alcohol damage, we have found decreased granule neurons in the dentate gyrus (DG) and altered microglia morphology in the mPFC. In contrast to binge drinking, exercise benefits neural health; we have shown voluntary exercise restores the binge-damaged DG. The beneficial actions of exercise appear to be due in part to its profound effect on glia. Thus, investigating the interactive effects of binge alcohol and exercise allows us to explore how glia respond to neural damage and recovery. This area of research is also clinically relevant due to the positive association between alcohol and exercise, such that drinkers tend to be more physically active than abstainers.
We are investigating the interactive effects of binge and exercise on the number and morphology of microglia in the mPFC. Adult female Long-Evans rats were gavaged with ethanol (25% w/v) in nutritionally complete diet every 8 hours for 4 days. Control animals received isocaloric control diet. After 7 days of abstinence, rats remained sedentary or exercised for 10 days. We found increased microglia in the mPFC of exercised control animals, and binged animals independent of exercise. Morphological analysis revealed a subpopulation of larger, thicker microglia consistent with descriptions of primed microglia. These primed microglia were found in all animals regardless of experimental condition, however, the binge exercise group had the most. In a separate cohort of animals that exercised for 4 weeks post binge, we found that binge exercise animals had a significant decrease in microglia compared to binge sedentary and control exercise animals. Thus, longer periods of post-binge exercise are associated with a decrease in microglia. Furthermore, computational arbor analytics of remaining microglia revealed that the binged animals (regardless of exercise) had microglia with thicker arbors and significantly less branching. It appears that despite a binge-induced increase in microglia, prolonged post-binge exercise significantly reduces microglia numbers. Furthermore, the remaining microglia display a phenotype indicative of partial activation, suggesting that it is the primed microglia that survive prolonged post-binge exercise. We are continuing to investigate how binge alcohol coupled with exercise alters microglia and the implications of this for overall neural health and functioning.

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Poster

037. Microglia I

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Topic: B.12. Glial Mechanisms

Support: JSPS KAKENHI Grant 26460094

JSPS KAKENHI Grant 26117504

Title: Hyperthermia activates microglia to engulf inhibitory synapses in early-life seizures

Authors: *R. KOYAMA[1], Y. KASAHARA[1], K. SHIBATA[1], S. UJITA[1], S. SUGIO[2], K. F. TANAKA[3], K. SHIBASAKI[2], Y. IKEGAYA[1];
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Abstract: Hyperthermia (typically greater than 38°C)-induced febrile seizures are the most common type of seizures in early childhood. Prolonged febrile seizures could afterwards initiate the development of acute encephalopathy consisting of a cluster of seizures and postictal coma, which is often followed by the development of epilepsy. The induction of delayed seizures after febrile seizures indicates the prolonged impairment in the excitatory versus inhibitory balance (E/I balance) of synapses; however the cellular and molecular link between hyperthermia and E/I imbalance is missing. Here we report that microglia, the brain-resident immune cells, disrupt the synapse E/I balance in dentate circuits with the phagocytic capacity. Microglia detected the increase in brain temperature during experimental febrile seizures by the $Ca2^{+}$-influx through activation of transient receptor potential vanilloid 4 (TRPV4), which is a thermosensor activated by >34°C. The TRPV4-mediated $Ca2^{+}$-influx led microglia to preferentially engulf inhibitory synapses, resulting in a decrease of the density of inhibitory synapses in the dentate gyrus. Finally, minocycline, an inhibitor of microglial activation, decreased the delayed seizure severity after febrile seizures. Thus, our study provides a novel mechanism by which the brain hyperthermia impairs the synapse E/I balance via activation of microglia.

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Poster

037. Microglia I

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Topic: B.12. Glial Mechanisms

Support: NIH-NIA and UCR Academic Senate funds

Title: Synaptic excitation and ltp are altered in juvenile trem2-deficient mice

Authors: *P. W. HICKMOTT[1], A. MADANY[2], M. CARSON[2];
[1]Psychology & Interdepartmental Neurosci. Pgm., Univ. California, Riverside, Riverside, CA;
[2]Div. of Biomed. Sci. and Interdepartmental Neurosci. Pgm., Univ. of California, Riverside, Riverside, CA

Abstract: Microglia are the resident tissue macrophage of the central nervous system. Within the brain, microglia are the only cells expressing the Triggering Receptor Expressed on Myeloid cells-2 (TREM2). Humans completely lacking a functional TREM2 pathway develop early onset dementia, while mutations in the ligand-binding pocket of TREM2 are associated with increased risk of Alzheimer's disease. Until recently, microglia were characterized primarily for their tissue defense and tissue repair functions but now microglia are realized to play key roles in modulating synaptic maturation. Here we examined the synaptic maturation within the CA1 region of the hippocampus. Histologically, we find that TREM2 deficient mice have reduced numbers of vGlut1+ puncta indicative of fewer excitatory synapses, without any apparent deficiencies in GAD65+ puncta indicative of unchanged numbers of inhibitory synapses. To determine whether these histologic measures were associated with physiologic changes, we recorded excitatory and inhibitory synaptic currents (EPSCs and IPSCs) from the CA1 region of hippocampus. In brief, we found alterations in EPSC amplitudes but not IPSC amplitudes. Long-term dynamics, measured by comparing the ability to induce long-term potentiation (LTP), were also affected. These data illustrate that primary defects in microglia are sufficient to cause alterations in synaptic development.

Disclosures: P.W. Hickmott: None. **A. Madany:** None. **M. Carson:** None.

Poster

037. Microglia I

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Topic: B.12. Glial Mechanisms

Title: TREM2 and CCR2 mediated regulation of hippocampal synapse development

Authors: *A. MADANY, Y. OTANI, D. DAVIS, S. SLONIOWSKI, I. ETHELL, M. J. CARSON;
Univ. of California Riverside, Riverside, CA

Abstract: Microglia are the resident brain macrophage, long recognized to support brain function in both health and disease. Recently, microglia are recognized to contribute to synaptic editing and/or maturation in a C1q dependent fashion. Triggering Receptor Expressed on

Myeloid cells-2 (TREM2) is an immunomodulatory receptor expressed on microglia within the brain. Individuals lacking a TREM2 pathway develop early onset cognitive dementia (Nasu-Hakola disease), whereas individuals with mutations in the ligand binding pocket of TREM2 have increased risk of Alzheimer's disease. Therefore, we hypothesized that synaptic maturation in TREM2 deficient mice or might be altered either inherently or as a consequence of potentially aberrant responses to systemic inflammation. Therefore, we quantified vGlut1+ and GAD65+ puncta in the hippocampus of TREM2KO and wild-type (WT) mice at postnatal day 15 +/- systemic immune challenge. While systemic inflammation causes a sustained decrease in vGlut1+ puncta in WT mice, we found that in untreated TREM2KO mice the numbers of vGlut1+ puncta were decreased to the levels similar to immune challenged WT mice brain. We found that both homeostatic (developmental) and immune challenge induced editing of vGlut1+ puncta was dependent on C1q in WT mice, while immune challenge induced editing was also dependent on CCR2 and an apparent influx of blood derived macrophages. By contrast, the numbers of vGlut1+ puncta in untreated TREM2KO mice were already reduced to levels similar to immune challenged WT mice. Furthermore, the decreased numbers of vGlut1+ in TREM2KO mice was not dependent on C1q nor CCR2. Taken together these data reveal that (1) both microglia and brain infiltrating macrophages can regulate excitatory synapse maturation during systemic inflammation; (2) TREM2 prevents non-C1q and macrophage mediated synaptic editing during normal development; (3) defects in microglia can alter developmental maturation of brain function in the absence of induced injury or infection.

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Poster

037. Microglia I

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Topic: B.12. Glial Mechanisms

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Title: Noradrenergic modulation of microglial dynamics and synaptic plasticity

Authors: *R. STOWELL, G. SIPE, A. MAJEWSKA;
Univ. of Rochester, Rochester, NY

Abstract: Microglia, the innate immune cells of the central nervous system (CNS), respond rapidly and dynamically to homeostatic perturbations of the CNS milieu. In the healthy unperturbed brain, microglial processes make frequent contracts with neurons at synapses, impacting synaptic remodeling and turnover of dendritic spines. However, it remains unclear what receptors and signaling pathways govern microglial surveillance and synapse monitoring. Noradrenaline is a powerful signal that can affect many aspects of synaptic function and plasticity. Because microglia express high levels of **β**2 adrenergic receptors (AR) compared to other cell types in the brain, we asked whether noradrenergic tone could alter microglial behavior with respect to synapses through **β**2 AR signaling. To begin to test this hypothesis we have manipulated **β**2 AR signaling pharmacologically using the following agents: Nadolol (BBB impermeant **β**AR antagonist), Clenbuterol (BBB permeant **β**2 AR agonist), and ICI 118-51 (BBB permeant **β**2AR antagonist). We paired nadolol with clenbuterol to stimulate **β**2 AR centrally without concomitant peripheral stimulation. We then evaluated changes in basic microglial physiology through a combination of *in vivo* two-photon microscopy and immunohistochemistry staining for Iba-1 and CD68. We have found that stimulation of **β**2 AR signaling *in vivo* reduces microglial motility and pseudopodia formation and causes microglia to assume a less ramified morphology. Thus our next question was if these changes in basic microglial function could impact microglial interactions with neurons and ocular dominance plasticity. Using intrinsic optical signal imaging we have shown that pharmacological manipulation of **β**2 AR signaling impairs ocular dominance plasticity during the visual critical period in mice. We are now testing the direct involvement of microglia in this impairment through cre-mediated excision **β**2 AR specifically in microglia. Based on our results we believe that **β**2 AR signaling serves important roles in modulating microglial physiology and our future experiments will begin to address how the endogenous ligand, norepinephrine, is involved in mediating the effects observed. These results and future findings will improve our understanding of the signaling mechanisms that govern microglial interactions with synapses and impact activity-dependent synaptic modification.

Disclosures: **R. Stowell:** None. **G. Sipe:** None. **A. Majewska:** None.

Poster

037. Microglia I

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Topic: B.12. Glial Mechanisms

Support: National Research Foundation

Title: Deficient autophagy in microglia results in impaired synapse pruning and social behavior

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Abstract: Autism spectrum disorders (ASDs) are neurodevelopmental disorders caused by various genetic and environmental factors resulting in the abnormalities of synapses. Microglia are suggested to be related with ASDs and play a role in refining synapses during development. Autophagy and related pathways are also suggested to be related with ASDs. However, the precise roles of autophagy in microglia on synapses and ASDs are unknown. Here we show that microglial autophagy refines synapses and regulates neurobehaviors. We found that atg7 deletion in the myeloid cell specific lysozyme-M cre mice showed social behavioral defects and repetitive behaviors, characteristic features of ASDs. These mice also showed increased dendritic spines, synaptic markers and altered connectivity between brain regions indicating defects in synapse refinement. Degradation of synaptosomes was impaired in atg7-deficient microglia and immature dendritic filopodia were increased in neuron cultures with atg7-deficient microglia. Our results first demonstrate the role of microglial autophagy in synapse regulation and neurobehaviors. We anticipate our results to be a starting point for more comprehensive understanding of microglial autophagy in ASDs and putative therapeutics development.

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Poster

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Topic: B.12. Glial Mechanisms

Support: NIH R21 NS080098

AHA16PRE27600010

Title: The inflammatory response following a laser-induced cortical microhemorrhage in a rodent model is dominated by migration of brain-resident microglia

Authors: *S. AHN[1], J. ANRATHER[2], N. NISHIMURA[1], C. B. SCHAFFER[1];
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Abstract: Clinical studies have linked microhemorrhages with cognitive decline, although how these small bleeds impact cognition is not understood at the cellular level. We used optical approaches to create microhemorrhages and image the cellular responses in the brain of mice. Irradiation with tightly-focused femtosecond laser pulses ruptured targeted penetrating arterioles, producing a hematoma of ~150-μm diameter in the cortex. Such lesions did not cause neural death, but did drive an increase in the number of nearby activated inflammatory cells. Such inflammation could have a profound impact on neural health and function. Here, we aim to understand the relative role of brain-resident and blood-derived inflammatory cells. We used chimeric transgenic animals with different inflammatory cell populations labeled with fluorescent proteins, and mapped the spatial and temporal profile of the cellular response after a microhemorrhage. We observed brain-resident microglia activate soon after the microhemorrhage and the doubling of their density adjacent to the lesion is the dominant aspect of the inflammatory response. A small number of blood-derived inflammatory cells were also found, with ~3 proinflammatory monocytes (CCR2+) entering the brain during the first two days after the lesion, and ~3 patrolling monocytes (CX3CR1+) entering after two days. The doubling of microglia density one day after the lesion could be due to migration and/or proliferation of microglia. Using time-lapse imaging, we observed microglia immediately send processes to, and then migrate over one day toward, the ruptured arteriole. For microglia within 150 μm of the targeted vessel, the migration speed was 0.9 μm/hr over the first 16 hours and then slowed. We used EDU staining to look for signs of microglia proliferation and found just one EDU positive microglia out of 163 (5 hemorrhages across 2 mice). Together these data suggest that the microglia density increase after a microhemorrhage is almost exclusively due to migration. Over two weeks the microglia density returned to baseline. To determine if apoptosis played a role in this decrease, we used TUNEL staining at four days after the lesion and found no TUNEL-positive microglia (0 out of 93 microglia; 6 hemorrhages across 2 mice). In summary, small brain bleeds trigger migration of nearby microglia toward the lesion, doubling the microglia density, and drive the infiltration of a very small number of inflammatory and patrolling monocytes. In ongoing work, we are exploring how this inflammation could affect the health and function of nearby brain cells and thus drive cognitive decline.

Disclosures: **S. Ahn:** None. **J. Anrather:** None. **N. Nishimura:** None. **C.B. Schaffer:** None.

Poster

037. Microglia I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 37.11/F53

Topic: B.12. Glial Mechanisms

Title: The role of APOE in MS-associated neuroinflammation

Authors: *E. CUDABACK;
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Abstract: Multiple sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system (CNS) that causes debilitating symptoms due to a loss of neuron conductivity. Encephalitogenic CD4+ T helper (Th) cells inappropriately respond to myelin antigens and invade the CNS, producing a variety of pro-inflammatory cytokines that activate resident innate immune cells, namely microglia and astrocytes, resulting in exacerbated or protracted neuroinflammatory responses that are thought to contribute to MS pathology. Autoreactive Th17 cells in particular are pathogenic in many inflammatory disorders, including MS, and make up a substantial portion of the inflammatory infiltrate in active lesions presenting in MS patients. While the molecular determinants influencing sensitivity and responsiveness of the affected brain to these inflammatory insults remain poorly understood, genetic studies have identified many potential disease risk alleles. Indeed, evidence suggests that *APOE* may act as an MS modifier, specifically influencing disease progression, severity, and cognitive outcomes, although observations in humans have been inconsistent. Because *APOE* genotype is known to differentially influence glial inflammatory responses to a broad range of insults, we decided to examine how *APOE* genotype modulates microglial and astrocyte activation in response to Th17 cell signaling cues. Our results support a role for *APOE* in MS neuroinflammation and expand our understanding of CNS inflammatory mechanisms, allowing for additional characterization of novel cellular and molecular therapeutic targets for MS.

Disclosures: E. Cudaback: None.

Poster

037. Microglia I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 37.12/G1

Topic: B.12. Glial Mechanisms

Support: CIHR

HSFC

FRSQ

MSFHR

Title: Microglia rapidly adopt a filopodia-rich phenotype during hypoxia by sensing tissue acidosis.

Authors: *L.-P. BERNIER, L. DISSING-OLESEN, J. K. HEFENDEHL, J. M. LEDUE, B. A. MACVICAR;
Psychiatry, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Microglia are highly motile cells that play a pivotal role in monitoring brain homeostasis by constantly probing the environment and responding to extracellular cues. They are involved in stroke-related pathologies by undergoing complex long-lasting transcriptional and functional changes. However the acute response of microglia to the metabolic stress of ischemia remains unclear. Here, we used two-photon imaging *in vivo* and in acute brain slices to monitor the initial effect of hypoxia on the morphological phenotype and dynamic properties of microglia. The highly ramified morphology of resting microglia is rapidly transformed during oxygen depletion with extension of fine actin-dependent filopodia followed by retraction of microtubule-dependent ramifications. This rapidly reversible switch in morphology drives significant changes in microglial sensing, affecting the capacity of microglial cells to respond to tissue damage. During short hypoxic insults, we show that this initial phenotypic switch is induced by microglia sensing the accompanying acidic shift in the extracellular environment. Concurrent observations indicate that rapid extension and retraction of actin filament-rich filopodia at the tip of major ramifications is an integral part of the microglial sensing behavior. This finely tuned random-searching dynamic process depends on compartmentalized cAMP signals, and overproduction of intracellular cAMP triggers dysregulated filopodia extension and retraction of ramifications, thereby leading to a phenotype similar to that observed during hypoxia. Characterizing the highly specialized sensing structures of microglia and defining the molecular cues responsible for the functional switch of microglial behaviour observed upon oxygen depletion will likely provide promising targets for stroke treatment.

Disclosures: **L. Bernier:** None. **L. Dissing-Olesen:** None. **J.K. Hefendehl:** None. **J.M. LeDue:** None. **B.A. MacVicar:** None.

Poster

037. Microglia I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 37.13/G2

Topic: B.12. Glial Mechanisms

Support: MH1RO1MH093362

Title: Transducing acidosis to panic: role of microglial and renin angiotensin system (RAS) mechanisms

Authors: ***A. WINTER**[1], R. SAH[2], L. VOLLMER[2], R. AHLBRAND[2], E. KRAUSE[3]; [1]Psychiatry, [2]Univ. of Cincinnati, Cincinnati, OH; [3]Univ. of Florida, Gainesville, FL

Abstract: Panic disorder (PD) is a complex anxiety disorder characterized by spontaneous, recurrent panic attacks consisting of intense fear, cardiovascular, and respiratory symptoms. Altered acid-base homeostasis has been observed in PD patients. CO_2 inhalation, an inducer of acidosis, evokes panic attacks in PD patients. Recent studies from our lab reported a role of microglial acid sensing and pro-inflammatory cytokine IL-1β in CO2-evoked fear (Vollmer et al Biological Psychiatry 2016). CO2 evoked microglial activation occurs within the subfornical organ (SFO), an area lacking the blood-brain-barrier and relevant to homeostasis. However, mechanisms transducing microglial acid sensing and inflammation to panic-relevant behavior are currently unknown. The current study investigated a potential role of renin angiotensin system (RAS) as an effector transducing microglial acid sensing to CO2-evoked fear. Microglial-RAS interactions have been recently reported, and importantly, RAS polymorphisms have been reported in PD patients . Angiotensin expressing neurons are abundant in the SFO and our recent studies show expression of IL-1b receptors on these neurons suggesting potential link between RAS and inflammatory mechanisms. Angiotensin II-type I receptor antagonist, losartan, was delivered intracerebroventricularly (ICV) in the vicinity of the SFO. Losartan or vehicle injected mice were exposed to CO_2 inhalation for 10 min. Fear (freezing) was measured during the 10 min CO_2 exposure and 24 hr post-CO_2 during exposure to context alone. A significant attenuation of freezing was observed in losartan treated mice during CO_2 (46%; $p<0.05$) and during conditioned contextual fear (47%; $p<0.05$). cFos immunohistochemistry revealed a significant decrease in cFos+ cells within the lateral hypothalamus (LH: 40%, $p<0.05$) and periaqueductal gray (PAG: 20%, $p<0.05$), brain regions connected to the SFO that regulate panic-relevant fear, cardiovascular and respiratory responses. Tracing studies are currently being

performed to validate this circuitry. Collectively, our data suggest SFO microglial inflammation-RAS interactions in transducing CO_2-evoked fear, providing a novel mechanistic basis for understating panic pathophysiology.

Disclosures: **A. Winter:** None. **R. Sah:** None. **L. Vollmer:** None. **R. Ahlbrand:** None. **E. Krause:** None.

Poster

037. Microglia I

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Topic: B.12. Glial Mechanisms

Support: NSFC Grant 31190061

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NSFC Grant 31501128

Title: Cytosolic immature cathepsin D controls F-actin-mediated lamellipodia extension in microglia

Authors: ***Y. LIU**[1], T. ZHANG[1], L. QIN[1], Y. ZHANG[2], X. LI[1], J. YANG[1], Y. HU[1], Z. GUO[1], H. LOU[1], M. HO[3], S. DUAN[1];
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Abstract: Rapid moving cells such as microglia evolve complex mechanisms that dynamically shape cytoskeleton to provide structural cues for injury-induced migration. During structural reorganization, endosome/lysosome-related enzymes, with their degradative function, have been implicated in signaling migration, debris uptake, and injury repair. However, to date, how these enzymes modulate cytoskeletal dynamics remain mysterious. Here we present evidence that the immature 48kDa lysosomal enzyme cathepsin D (cathD) translocates to the microglial cytosol via a mechanism that depends on Hsp70-mediated membrane permeability. Upon ATPγS stimulation, cytosolic cathD localizes to the F-actin lamellipodia at the leading edge, where directional migration begins to occur. Down regulation of cathD expression using siRNA, drug inhibitor, or in cathD-deficient microglia leads to the maintenance failure of lamellipodia protusions, albeit initial extensions remain unaffected. Intriguingly, cathD interacts with the actin-severing protein cofilin and controls the availability of G-actin monomers via cofilin phosphorylation. On the other hand, treatment with cytochalasin D rescues the cathD-mediated

lamellipodia maintenance defects, pinpointing a role for cathD in controlling the amount of available raw materials, rather than the functional extension for actin filament assembly. Taken together, our results identify a key lysosomal factor that exhibits an unconventional cytosolic localization during ATP-evoked responses in microglia. Cytosolic cathD contributes to actin filament assembly and cytoskeleton reorganization via regulating cofilin activity. These findings underlie the mechanism of microglial migration and significantly advance our understanding in how microglia respond to pathological injury.

Disclosures: **Y. Liu:** None. **T. Zhang:** None. **L. Qin:** None. **Y. Zhang:** None. **X. Li:** None. **J. Yang:** None. **Y. Hu:** None. **Z. Guo:** None. **H. Lou:** None. **M. Ho:** None. **S. Duan:** None.

Poster

037. Microglia I

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Topic: B.12. Glial Mechanisms

Support: European Research Council (309788)

Israeli Science Foundation (1782/11)

Title: Microglia development follows a stepwise program to regulate brain homeostasis

Authors: ***O. MATCOVITCH-NATAN**[1], D. R. WINTER[2], S. ITZKOVITZ[3], E. ELINAV[2], M. SIEWEKE[5], M. SCHWARTZ[4], I. AMIT[2];
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Abstract: Microglia, the brain resident macrophages, play important roles in life-long brain maintenance and in pathological conditions. In addition, these cells are crucial players during central nervous system development. Yet, the mechanisms that regulate the dynamics during this maturation process has not been fully elucidated. Using genome-wide single-cell transcriptomic analysis and chromatin profiling we revealed that microglia undergo three temporal developmental stages in synchrony with the brain: early, pre-, and adult microglia, which are under the control of distinct regulatory circuits. Environmental perturbations, such as disrupted microbiome or prenatal immune activation, led to dysregulation of the developmental program, particularly in terms of inflammation. Knockout of MafB, a transcription factor which is predominantly expressed in the adult microglia, resulted in disturbance of homeostasis and increased expression of inflammatory genes. Together, our work identifies the circuits of a

microglia developmental programs that maybe associated with susceptibility to neurodevelopmental disorders.

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Poster

037. Microglia I

Location: Halls B-H

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Topic: B.12. Glial Mechanisms

Support: NIH

Title: Nuclear GAPDH cascade and cellular autofluorescence: microglia-mediated novel mechanisms involved in cognitive deficits in schizophrenia

Authors: *A. RAMOS[1], N. ELKINS[1], T. TSUJIMURA[1], T. SAITO[2], F. EMILIANI[1], N. GAMO[1], C.-Y. LI[1], T. SEDLAK[1], C. KORTH[3], K. ISHIZUKA[1], A. SAWA[1];
[1]Psychiatry and Behavioral Sci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Aomori Univ., Aomori, Japan; [3]Univ. of Duesseldorf Med. Sch., Duesseldorf, Germany

Abstract: Behavioral flexibility is one of the most important behavioral constructs that is required for adaptability. This behavior is affected under stress conditions, and its disturbance is involved in many neuropsychiatric disorders, in particular schizophrenia. Nonetheless, molecular and cellular mediators for this behavior are unclear.
To address this question, we used LPS-treated mice (a model frequently used to represent brain pathology upon stressors such as immune inflammatory or oxidative stress). We found that the mice displayed behavioral inflexibility in a paradigm using rule shifting. Interestingly, the behavioral deficits were ameliorated by a compound (1R,3R)-1,3-dymethyl-2-propargyl-1,2,3,4tetrahidrosiquinoline (called RR). RR is a potent blocker of the nuclear GAPDH cascade (Hara *et al*. Nat Cell Biol. 2005). Therefore, we next validated whether the nuclear GAPDH cascade was indeed activated after LPS treatment. We found that the cascade was activated selectively in microglia. Several studies in behavioral neuroscience have indicated the involvement of the prefrontal cortex for behavioral flexibility. Consistent with this notion, we observed selective activation of the nuclear GAPDH cascade in cortical, but not striatal, microglia. Together, we now propose that a nuclear pool of GAPDH that is known to modulate epigenetic and transcriptional machinery specific to microglia may be a key molecular and cellular mediator for behavioral flexibility.

To extend this preclinical observation into clinical settings, we have looked for molecular and cellular markers that correlate with behavioral inflexibility (e.g., deficits in Wisconsin Card Sorting Test) in patients with schizophrenia (SZ). We found that autofluorescence in lymphoblasts is significantly higher in SZ, compared with matched controls, which is independent of medication. In particular, the extent of the behavioral inflexibility was correlated with the levels of impairment in the cellular autofluorescence. As far as we have tested multiple independent behavioral constructs in humans, behavioral flexibility is the only one that shows correlation with autofluorescence. Importantly, the aberrant autofluorescence has been normalized by RR, the blocker of the nuclear GAPDH cascade.
Together, we propose that: 1) The activation of the nuclear GAPDH cascade in cortical microglia is involved in a mechanism responsible for behavioral flexibility. 2) Autofluorescence that may reflect the activation of the nuclear GAPDH cascade pathway is a possible high throughput biomarker for behavioral inflexibility in schizophrenia.

Disclosures: **A. Ramos:** None. **N. Elkins:** None. **T. Tsujimura:** None. **T. Saito:** None. **F. Emiliani:** None. **N. Gamo:** None. **C. Li:** None. **T. Sedlak:** None. **C. Korth:** None. **K. Ishizuka:** None. **A. Sawa:** None.

Poster

037. Microglia I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 37.17/G6

Topic: B.12. Glial Mechanisms

Title: THIK-1 regulates cytokine release by microglia

Authors: V. KYRARGYRI[1], C. MADRY[1], R. JOLIVET[1,2,3], *D. ATTWELL[1];
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Abstract: Microglia are the resident macrophages of the central nervous system (CNS), which continually survey the brain for infectious agents, amyloid plaques and unnecessary neurons and synapses. Upon activation, microglia generate an inflammatory response, which can lead to neuronal cell death in a number of neurodegenerative diseases. This involves activation of the NLRP3 inflammasome and subsequent release of proinflammatory cytokines (Guo et al., Nat. Med., 2015). We recently found that microglial surveillance is regulated by the resting membrane potential, which is set by the tonic activity of THIK-1, an anaesthetic-sensitive two-pore domain K^+ channel highly expressed by microglia in the brain (Madry et al., submitted). In addition to their tonic activity, THIK-1 channels are activated by brain damage or ATP, causing

a strong hyperpolarisation via K^+ efflux, which may be an important trigger for inflammasome activation. Indeed, we now report that THIK-1 also plays an important role in regulating the microglial inflammatory response. Acute hippocampal slices from P12 rats were exposed for 3 h to lipopolysaccharide (LPS) followed by 3 h incubation with LPS and ATP. The supernatants were collected and the amounts of released interleukin (IL)-1β and TNFα were quantified by ELISA. LPS alone (but not ATP) induced a release of IL-1β and TNFα, however the production of IL-1β (but not of TNFα) was greatly increased after application of LPS and ATP together. Blocking THIK-1 with tetrapentylammonium (TPA) abolished the release of IL-1β and TNFα in response to LPS alone, or to LPS with ATP, whereas 4-AP and charybdotoxin had no effect, excluding a role for voltage- and Ca^{2+}-activated K^+ channels. Pharmacological inhibition of THIK-1 channels by TPA also abolished the cell death induced by LPS and ATP, as measured by assaying LDH released into the supernatant. Our results show that, in addition to controlling microglial surveillance, THIK-1 is also necessary for microglia to generate an inflammatory response, and raise the possibility of therapeutic targeting of THIK-1 in neuroinflammation-driven diseases.
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Disclosures: **V. Kyrargyri:** None. **C. Madry:** None. **R. Jolivet:** None. **D. Attwell:** None.

Poster

037. Microglia I

Location: Halls B-H

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Topic: B.12. Glial Mechanisms

Support: The Research Support Center, Research Center for Human Disease Modeling, Kyushu University Graduate School of Medical Sciences

Title: Neuroprotective effect of nicotine by inhibition of microglial proton currents via alpha7 nAChR

Authors: ***M. NODA**[1], A. KOBAYASHI, 812-8582[2];
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Abstract: Alpha 7 subunits of nicotinic acetylcholine receptors (nAChRs) are expressed in microglia and are involved in the suppression of neuroinflammation. Over the past decade, many reports show beneficial effects of nicotine, though little is known about the mechanism. Here we show that nicotine inhibits lipopolysaccharide (LPS)-induced proton (H^+) currents by using primary cultured microglia. (Methods) The H^+ channel currents were measured by whole-cell

patch clamp method under voltage-clamp condition. The morphological change was observed by immunocytochemical method. The expression level of H^+ channels was measured by western blotting. Neurotoxicity was tested by using primary cultured neurons from mouse hippocampus and cortex and LPS-treated microglial conditioned medium. The number of live neuronal cells with or without LPS application was checked by using Cell Counting Kit-8. (Results) H^+ currents in cultured newborn mouse brain microglia recorded at physiological temperature were specifically activated at positive potentials by 24-h LPS pretreatment. Increased H^+ current in activated microglia was attenuated by blocking NADPH oxidase, confirming its physiological role of electrical charge compensation following negatively charged superoxide generation in the extracellular compartment. Nicotine produced a dose-dependent inhibition of LPS-activated H^+ current, with an apparent IC_{50} of 112.1 nM. Morphological changes associated with LPS-induced microglial activation were almost completely reverted by nicotine. Nicotine reverted only H^+ current levels but not increased HVCN1 protein expression upon LPS exposure of cultured microglia. The inhibitory effect of nicotine was exerted via nAChRs, being completely counterbalanced by pretreatment with specific alpha7 nAChRs inhibitors such as alpha-bungarotoxin or methyllicaconitine. Nicotine pretreatment of LPS-stimulated microglia was able to inhibit neurotoxic effects of microglial-conditioned cell culture medium. (Discussion) These results suggest that alpha7 nAChRs in microglia may be a therapeutic target in neuroinflammatory diseases.

Disclosures: M. Noda: None. **A. Kobayashi:** None.

Poster

037. Microglia I

Location: Halls B-H

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Topic: B.12. Glial Mechanisms

Title: Elimination of microglia in adult mouse forebrain does not alter kynurenine 3-monooxygenase activity

Authors: *K. V. SATHYASAIKUMAR[1], P. SEVERSON[2], B. L. WEST[2], R. SCHWARCZ[1]; [1]Univ. of Maryland Sch. of Med., Baltimore, MD; [2]Plexxikon Inc., Berkeley, CA

Abstract: Kynurenine 3-monooxygenase (KMO) plays a key regulatory role in the kynurenine pathway (KP) of tryptophan degradation, which contains several neuroactive metabolites. Impairment of KMO has been implicated in several major brain diseases including Huntington's disease and schizophrenia. In the mammalian brain, KMO has been suggested to be predominantly localized in microglial cells (Alberati-Giani et al., 1996; Heyes et al., 1996;

Guillemin et al., 2001), but verification *in vivo* has not been provided so far. The present study was designed to examine possible impairments in KP metabolism in the mouse brain after depleting microglial cells in the central nervous system through pharmacological inhibition of the colony stimulating factor 1 receptor (CSF1R). The CSF1R inhibitor PLX5622 depletes microglia in normal mice by 80% after 1 week of treatment, as determined by anti-IBA-1 immunohistochemistry (Dagher et al., 2015). In the current tests, separate male C57BL/6J mice (~2-month-old) were fed PLX5622 (1200 ppm in chow) for 21 days, while controls received normal chow. Animals were euthanized either on day 22 (control: n=6; treated: n=6) or, after receiving normal chow for an additional 21 days, on day 43 (n=3). To verify the depletion of microglia in the forebrain, the expression of several marker genes was assessed in one hemisphere using real-time PCR. *KMO* expression, KMO activity, KYNA and 3-hydroxykynurenine (3-HK) levels were determined in parallel using the second hemisphere. As expected, expression of the marker genes *Aif1* (-89%), *Csf1r* (-97%), *Cx3cr1* (-97%), *Siglech* (-97%) and *Tmem119* (-90%) was dramatically reduced on day 22 but had recovered by day 43. In contrast, PLX5622 did not cause any significant changes in either *KMO* expression or KMO activity at any timepoint. The brain levels of KYNA and 3-HK were also unchanged. Taken together, our data suggest that microglial cells do not harbor the majority of KMO in normal adult mouse brain. Possible compensatory events following PLX5622 treatment, as well as alternative scenarios in dysfunctional states, are currently under investigation.

Disclosures: K.V. Sathyasaikumar: None. **P. Severson:** None. **B.L. West:** None. **R. Schwarcz:** None.

Poster

037. Microglia I

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Topic: B.12. Glial Mechanisms

Support: EU H2020-MSCA-IF 2014 Grant 657733-ISSVS

Title: Sex-specific vulnerability to maternal immune activation - Are microglia involved?

Authors: *S. M. SCHAAFSMA, K. E. LAUPMAN, E. M. HOL, M. JOËLS;
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Abstract: Prenatal stress, including maternal immune activation (MIA), has long term effects on brain and behavior. It is becoming increasingly clear that environmental factors such as prenatal

stress, in addition to genetic factors, play an important role in the etiology of neurodevelopmental and neuropsychiatric disorders. Prenatal stress has sex-specific effects, with males showing the most pronounced deficits in behaviors when exposed to early prenatal stress. Many neurodevelopmental and neuropsychiatric disorders show a sex bias in incidence. E.g. for every girl diagnosed with an autism spectrum disorder, there are 4 males. Microglia are the main innate immune cells of the brain and respond quickly to injury and inflammation. Chronic activation of microglia can have detrimental effects and is observed in patients diagnosed with a neurodevelopmental disorder. Prenatal stress can activate microglia which can last well into adulthood. Besides microglia´s immune function, these cells are also involved in the masculinization process. Blocking male-specific neonatal microglia activation prevents masculinization of brain and behavior in male rodents (Lenz *et al.* J. Neurosci. 2013). We hypothesize that males that are exposed to MIA experience two hits on their neonatal microglia (due to MIA and due to masculinization) - versus one hit in females - which may explain the male-specific effects of prenatal stress on behavior later in life. Time pregnant C57BL/6J mice were sc injected with 0.30 mg/kg LPS in saline, or vehicle only at gestational day 7. Neonatally, 6 experimental groups were created within each litter. To induce masculinization in females 0.625µg PGE2 in 1µl saline (or vehicle only for controls) was injected icv in each hemisphere at postnatal day (PD)0 and PD1. To block masculinization in males 25µg indomethacin in DMSO in saline (or vehicle only for controls) was injected sc at PD0 and PD1. One additional male and female were left in the nest to serve as full controls. At PD4 the pups were sacrificed and their brains, fixed in PFA, used for immunohistochemistry. Data will be presented on quantitative (# microglia) and qualitative measures of microglia activation (cell morphology and excretion profile indicating M1 or M2 activation) in brain areas relevant to neurodevelopmental disorders (amygdala, hippocampus, mPFC). Additionally, preliminary behavioral data, focusing on anxiety, cognition, repetitive, and social behaviors will be presented. Our data may help elucidate the underlying mechanism of the sex-specific effects of prenatal stress and contribute to the understanding of the male-biased incidence of neurodevelopmental disorders.

Disclosures: **S.M. Schaafsma:** None. **K.E. Laupman:** None. **E.M. Hol:** None. **M. Joëls:** None.

Poster

037. Microglia I

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Topic: B.12. Glial Mechanisms

Support: TÜBİTAK Grant 114S378

Title: Alterations in glial iron homeostasis in hypoxic conditions and effects of induced hypothermia on iron regulatory proteins in cell culture

Authors: *A. L. ARAL[1], M. A. ERGUN[2], B. ENGIN[5], A. Ö. BÖRCEK[3], L. PINAR[4], M. K. BAYKANER[3], H. BOLAY[6];
[1]Dept. of Immunol., [2]Med. Genet., [3]Neurosurg., [4]Physiol., Gazi Univ. Fac. of Med., Ankara, Turkey; [5]Toxicology, Gazi Univ. Fac. of Pharmacy,, Ankara, Turkey; [6]Neurol. & Neuropsychiatry Ctr., Gazi Univ., Ankara, Turkey

Abstract: Fetal and neonatal inflammatory responses may contribute towards neonatal brain injury and developmentally related disabilities such as cerebral palsy as well as cognitive deficits later in life. Pathogenesis of the brain white matter lesions in newborns involves damage related to hypoxia with inflammation-induced brain injury. Microglia and astrocytes do not only initiate, but also contribute to the growth of lesions within the white matter as well as representing a potential strategic target for treatment in hypoxic disorders. The proper homeostasis of cellular iron transporters and proteins involved in iron storage, are important to prevent excess iron overload or iron starvation in cells. In this study we aimed to show the effects of hypoxia on iron related proteins and cytokine production in glial cells, and assess if hypothermic preconditioning has any effect on iron levels and molecules involved in regulating iron homeostasis.Microglia and astrocyte cell cultures were prepared from the cerebral cortex of neonatal C57BL/6 mice (n=3). Both cell types were incubated in hypoxic conditions for 12 hours. Effects of hypoxia with glucose deprivation on iron transport protein expression were evaluated at the mRNA level by quantitative real-time PCR. Cellular iron accumulation was evaluated using Perl's histochemistry. Cytokine levels have been studied using commercial ELISA kits. Hypoxia stimulated the expression of the ferritin in both cell types. Histochemical staining of accumulated iron in the cells correlated with ferritin expression. In addition to proinflammatory cytokine levels, iron-influx proteins TfR1 and DMT-1 increased after hypoxia especially in microglia. Iron-efflux protein ferroportin increased after hypoxic conditions in both cells. Hypothermic preconditioning prior to hypoxia decreased the cellular iron significantly in microglia. Hypothermia increases the expression of transport protein levels differently. In conclusion, in our study, hypoxic conditions accompanying glucose deprivation stimulated iron accumulation in microglia and astrocytes and triggered the expression of iron homeostasis proteins differently. Increased cellular iron and its efflux from glial cells might create a signal to trigger inflammatory responses which play role in hypoxic ischemic injury. Hypothermic preconditioning decreased the ferritin expression especially in microglia, which might suggest that the hypothermic control of cellular iron transporters and iron levels has a regulatory effect especially on the inflammatory pathways stimulated with hypoxic injury.

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Poster

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Topic: B.12. Glial Mechanisms

Support: Ministry of Science 2013R1A1A2074231

SSTF-BA1502-13

Title: Role of spinal microglia in DCP-induced contact dermatitis.

Authors: *B. CHOI, H. MIN, S. LEE;
Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Studies indicate an important role of spinal cord glial cells in central sensitization of not only pain but also itch sensation. In diphenylcyclopropenone (DCP)-induced contact dermatitis leading to chronic itch state, astrocyte activation caused by scratching stimulus triggers activation of STAT3/LCN2 signaling cascade and contributes to enhancement of itch signal sensation at the spinal cord level. Meanwhile, it was also reported that spinal microglia are activated through CX3CL1/CX3CR1 signaling in 2,4-dinitrofluorobenzene (DNFB)-induced contact dermatitis model. In this regard, we tested if spinal cord microglia activation is involved in chronic itch in DCP-induced contact dermatitis model. After 5 consecutive days of DCP challenge, a significant activation of spinal cord microglia was detected in the spinal dorsal horn. Inhibition of the DCP-induced microglia activation by intrathecal injection of minocycline attenuated scratching behavior, suggesting putative involvement of spinal cord microglia activation in itch sensitization. The mechanisms of spinal cord microglia activation after chronic DCP treatment have been further explored. Our data suggest that, similar to spinal cord astrocytes, spinal cord microglia activation affects chronic itch sensation.

Disclosures: B. Choi: None. **H. Min:** None. **S. Lee:** None.

Poster

037. Microglia I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 37.23/G12

Topic: B.12. Glial Mechanisms

Title: Transforming growth factor - beta1 attenuates pro-inflammatory activation in microglia through ALK5-dependent signaling

Authors: *Q. CHEN[1], B. V. CHEUNG[2], T. SHIMADA[1], M. MATSUMOTO[3], H. ITO[1], K. TAJINDA[1];
[1]Astellas Res. Inst. of America LLC, Skokie, IL; [2]Master of Biotech. program, Northwestern Univ., Evanston, IL; [3]Neurosci. Res. Unit, Drug Discovery Research, Astellas Pharma Inc., Tsukuba, Japan

Abstract: Microglia are the residential immune cells in the brain and play important roles in the maintenance of tissue homeostasis under physiological conditions. The abnormally activated microglial cells have been found in a variety of neuropsychiatric disorders and neurodegenerative diseases. During the neuroinflammation, over-activated microglia undergo a morphological change, synthesize and release various cytotoxic mediators such as pro-inflammatory cytokines and reactive oxygen species. Overproduction of these cytotoxic mediators could disrupt the normal brain development and neuronal function which are considered to be pathophysiological mechanisms in psychiatric disorders. Transforming growth factor - beta 1 (TGFβ1) is a pleiotropic cytokine and a potent regulator of neuroinflammation and cytotoxicity. TGFβ1 plays a critical role in supporting microglial development and maintaining functional microglial signature in the brain. Here, we investigated the anti-inflammatory mechanism of TGFβ1in LPS- stimulated primary microglial cells. We examined both the canonical and non-canonical pathways in TGFβ1-treated microglial cells. Western blot and immunocytochemistry results showed that Smad2/3 signaling was selectively activated in primary microglia. TGFβ1 treatment could effectively reduce proinflammatory cytokines and iNOS expression in LPS-activated microglial cells. The further mechanism studies found that TGFβ1 induced TGFβ receptor ALK5 expression in microglia cells. In addition, pharmacological inhibition of ALK5 signaling partially diminished the anti-inflammatory effects of TGFβ1 in LPS stimulated microglia. Further experiments are ongoing to investigate the role of TGFβ1 in regulation of inflammatory signaling in microglial cells. Our data suggested that activation of TGFβ/ALK5 signaling in microglia could effectively alleviate microglia mediated neuroinflammation.

Disclosures: **Q. Chen:** A. Employment/Salary (full or part-time): Astellas Research Institute of America, Astellas Pharma. **B.V. Cheung:** A. Employment/Salary (full or part-time): intern in Astellas Research Institute of America. **T. Shimada:** A. Employment/Salary (full or part-time): Astellas Research Institute of America, Astellas Pharma. **M. Matsumoto:** A. Employment/Salary (full or part-time): Neuroscience Research Unit, Drug Discovery Research, Astellas Pharma Inc. **H. Ito:** A. Employment/Salary (full or part-time): Astellas Research Institute of America, Astellas Pharma. **K. Tajinda:** A. Employment/Salary (full or part-time): Astellas Research Institute of America, Astellas Pharma.

Poster

037. Microglia I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 37.24/G13

Topic: B.12. Glial Mechanisms

Support: NUS Startup Grant R-181-000-155-133

NUS Startup Grant R-181-000-155-733

Title: Piwi-like 4 regulates neuroinflammation via the nf-κb and p38 mapk pathways

Authors: *Q. HU[1], C. SUBHRAMANYAM[2], C. WANG[2], Q. CAO[2];
[1]Anat., [2]Natl. Univ. of Singapore, Singapore, Singapore

Abstract: In neurodegenerative diseases, chronic inflammation hampers regeneration and exacerbates the pathology. It is mainly caused by excess activation of microglia, the immune cells in the central nervous system (CNS). Recently, small RNAs have been shown to regulate gene expression at multiple levels. In particular, the Piwi subclass of the Argonaut family binds to a cohort of small RNAs called piwi-interacting RNAs (piRNAs). Though pilot studies have established that piRNA/Piwi mainly function to silence transposons in germline cells, it is found that mouse hippocampal neurons express piRNAs and that numerous piRNAs were differentially regulated in a rat stroke model. Hence, we investigated the role of Piwi in regulating the activation of microglia. Our data showed that inflammatory signals could strongly induce the expression of Piwil4 in murine BV-2 cells, a microglial cell model. The induced Piwil4 protein was mainly localized in the cell nucleus. In the loss-of-function assays, we detected a significant decrease in the LPS-induced expression of pro-inflammatory genes. On the other other hand, some anti-inflammatory and wound healing factors were upregulated by Piwil4 knockdown. In addition, we also observed that in LPS-treated microglia, the depletion of Piwil4 led to decreased production of reactive oxygen species and nitric oxide, both of which not only eliminate pathogens but also damage healthy nerve tissue if not contained properly. Accordingly, when dopaminergic neuronal cells were exposed to conditioned medium collected from Piwil4-depleted LPS-activated microglia, less cell toxicity and death were observed. We further found that Piwil4 could regulate the nuclear accumulation of NF-κB and its chromatin binding to target genes. In addition, the activation of p38 MAPK, as well as JNK, was also affected by Piwil4 knockdown. As a result, the AP-1-regulated gene expression could be attenuated. Hence, our study reveals that Piwil4 can modulate microglial activation at the transcriptional level, providing a novel strategy to fight against chronic inflammation in neurodegenerative diseases.

Disclosures: Q. Hu: None. **C. Subhramanyam:** None. **C. Wang:** None. **Q. Cao:** None.

Poster

037. Microglia I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 37.25/G14

Topic: B.12. Glial Mechanisms

Support: T32 NS 082145

R01 MH090127

1F31 MH102070-01A1

Title: Brain-derived neurotrophic factor promotes a neuroprotective phenotype in microglia during stress or inflammation

Authors: *A. M. DUGAN[1], J. M. PARROTT[1], L. REDUS[1], J. G. HENSLER[1], J. C. O'CONNOR[1,2];
[1]Pharmacol., Univ. of Texas Hlth. Sci. Ctr. at San Antonio, San Antonio, TX; [2]Audie L. Murphy VA Hosp., San Antonio, TX

Abstract: Both chronic stress and peripheral inflammation reduce expression of brain-derived neurotrophic factor (BDNF) in the brain and may contribute to depression. We have previously observed that BDNF deficient ($BDNF^{+/-}$) mice are more susceptible to both inflammation- and stress-induced depressive-like behaviors. In both cases, $BDNF^{+/-}$ mice exhibited a significantly greater shift in kynurenine metabolism toward overproduction of neurotoxic metabolites when compared to wild type (WT) controls. $BDNF^{+/-}$ mice also failed to up-regulate the anti-inflammatory cytokine interleukin-10 (IL-10) after stress when compared to WT controls. We aimed to investigate whether BDNF is directly involved in the cellular regulation of these neuroinflammatory factors in the context of stress or immunological challenge. Because microglia are the principle cells responsible for neurotoxic metabolism of kynurenine within the brain, we first sought to determine whether BDNF directly regulates microglial expression of kynurenine pathway enzymes. BV-2 mouse microglial cells were treated with vehicle or corticosterone (CORT, the major hormone involved in stress responses) in the presence or absence of BDNF in order to model the effects of stress in BDNF competent or deficient conditions. After 6h, CORT increased microglial kynurenine monooxygenase (KMO) expression with and without BDNF. However, after 24h, BDNF treatment reduced indoleamine-2,3-dioxygenase (IDO) expression in CORT-treated microglia. This pattern of expression in response to CORT and BDNF suggests that BDNF reduces the activity of the KP in microglia. In similar experiments aimed to address the role of BDNF regulation of microglia under inflammatory conditions, BV-2 microglia were treated with vehicle or lipopolysaccharide (LPS, an inflammatory stimulus) in the presence or absence of BDNF. BDNF treatment reduced LPS-

induced expression of microglial pro-inflammatory cytokines tumor necrosis factor-α (TNFα), interleukin-1β (IL-1β), and the activation marker ionized calcium-binding adapter molecule-1 (IBA1). Importantly, BDNF treatment increased microglial IL-10 expression after exposure to LPS. These data suggest that BDNF modulates the metabolic and inflammatory responses of microglia, which may be involved in conferring vulnerability/resilience to stress- or inflammation-induced psychiatric disorders.

Disclosures: **A.M. Dugan:** None. **J.M. Parrott:** None. **L. Redus:** None. **J.G. Hensler:** None. **J.C. O'COnnor:** None.

Poster

037. Microglia I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 37.26/G15

Topic: B.12. Glial Mechanisms

Title: Maternal immune activation and later-life behavioral deficits: is there a link with embryonic microglia migration?

Authors: C. LACABANNE[1,2], A. BENMAMAR--BADEL[3], S. LAYE[2], *G. N. LUHESHI[1]; [1]Douglas Mental Hlth. Univ. Inst., McGill Univ., Verdun, QC, Canada; [2]INRA-Université de Bordeaux, Bordeaux, France; [3]Ecole Normale Supérieure de Lyon, Univ. de Lyon, Lyon, France

Abstract: Microglia, the resident immune cells of the central nervous system (CNS), have recently been demonstrated to have a central role in a number of aspects of neural development, including cell proliferation, neurogenesis, synaptogenesis and programmed cell death. Microglial function in the foetal brain has been shown to be sensitive to environmental insults with some suggesting a link with neurodevelopmental defects. Of the environmental insults, maternal immune activation (MIA) has been repeatedly associated with the pathogenesis of several neurodevelopmental conditions in the offspring. We thus hypothesized that microglia could be involved in neuronal and behavioural defects in the offspring that have been exposed to MIA. In addition to the type and severity of infection, the stage of embryonic development is a significant factor in this hypothesis and to test the signfiicance of this we decided to target a very early stage of gestation, namely the time when microglia first migrates to the fetal brain. Microglial precursors originate from the yolk sac and invade the brain very early (Embryonic day (E) 9.5) in the developmental process. In the present study, we tested the effect of an immune challenge (lipopolysaccharide, LPS) at E9.5 on the microglial migration process conducting a combination of molecular (gene expression), imaging (two-photon, confocal imaging and 3D reconstruction) and a battery of behavioural approaches. The disruption of early microglia migration by maternal

inflammation resulted in dysfunctional microglia and subsequent neuronal and behavioural defects in offspring. Our results will contribute to our understanding of the role of microglia in early prenatal infection on later-life behavioral deficits.

Disclosures: C. Lacabanne: None. **A. Benmamar--badel:** None. **S. Laye:** None. **G.N. Luheshi:** None.

Poster

037. Microglia I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 37.27/G16

Topic: B.12. Glial Mechanisms

Support: Fundacion Marcos Moshinsky

CONACYT-117

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CONACYT-181323

GAPA-UNAM IN200715

CONACYT-105807

Title: Measurements of hydrogen peroxide in line and their change by hypoxia and microglia modulation

Authors: *K. PARDO-PEÑA[1,2], F. PEÑA-ORTEGA[2], A. MORALES-VILLAGRÁN[3], N. CAMACHO-HERNÁNDEZ[2], J. LOREA-HERNÁNDEZ[2];
[2]Developmental Neurobio. and Neurophysiol., [1]UNAM, Queretaro, Mexico; [3]Mol. and Cell Biol., CUCBA, Univ. de Guadalajara, Guadalajara, Mexico

Abstract: Hydrogen peroxide (H_2O_2) is a prominent member of the Reactive Oxigen Species (ROS) family, which can be produced and or release by different cell-types. Episodes of hypoxia, and the subsequent reoxygenation, result in the accumulation of ROS and the induction of oxidative stress. These hypoxic conditions can promote the increase of extracellular H_2O_2 concentrations. On the other hand, microglia can modulate circuit activity and the response to hypoxic events. Thus, dynamic measurements of H_2O_2 concentrations are a valuable tool for the

understanding of microglia function during hypoxia. In this study, we developed a new technique for measuring H_2O_2 concentration in brainstem slices with one second resolution. This method consists of mixing the extracellular fluid obtained from the brainstem slices with an enzymatic reactor composed by horseradish peroxidase and Amplex UltraRed, which generates a derivate (resorufin) that fluoresces at 590 nm when excited at 560 nm. Resorufin excitation was induced with a laser beam whereas the fluorescence intensity was determined with a photomultiplier. By this procedure the fluorescent signal intensity was proportional to H_2O_2 concentration. Our data show that both hypoxia and microglial-activation with lipopolysaccharide (LPS) increase extracellular H_2O_2 concentration. When microglia is activated by LPS (500 ng/ml), the H_2O_2 concentration increased dramatically to 18.3±0.5 μM, compared to baseline levels which were 1.6±0.4 μM. This increase lasted as long as LPS was present in the bath and did not return to normal levels upon the application of Minocycline (30 μM; a microglial inhibitor; 17.8±0.6 μM). However, when Minocycline was administered as pretreatment, LPS-induced microglia activation produced a reduced release of H_2O_2, only reaching a concentration of 4.7±1.7 μM. On the other hand, hypoxic conditions sustained for 15 consecutive minutes produced an increased in H_2O_2 concentration of 5.0±0.3 μM, whereas during reoxygenation the H_2O_2 concentration remained elevated at 5.6±1.0 μM. Interestingly, this increase produced by transient hypoxia lasted for at least 90 minutes.Pretreatment with Minocycline, 60 minutes before hypoxia, reduces by 64% the increase in H_2O_2 concentration during hypoxia (1.8±0.6 μM) but only by 17% during reoxygenation (4.65±0.2 μM). Interestingly, Minocycline reduced H_2O_2 concentration by itself. These data support the use of this technique for the determination of H_2O_2 release from microglia with high temporal resolution, *in vitro*. Furthermore it would allow to correlate changes in neural function with the release of this ROS, both under normal and pathological conditions.

Disclosures: **K. Pardo-Peña:** None. **F. Peña-Ortega:** None. **A. Morales-Villagrán:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 309696-335409. **N. Camacho-Hernández:** None. **J. Lorea-Hernández:** None.

Poster

037. Microglia I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 37.28/G17

Topic: B.12. Glial Mechanisms

Support: NIH R01 HD069899

Title: Effects of early life infection and iron deficiency upon neurodevelopment in the neonatal piglet

Authors: *B. J. LEYSHON[1], R. W. JOHNSON[1,2];
[1]Div. of Nutritional Sci., [2]Dept. of Animal Sci., Univ. of Illinois at Urbana-Champaign, Urbana, IL

Abstract: During the postnatal period the developing brain is vulnerable to insults including nutrient insufficiency and infection that may lead to disrupted development and cognitive dysfunction. Iron deficiency (ID) is the most common micronutrient deficiency worldwide. Pregnant women and young children are most vulnerable to ID. Iron is a necessary cofactor for a variety of biological processes in both the immune and neurodevelopmental domains, including synthesis of lipids for myelination and meeting the metabolic demands of the growing brain. Since ID and the associated anemia often presents with immunodeficiency, the objective of this study was to investigate negative impacts on neurodevelopment, microglial phenotype and immune function manifested by early life ID coupled with respiratory infection using a neonatal piglet model. On postnatal day 2 (PD 2) piglets were divided into four groups, and fed either control or ID milk replacer. Piglets were inoculated with either vehicle or porcine reproductive and respiratory syndrome virus (PRRSV; P-129) on PD 8. Baseline blood samples were collected prior to inoculation (PD 7) and repeated once weekly. Rectal temperatures, feeding score and sickness behaviors were measured daily until piglets were sacrificed on PD 28 for tissue collection. Hematocrit and hemoglobin were reduced by ID but not PRRSV infection. Both groups of PRRSV infected piglets displayed increased serum viremia on PD 14. However, PRRSV piglets fed control diet displayed decreased viremia on PD 21 and PD 28, while those fed an ID diet displayed similar viremia throughout, suggesting that ID impaired immune function necessary for viral clearance. Similarly, ID piglets infected with PRRSV initially displayed reduced sickness behavior score compared to those fed control diet, which progressively worsened while PRRSV piglets fed control diet displayed improved sickness score after PD 20. Brain weight was reduced by PRRSV infection but not ID. Primary microglia isolated from brain displayed increased MHCII expression and phagocytic activity in PRRSV infected piglets compared to uninfected piglets. However, ID did not impair microglial MHCII expression or phagocytosis. Taken together, these data suggest that ID decreases peripheral immune function leading to decreased viral clearance, but immune activity in the brain is protected from acute ID. Future areas of inquiry for this project include evaluating microglial cytokine production and gene expression in the context of ID and PRRSV infection, especially genes pertaining to glycolytic and oxidative metabolism, a key difference between pro-inflammatory (M1) and pro-resolving (M2) phagocytes.

Disclosures: B.J. Leyshon: None. **R.W. Johnson:** None.

Poster

037. Microglia I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 37.29/G18

Topic: B.12. Glial Mechanisms

Support: NIH Grant R01AT007429

NIH Grant R01NS046400

Title: Activation of the PGE2 EP1 receptor promotes microglial phagocytosis through CD36 receptor recycling

Authors: *B. MA[1], B. SLOOTSKY[2], H. PHILLIPS[3], K. KOLAROVA[3], S. DORÉ[3];
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Abstract: We have previously documented that the deletion of the prostaglandin E2 (PGE2) EP1 receptor undermined microglial phagocytosis in vivo. However, the related mechanism still remains unknown. CD36 is a type II scavenger receptor that mediates microglial phagocytosis. Moreover, it was reported that microglial CD36 contributes to hematoma clearance after intracerebral hemorrhage (ICH). In this study, we sought to identify the effects of two EP1 receptor agonists on phagocytosis and CD36 receptor recycling in microglial cultures.
The EP1 agonists 17-phenyl trinor-PGE2 (17-pt-PGE2) and ONO-DI-004 were used in our study to treat mouse primary microglial cultures. Fluorescent latex beads or CFSE-labeled red blood cells (CFSE-RBCs) were added to microglial cells at a concentration of 10 beads/RBCs per cell for live-cell imaging. Immunocytochemistry was also performed with antibodies against ionized calcium-binding adapter molecule 1 (Iba1, a microglial marker). Furthermore, we analyzed CD36 receptor recycling in primary microglial cells by using an established receptor recycling assay.
The primary microglial cells were incubated with fluorescent latex beads and treated with the EP1 agonist 17-pt-PGE2 for 120min. We found that 10uM of 17-pt-PGE2 significantly increased the number of attached beads to microglial cells from 40 to 120min of incubation. Furthermore, we analyzed CD36 receptor recycling using an established receptor recycling assay and the quantitative results showed that 10uM of 17-pt-PGE2 treatment markedly increased the positive-staining area of recycled CD36 receptor in microglial cells. We then treated primary microglial cells with another EP1 agonist, ONO-DI-004, for 120min at the concentration of 5 and 10uM, respectively. The fluorescent area of CFSE-RBCs after ice-cold PBS washing was markedly increased with 10uM ONO-DI-004 treatment. In the live-cell imaging experiment, 10uM of

ONO-DI-004 treatment also significantly increased the number of attached CFSE-RBCs to microglial cells from 25 to 60min of incubation.
Together, this study demonstrates that the PGE2 EP1 agonists 17-pt-PGE2 and ONO-DI-004 can promote microglial phagocytosis in vitro and that enhanced CD36 receptor recycling could mediate these effects.

Disclosures: **B. Ma:** None. **B. Slootsky:** None. **H. Phillips:** None. **K. Kolarova:** None. **S. Doré:** None.

Poster

037. Microglia I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 37.30/G19

Topic: B.12. Glial Mechanisms

Support: NIEHS grant ES007062

Title: A putative interaction of tspo with nadph oxidase in primary microglia

Authors: ***M. K. LOTH**[1,2], S. R. GUARIGLIA[2], J. CHOI[2], D. B. RE[2], T. R. GUILARTE[1,2]; [1]Envrn. & Occup. Hlth., FIU, Miami, FL; [2]Envrn. Hlth. Sci., Columbia Univ., New York, NY

Abstract: Translocator protein 18 kDa (TSPO) is a glial protein that is extensively used as a biomarker of neuroinflammation. We have previously shown that TSPO ligands (TSPO-L) induce cellular functions in microglia consistent with an activated state, suggesting an important role of TSPO in the inflammatory response to brain injury. In particular, exposing microglia to TSPO-L (1-100 nM) induces ROS production that can be abrogated by NADPH oxidase (NOX) inhibitors, suggesting an association between TSPO and NOX, a multi-subunit enzyme that is highly enriched in microglia and is a major source of ROS in the CNS. To further elucidate the relationship between TSPO and NOX, we determined the source of ROS production resulting from microglia exposure to TSPO-L. Intracellular and extracellular ROS production was inhibited by NOX inhibitors, but not by a mitochondria permeability transition pore inhibitor, indicating that the source of ROS production is from NOX and not from mitochondria. These findings were confirmed using the mitochondria specific ROS probe MitoSOX. To assess if TSPO and NOX are interacting, we performed co-immunoprecipitation experiments. The results indicate that gp91phox and p22phox co-immunoprecipitated with TSPO supporting a functional protein-protein interaction. Our findings provide novel insight on the potential function and subcellular localization of TSPO in microglia. To further explore the subcellular localization of TSPO, we performed triple-label immunofluorescent confocal imaging of TSPO with the

mitochondrial protein VDAC and the principal NOX subunit gp91phox, both of which revealed significant colocalization. We are currently confirming our results using immuno-EM labeling. Together our findings suggest a novel interaction of TSPO with NOX in primary microglia with significant implications for a new understanding of TSPO glial cell biology and for the development of therapeutic strategies [supported by NIEHS grant ES007062 to TRG].

Disclosures: **M.K. Loth:** None. **S.R. Guariglia:** None. **J. Choi:** None. **D.B. Re:** None. **T.R. Guilarte:** None.

Poster

038. Immune Responses in Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 38.01/G20

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Mitochondrial fragmentation induced ROS triggers p62 mediated autophagy in microglia

Authors: *U. CHAE[1], H. LEE[2], D.-S. LEE[1];
[1]Kyungpook Natl. Univ., Taegu-City, Korea, Republic of; [2]Biomed. Res. Institute, Chung-Ang Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Autophagy have a critical role in many process, including elimination of dysfunctional organelles, repair mechanism and programmed cell death. Reactive Oxygen Species (ROS) which is one of the autophagy inducer is not clearly elucidated in microglia. In this study, we demonstrated that mitochondrial ROS which was induced by mitochondrial fission can promote autophagy in microglia. Moreover, inhibition of mitochondrial fission or mitochondrial ROS could downregulates the formation of autophagosome. Notably, regulatory effect between mitochondrial ROS and autophagy is mediated by only p62 not LC3B. Taken together, these results suggest that the increased autophagy induced by LPS can be decreased by inhibition of mitochondrial ROS through downregulation of p62. Our results indicate that concrete regulatory effect between mitochondrial ROS and autophagy in microglia.

Disclosures: **U. Chae:** None. **H. Lee:** None. **D. Lee:** None.

Poster

038. Immune Responses in Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 38.02/G21

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Swiss National Science Foundation

Title: Role of adult neurogenesis in increased sensitivity of ArcticAbeta mice to kainic acid

Authors: ***M. ZAICHUK**, T. GSCHWIND, I. KNUESEL, J.-M. FRITSCHY;
Univ. of Zürich, Zürich, Switzerland

Abstract: Overlap between Alzeimer's disease (AD) and epilepsy is becoming widely accepted. Going in line with the evidence of co-occurrence of both conditions in human patients, a number of transgenic AD mouse models were shown to have spontaneous epileptiform discharges, disturbed rhythmic activity or higher sensitivity to seizure inducing drugs. We used unilateral intrahipocampal injection of kainic acid to mimic temporal lobe epilepsy in a familial AD mouse model. In the hippocampus, kainic acid-mediated induction of an epileptic focus causes progressive neurodegeneration of CA1 pyramidal cells, granule cell dispersion and non-convulsive recurrent seizures that persist for months. Adult ArcticAbeta mice, that overexpress human APP with Arctic and double Swedish mutations showed increased sensitivity to kainic acid in comparison to the wild-type controls. Increased mortality of the transgenic mice was observed in the first few weeks after the injection. Adult neurogenesis was previously implicated in the epileptogenesis and was separately shown to be affected in familial AD mouse models. We studied adult neurogenesis in ArcticAbeta mice to understand their sensitivity to kainic acid. Adult transgenic mice had increased rate of cell proliferation and reduced cell survival in the subgranular zone of the dentate gyrus. Adult born neurons in ArcticAbeta mice were shown to have decreased number of dendritic spines and reduced dendritic branching during maturation. Those findings are another set of evidence showing that adult neurogenesis can be affected by the overexpression of mutated forms of APP. In addition to other factors (see Tilo Gschwind´s poster) those changes in adult neurogenesis could contribute to the epileptogenesis in AD-mice.

Disclosures: **M. Zaichuk:** None. **T. Gschwind:** None. **I. Knuesel:** None. **J. Fritschy:** None.

Poster

038. Immune Responses in Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 38.03/G22

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: SNF Grant 144199

Title: The contribution of Alzheimer's disease-like pathology to epileptogenesis in a mouse model for temporal lobe epilepsy

Authors: *T. GSCHWIND[1], T. GFELLER[1], C. LAFOURCADE[1,2], M. ZAICHUK[1], I. KNUESEL[1], J.-M. FRITSCHY[1];
[1]Univ. of Zurich, Zürich, Switzerland; [2]Univ. de los Andes, Santiago, Chile

Abstract: The most prevalent age-related dementia, Alzheimer's disease (AD), causes synaptic dysfunction and neuronal reorganization, along with a chronic neuroinflammation. These features are similar to those seen in temporal lobe epilepsy (TLE). As observed in AD patients, marked variations in cognitive performance can occur due to undetected epileptic seizures of limbic origin.
The aim of this study is to investigate how a genetic predisposition for AD affects epileptogenesis in an animal model for TLE. For this purpose, transgenic mice overexpressing human $APP_{swe,arc}$ (arctic Aβ mice; a model of familial AD) were injected with kainic acid (KA) into the dorsal hippocampus to induce an epileptic focus, which leads to local inflammation and non-convulsive spontaneous recurrent seizures. Intrahippocampal electrodes were implanted to characterize seizure activity after KA injection. Neurodegeneration, loss of interneurons, axonal sprouting, endothelial inflammation as well as activation of innate and adaptive immune cells were assessed immunohistochemically to determine the contribution of inflammation and immune responses on the histopathological alterations caused by KA-injection. The acute effect of KA on hippocampal circuitry was analyzed using a combination of local field potential and whole cell patch clamp recordings in acute hippocampal slice.
Compared to wildtype littermates, AD transgenic mice exhibit a much higher vulnerability towards KA, in vivo and in slices. The causal investigation for this phenotype unraveled a possible link between AD-like pathology and epilepsy.

Disclosures: **T. Gschwind:** None. **T. Gfeller:** None. **C. Lafourcade:** None. **M. Zaichuk:** None. **I. Knuesel:** None. **J. Fritschy:** None.

Poster

038. Immune Responses in Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 38.04/G23

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NINDS P30 NS069375

Title: Beta1 adrenergic receptors modulate neurocognitive function, neuroinflammation and pathology in a mouse model of Alzheimer's Disease

Authors: *A. K. EVANS, B. YI, M. SHAMLOO;
Neurosurg., Stanford, Stanford, CA

Abstract: Severe degeneration of Locus Coeruleus noradrenergic (NA) neurons has been reported in Alzheimer's Disease (AD), and loss of noradrenergic tone may contribute to progression of pathology, neuroinflammation and cognitive dysfunction. Beta adrenergic receptors (ADRB1 and ADRB2) on neurons, microglia and astrocytes modulate learning and memory, regulate neuroinflammation and govern compensatory and protective mechanisms for neuronal function and survival. Pharmacological and genetic disruption of noradrenergic tone 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 or antagonism of the ADRB1 receptor on behavioral, pathological, and neuroimmune endpoints in mouse models of AD. Using selective beta-adrenergic pharmacology in a transgenic model in which mice express a mutated form of human amyloid precursor protein, transgenic and wildtype male mice were chronically dosed with vehicle or with either a selective ADRB1 partial agonist, xamoterol (0.3-1.0 mg/kg daily s.c. injection) or a selective ADRB1 antagonist, metoprolol (5 mg/kg daily s.c. injection). Mice were dosed for approximately 3 months and run through a series of behavioral tests during the last 6 weeks of dosing (Activity Chamber, Y-maze, Morris Water Maze, and Fear Conditioning). At conclusion, plasma was collected and brains were perfused with saline and hemi-brains were flash-frozen or paraformaldehyde-fixed for neurobiological analyses. Chronic dosing with a selective ADRB1 partial agonist, xamoterol, improved and metoprolol impaired contextual fear conditioning. Chronic dosing with metoprolol exacerbated deficits in spatial learning in transgenic mice. Modulation of ADRB1 altered amyloid beta pathology and modulated indices of neuroimmune activation (microglia/macrophage immunoreactivity, and immune-related mRNA and protein expression). These data support the hypothesis that ADRB1 activation regulates pathology and cognitive function in mouse models of AD and may do so by modulating neuroimmune function.

Disclosures: **A.K. Evans:** None. **B. Yi:** None. **M. Shamloo:** Other; Cortice Biosciences has licensed a patent for xamoterol from Stanford University..

Poster

038. Immune Responses in Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 38.05/G24

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: L'Oreal Fellowship for Women in Science

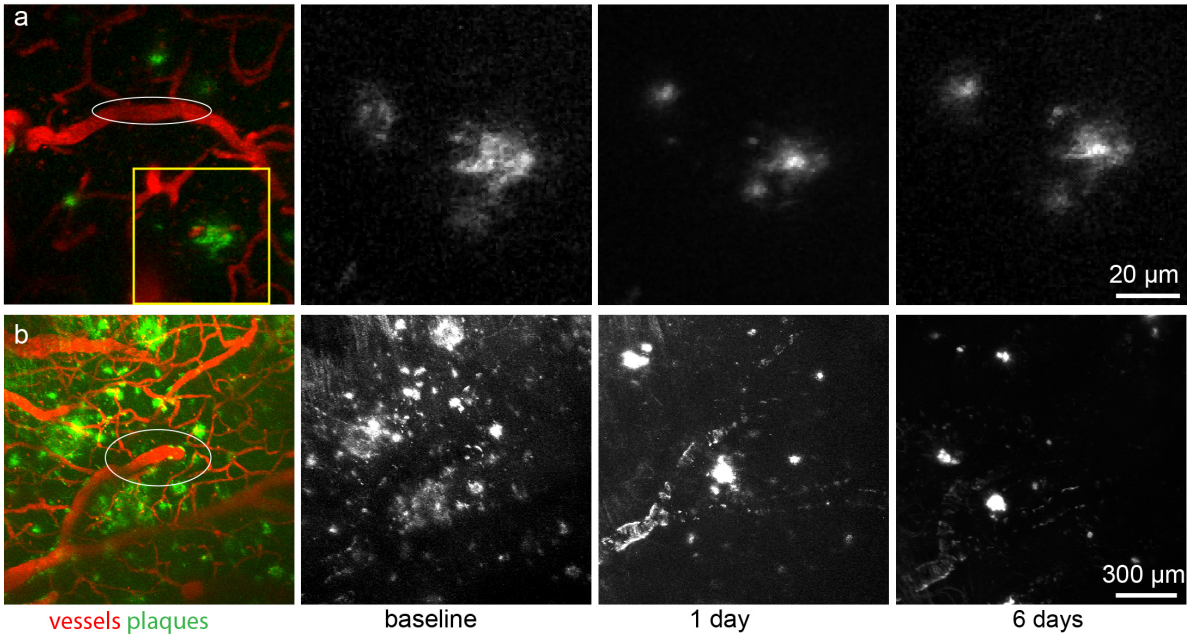
AHA 09POST2250177

NIH 1F32AG031620-01A2

Title: Microvessel occlusions alter amyloid-beta plaque morphology in mouse models of Alzheimer’s disease

Authors: ***N. NISHIMURA**, E. D. BANDER, Y. LEE, C. MUOSER, C. B. SCHAFFER; Biomed. Engin., Cornell Univ., Ithaca, NY

Abstract: Vascular health is correlated to the incidence and severity of Alzheimer’s disease (AD). In mouse models of AD (APP/PS1) we tested the hypothesis that occlusions of the microvasculature could alter amyloid-beta (Aβ) accumulation using in vivo time-lapse multiphoton microscopy of amyloid plaques and vasculature. We found that femtosecond laser-induced occlusions in single capillaries or small arterioles beneath the brain surface generated a shift in the type of Aβ deposits observed over the period of one week. For larger pre-existing plaques, occlusions caused an increase in the intensity of Aβ labeling with methoxy-X04 (labels fibrillar Aβ, Fig. a). We then varied the types of microvascular lesions to confirm that these results were not artifacts of our laser-induced lesion model. We used photothrombosis with intravenously-injected rose bengal to occlude several arterioles and capillaries in a region (Fig. b). This larger injury demonstrated a similar increase in labeling intensity in pre-existing plaques after injuries. Some plaques also disappeared after the lesion. This suggests that “diffuse” plaques can turn into to dense plaques, potentially explaining the observation of both types of plaque in humans and animals. These results suggest that microvascular lesions can alter the deposition and clearance of Aβ and confirm that Aβ plaques are not static, but can change in response to vascular lesions.



Disclosures: **N. Nishimura:** 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; L'Oreal Fellowship for Women in Science, American Heart Association 09POST22250177, NIH F32AG031620. **E.D. Bander:** None. **Y. Lee:** None. **C. Muoser:** None. **C.B. Schaffer:** 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 Grant AG049952.

Poster

038. Immune Responses in Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 38.06/G25

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR

CCNA

CFI

OGS

Title: Investigating the effects of hypertension on a transgenic model of Alzheimer disease

Authors: *A. LEVIT[1], V. HACHINSKI[2], S. WHITEHEAD[1];
[1]Dept. of Anat. & Cell Biol., Univ. of Western Ontario, London, ON, Canada; [2]Clin. Neurolog. Sci., London Hlth. Sci. Ctr., London, ON, Canada

Abstract: Characterizing the role of hypertension in neurodegeneration could have important clinical implications and may be crucial for the study of Alzheimer disease (AD) etiology. Neurovascular pathology, which can result from systemic vascular disease, disrupts CNS proteostasis and can lead to AD. Inflammation, BBB dysfunction, and neurovascular uncoupling have all been proposed as mechanisms by which neurovascular pathology can disrupt CNS glia and neurons, but it remains to be resolved whether hypertension can cause these pathogenic processes. We hypothesize that hypertension can increase inflammation, astrogliosis, and BBB breakdown in a transgenic rat model of Alzheimer disease, and initiate cognitive impairment. This transgenic strain of Fischer 344 rats (TgF344) overexpresses mutant amyloid precursor protein with Swedish and Indiana mutations but does not develop overt pathology; this presents a model that can be used to test whether hypertension can induce early stages of AD-related changes. The TgF344 rats received 8 weeks of continuous angiotensin II (Ang-II) infusion by subcutaneous osmotic minipump to induce hypertension. Control groups for strain and Ang-II were also studied (n=13-15). Elevated blood pressure impaired both wildtype and transgenic rats in delayed match-sample testing in the Morris Water Maze, with a trend of greater impairment in the transgenic strain. Brain tissue cross-sections were analyzed for activation of microglia, astrogliosis, and vascular associated pathology. Tissue analysis indicates that the transgenic strain has increased activation of microglia in white matter which is further exacerbated by hypertension. Spontaneous neuropathology has not been previously reported in the transgenic strain, but increased microglia activation suggests the presence of abnormal white matter inflammation. White matter pathology may account for the greater cognitive effect that hypertension had on the transgenic group. These findings prompt further investigation of the pathological interaction between elevated blood pressure and overexpression of the mutant human amyloid precursor protein.

Disclosures: A. Levit: None. **V. Hachinski:** None. **S. Whitehead:** None.

Poster

038. Immune Responses in Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 38.07/G26

Topic: C.02. Alzheimer's Disease and Other Dementias

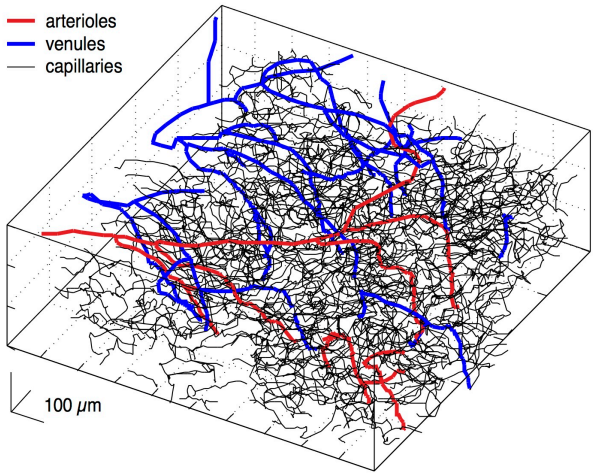
Support: ERC Brain Micro Flow Grant GA 615102

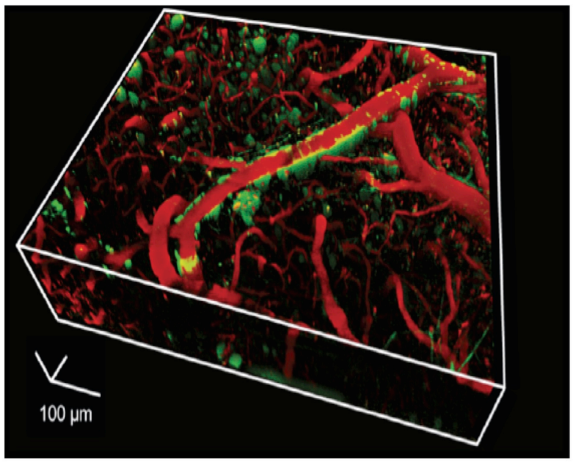
NIH Grant AG049952

Title: Comparison of brain vasculature network characteristics between wild type and Alzheimer's disease mice using topological metrics

Authors: *M. HAFT JAVAHERIAN[1], V. MUSE[1], J. C. CRUZ HERNÁNDEZ[1], C. KERSBERGEN[1], I. IVASYK[1], Y. KANG[1], G. OTTE[1], S. LORTHOIS[2], C. B. SCHAFFER[1], N. NISHIMURA[1];
[1]Biomed. Engin., Cornell Univ., Ithaca, NY; [2]Inst. de Mécanique des Fluides de Toulouse, Toulouse, France

Abstract: There is a strong clinical correlation between Alzheimer's disease (AD) and microvascular disorders. In mouse models of AD, our lab has found blood flow dysfunction in brain capillaries, suggesting the need to study the function of vascular networks at the capillary level. However, the ability to deliver blood flow continuously to all neurons also depends on connections between vessels, requiring that we also characterize the topology of brain vascular networks. Here, we use graph theory and topological metrics to characterize the connectivity of brain capillary networks in AD and control mice.
We imaged cortical vascular networks using *in vivo* two-photon excited fluorescence microscopy in APP/PS1 (AD) and littermate, wild type (WT) mice (3 mice per group; ~1,100 vessels per mouse; Fig. a). Then, we represented the vascular network using graphs in which individual vessels are defined by the path they take through the tissue and their connections to other vessels (Fig. b).
Two metrics suggested interesting differences between WT and AD mice. The average shortest path length is the mean of the smallest number of vessel segments that joins all pairs of vessel junctions. In AD animals this metric was 10.5 ± 0.6 vessels (mean ± standard deviation), which was just 8% lower ($p = 0.07$, t-Test) than in WT animals (11.4 ± 0.7 vessels). The average clustering measures the tendency of vessels in the network to group together; higher numbers imply that vessels and their neighbors have more connections to each other. In AD animals this metric was 37% ($p=0.09$) lower than WT animals. This metric is related to the redundancy of networks connections, which enables the vascular system to maintain blood supply even with occluded vessels, suggesting that capillary networks in AD mice are less connected and redundant than in WT animals.
In order to study how the brain blood flow is affected during the progression of AD, we developed a novel formalism to compare and describe the differences in the brain capillary vascular networks.





Disclosures: **M. Haft Javaherian:** None. **V. Muse:** None. **J.C. Cruz Hernández:** None. **C. Kersbergen:** None. **I. Ivasyk:** None. **Y. Kang:** None. **G. Otte:** None. **S. Lorthois:** None. **C.B. Schaffer:** None. **N. Nishimura:** None.

Poster

038. Immune Responses in Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 38.08/G27

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Genetic evidence for the substantial contribution of the trail/trail-receptor system to amyloid-beta neurotoxicity in the mouse

Authors: G. DI BENEDETTO[1], L. LEMPEREUR[2], F. SERAPIDE[1], O. VALERIO[1], H. WALCZAK[3], *R. BERNARDINI[1,4], G. CANTARELLA[1,4];
[1]Univ. Catania Med. Sch., Catania, Italy; [2]Eli-Lilly Lab., Catania, Italy; [3]Dept of Cancer Biology, CRUK-UCL Cancer Ctr., UCL Cancer Inst., London, United Kingdom; [4]Clin. Toxicology Unit, Policlinico, Univ. of Catania, Catania, Italy

Abstract: We have shown that TRAIL, a proapoptotic cytokine belonging to the TNF superfamily, is a potent inducer of neuronal death in animal models of Alzheimer's disease (AD). TRAIL effects appear mediated by its death receptor, mouse TRAIL-receptor (TRAIL-R), also known as mouse death receptor 5 (DR5). In fact, immunoneutralization of TRAIL results in restored cognitive behavior, as well as in silencing of an array of inflammatory molecules in the AD 3x transgenic mouse brain. To further dissect the role of the TRAIL system in the AD

neuroinflammatory setting, we employed Trail-r-deficient mice. In initial in-vitro experiments, we analysed the impact of treatment with amyloid-beta (AB) as compared to TRAIL on survival of dispersed Trail-r-deficient embryonal hippocampal cells. Subsequently, we evaluated the expression of TRAIL, TRAIL-R and markers for microglia and astrocytes in hippocampi of animals following stereotactical treatment with fragment 1-42 of AB. Hippocampal cells from Trail-r-deficient mice showed significant resistance to death induction by both AB 1-42 or TRAIL as compared to cells Trail-r-proficient mice. Immunofluorescence experiments performed on brains indicated that activation of microglia (Iba-1) and astrocytes (GFAP) was blunted following stereotactical treatment of Trail-r-deficient mice with AB 1-42. Consistently, TRAIL immunoreactivity was absent in the same animals. Moreover, western blot analysis of hippocampal homogenates from Trail-r-deficient mice treated with AB, showed decreased expression of IL-1, as well as of the phosphorylated form of tau protein, along with reduced activation of caspase-3. Together, these results show that the lack of TRAIL-R is associated with a dramatic reduction of detrimental effects of AB, providing genetic evidence that TRAIL released during neuroinflammatory processes plays a major role in AB-induced neurotoxicity in the mouse. On the basis of these results the TRAIL system may be envisioned as a potential candidate for therapeutic intervention in AD.

Disclosures: **G. Di Benedetto:** None. **L. Lempereur:** None. **F. Serapide:** None. **O. Valerio:** None. **H. Walczak:** None. **R. Bernardini:** None. **G. Cantarella:** None.

Poster

038. Immune Responses in Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 38.09/G28

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: JSPS KAKENHI Grant 16K08328

Title: RXR agonist with Am80 improved memory deficits in a mouse model of Alzheimer's disease

Authors: *K. KAWAHARA[1], H. TAKASE[1], Y. YAMADA[1], D. YAMADA[1], T. MAEDA[1], H. NAKAYAMA[2], H. FUKASAWA[3], K. SHUDO[4];
[1]Niigata Univ. of Pharm. and Applied Life Sci., Niigata, Japan; [2]Kumamoto Univ., Kumamoto, Japan; [3]Tokyo Med. and Dent. Univ., Tokyo, Japan; [4]Kemphys Ltd, Tokyo, Japan

Abstract: Alzheimer’s disease (AD) is a neurodegenerative process involving amyloid-β (Aβ) peptide deposition and progressive memory loss. Bexarotene, a retinoid X receptor (RXR)

agonist, has been proposed as a promising therapy for AD, resolving both the amyloid pathology and memory loss. Despite the first compelling report (Cramer et al., Science 335:1503-1506, 2012), however, multiple failures have been documented (Landreth et al., Science 340:924-g, 2013), raising concern about whether RXR agonist could in fact become a novel disease-modifying strategy for AD. To help clarify this, we first investigated the effect of specific RXR pan agonist HX630 in APP23 transgenic mice. Oral administration of HX630 to the 8.5-month-old APP23 mice (5 mg/kg/day, for 17 days) did not achieve any significant memory improvement, or brain Aβ reduction. In contrast, oral co-administration of HX630 (5 mg/kg) with retinoic acid receptor (RAR)α,β agonist Am80 (tamibarotene, 0.5 mg/kg) for 17 days significantly improved memory deficits (Morris water maze) in APP23 mice and reduced the level of insoluble Aβ peptide in the brains. Am80 alone produced no effect. These results thus indicate that effective memory improvement via reduction of insoluble Aβ peptide in 8.5-month-old APP23 mice requires co-activation of RARα,β and RXRs. It is suggested by our group that higher dose of bexarotene binds to RXR in RAR-RXR heterodimer and enhances RAR signaling. The effect of bexarotene by combinational administration with Am80 in APP23 mice, is under investigation.

Disclosures: **K. Kawahara:** None. **H. Takase:** None. **Y. Yamada:** None. **D. Yamada:** None. **T. Maeda:** None. **H. Nakayama:** None. **H. Fukasawa:** None. **K. Shudo:** None.

Poster

038. Immune Responses in Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 38.10/G29

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Yonsei University Future-leading Research Initiative (Yonsei Challenge) of 2015 (2015-22-0137)

Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (2015R1C1A1A02036851)

Title: Placenta-derived mesenchymal stem cells facilitate neural and cognitive recovery in dementia rat model

Authors: ***J. CHO**[1], J. LEE[1,2], D. JEONG[1], H. KIM[3], W. CHANG[1], J. MOON[3,4], J. CHANG[1,2]; [1]Dept. of Neurosurg., Yonsei Univ. Col. of Med., Seoul, Korea, Republic of; [2]Brain Korea 21 PLUS Project for Med. Sci. and Brain Res. Institute, Yonsei Univ. Col. of Med., Seoul, Korea,

Republic of; [3]Gen. Res. Institute, Gangnam CHA Gen. Hosp., Seoul, Korea, Republic of; [4]Dept. of Bioengineering, Col. of Life Science, CHA Univ., Seoul, Korea, Republic of

Abstract: Introduction: Dementia is a term that encompasses various types of neurodegenerative diseases of the brain that cause a gradual decline in mental abilities. Loss of cholinergic neurons in the brain cholinergic system including the hippocampus is a hallmark of many dementia cases. In this study, we report the therapeutic effects of administration of human placenta-derived mesenchymal stem cells (pMSCs) in dementia model Sprague-Dawley (SD) rats using two different cell injection methods: intracerebroventricular (ICV) and intravenous (IV) injections.
Methods: Dementia modeling was carried out by intraventricular injection of 192 IgG saporin, which causes lesion of cholinergic neurons. Fifty male SD rats were divided into four groups: normal (n=9), lesion (n=9), ICV (n=12) and IV (n=12). All rats were then subject to Morris water maze test and subsequent immunostaining analyses using markers for human cytoplasm, acetylcholinesterase (AChE), choline acetyltransferase (ChAT) and microglial cells at the hippocampus.
Results: Lesioned rats showed poor performance in the Morris water maze test compared to the normal rats. Both ICV and IV pMSC administration allowed significant cognitive recovery compared to the lesioned rats. AChE was also significantly recovered back to normal levels at the hippocampus in rats injected with pMSCs post-lesion. ChAT did not co-localize with pMSCs, showing that pMSCs did not directly differentiate into cholinergic cells. Stem cell count showed a significantly greater number of pMSCs at the hippocampal dentate gyrus in IV group rats compared to ICV group rats. Number of microglial cells increased in lesioned rats, and was significantly reduced back to normal levels after pMSC injection.
Discussion: Our results demonstrate that injection of pMSCs facilitates recovery of cholinergic neuronal population and function, as well as cognitive behavior. The mechanism through which such recovery happens does not seem to be direct differentiation of injected pMSCs into cholinergic neurons, but rather seems to be through paracrine effects that resemble microglial function. Further research will be necessary for elucidation of the exact mechanisms involved and establishment of optimal parameters for successful cell homing.
Acknowledgements: This study was supported by the grant from the Yonsei University Future-leading Research Initiative (Yonsei Challenge) of 2015 (2015-22-0137) and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (2015R1C1A1A02036851).

Disclosures: **J. Cho:** None. **J. Lee:** None. **D. Jeong:** None. **H. Kim:** None. **W. Chang:** None. **J. Moon:** None. **J. Chang:** None.

Poster

038. Immune Responses in Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 38.11/G30

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01AG043788

NIH R01GM076063

NIH P30AG010129

BrightFocus Foundation

Title: Microglial kca3.1 channels as a potential therapeutic target for alzheimer's disease

Authors: *I. MAEZAWA[1], H. M. NGUYEN[2], J. DI LUCENTE[3], V. SINGH[2], L. SINGH[2], M. CHAVEZ[3], H. WULFF[2], L.-W. JIN[1];
[1]M.I.N.D. Inst, UC Davis, Sacramento, CA; [2]Pharmacol., [3]Pathology, UC Davis, Davis, CA

Abstract: Microglia play a pivotal role in the initiation and progression of Alzheimer's disease (AD) by either clearing amyloid-β (Aβ) deposits or releasing cytotoxic substances in response to activation by various stimuli including Aβ aggregates such as oligomer (AβO). We here propose microglial KCa3.1 channels as a novel pharmacological target for curbing the harmful effects of Aβ-activated microglia. In the forebrain, KCa3.1 is predominantly expressed in microglia, where its activation by cytoplasmic Ca^{2+} provides the driving force for further Ca^{2+} entry, thus modulating Ca^{2+} signaling and microglial activation. KCa3.1 has been shown to be involved in oxidative burst, nitric oxide (NO) production, and microglia-mediated neuronal killing. We previously reported that AβO-induced microglial activation and microglial neurotoxicity *in vitro* required KCa3.1 activity and could be inhibited by the specific KCa3.1 blocker TRAM-34 (*J Biol Chem* 286:3693, 2011). To further investigate the potential of KCa3.1 inhibition for AD therapy, we generated the following lines of evidence: (1) A seven-day course of intraperitoneal TRAM-34 (40mg/kg/day) inhibited microglial activation induced by intrahippocampal injection of AβO in rats. (2) A three-week course of TRAM-34 administration to mice with induced Aβ accumulation improved their performance in a hippocampus-dependent novel objection recognition task to the control level. (3) TRAM-34 prevented AβO-induced reduction of hippocampal long-term potentiation (LTP), an effect related to the ability of TRAM-34 to reduce microglial production of NO and IL-1β. (4) TRAM-34 did not affect the ability of microglia to phagocytose AβO. (5) Microglial KCa3.1 expression was upregulated in the brains of 5xFAD mice and patients with AD. For future clinical development, we resynthesized senicapoc, which is orally available, structurally similar to TRAM-34, and was shown to be safe and well tolerated

in Phase-2 and Phase-3 clinical trials for sickle cell anemia. We found that senicapoc had excellent brain permeability with a C_{brain}/C_{plasma} value about 6 in mice and rats. Senicapoc inhibited AβO-induced microglial activation and microglial neurotoxicity similar to TRAM-34, and mitigated microglial activation *in vivo* in 5xFAD mice when it was administered by medicated diet for six months. Senicapoc-treated 5xFAD mice also showed hippocampal LTP indistinguishable from that of wild-type mice. Taken together, these observations support that KCa3.1 is a potential therapeutic target for AD.

Disclosures: I. Maezawa: None. **H.M. Nguyen:** None. **J. Di Lucente:** None. **V. Singh:** None. **L. Singh:** None. **M. Chavez:** None. **H. Wulff:** None. **L. Jin:** None.

Poster

038. Immune Responses in Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 38.12/G31

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Cure Alzheimer's Fund

DUKE ENERGY SPECIAL INITIATIVES FUND

Title: A 3D Alzheimer’s disease brain model recapitulates microglial activation, recruitment and neuronal loss

Authors: *J. PARK[1,2], D. KIM[3], R. E. TANZI[3], H. CHO[1,2];
[1]Mechanical Engin. and Engin. Sci., UNC Charlotte, Charlotte, NC; [2]Ctr. for Biomed. Engin. and Sci. (CBES), Charlotte, NC; [3]MassGeneral Inst. for Neurodegenerative Dis., Massachusetts Gen. Hospital, Harvard Med. Sch., Charlestown, MA

Abstract: Microglia are a type of resident brain immune cells, which are neuroprotective by phagocytosing beta-amyloids (Abeta), secreting growth factors and anti-inflammatory cytokines in Alzheimer’s disease (AD), whereas their chronic activation leads to the secretion of proinflammatory mediators that cause neurodegeneration. However, it has been challenging to study the impact of microglia contribution on AD pathogenesis due to lack of relevant human cellular models. Here, we propose a 3D human AD brain model based on a novel microfluidic device that makes it possible to co-culture human AD neuron cells and human microglia in a 3D microenvironment. Human neural stem cells, which were genetically engineered to overexpress FAD-related genes, were grown in a Matrigel-filled central chamber up to 9 weeks and generated an AD pathogenic signature, including excessive accumulation of pathogenic Abeta species.

Immortalized adult human microglia cells were co-cultured for a week in an angular chamber connected to the neuronal central chamber and their interaction were monitored in a real-time and at a single cellular resolution. Using this unique 3D oranotropic culture system, we found that microglial recruitments toward 3D AD cultures are robustly elevated (~10 fold) as compared to control wild-type 3D cultures. The elevated migratory activity was proportional to the levels of pathogenic Abeta species. Notably, AD neuron signaling impacted broadly on microglial immune responses by inducing more chemokines: CCL2 (2.1-fold), CCL5 (26-fold), CXCL10 (2.6-fold), CXCL12 (1.2-fold) compared to normal neurons, and leukocyte growth factors: GM-CSF, G-CSF, which were not induced by normal neurons. Furthermore, we observed increases in neuronal loss in 3D AD cells with microglial cells as compared to the control cells or to AD cells without microglial cells. Finally, we also found increases in cytokine levels including IL-1beta (2.1-fold increase) and caspase-3 activation on AD neurons only in the case of co-culturing with 9-week differentiated AD neurons. Taking together, these clearly demonstrate that our AD brain model is a valid AD cellular model particularly in recapitulating microglia-neuron interaction.

Disclosures: **J. Park:** None. **D. Kim:** None. **R.E. Tanzi:** None. **H. Cho:** None.

Poster

038. Immune Responses in Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 38.13/G32

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG049952

DFG German Research Foundation

Title: Reducing the incidence of stalled blood flow in brain capillaries leads to improved cognitive function in a mouse model of Alzheimers disease

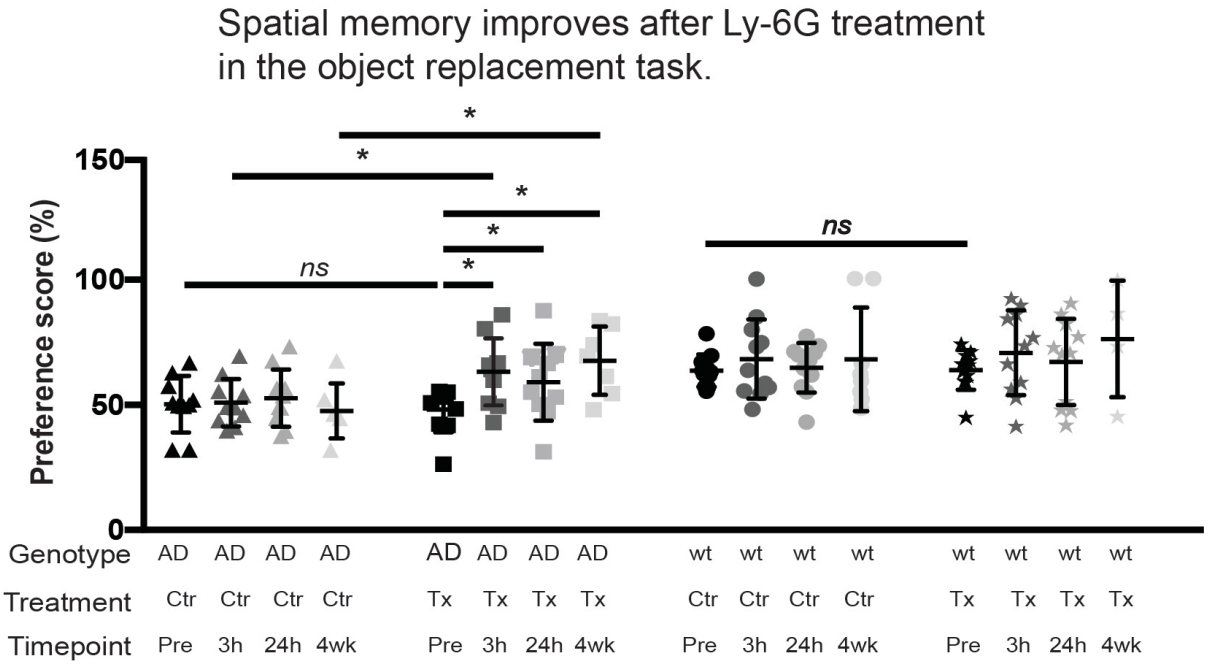
Authors: *O. BRACKO, J. C. CRUZ HERNÁNDEZ, L. VINARCSIK, N. NISHIMURA, C. B. SCHAFFER;
Biomed. Engin., Cornell Univ., Ithaca, NY

Abstract: Alzheimer’s disease (AD) is the leading cause of dementia. AD is accompanied by a ~30% reduction in cerebral blood flow in both patients and mouse models, which likely contributes to the cognitive decline and pathology. No physiological explanation for the blood flow deficit has emerged.
We have shown that there is a five-fold elevation of the number of stalled capillaries with no

blood flow (to 1.6%) in a mouse model of AD (APP/PS1) as compared to age-matched controls. The majority of these capillary stalls were caused by neutrophils adhered to the endothelium. Administration of antibodies that reduce neutrophil adhesion (anti-Ly6G) led to a ~60% reduction in the number of stalled capillaries and a ~40% increase in blood flow in penetrating arterioles within minutes, while isotype control antibodies had no impact.

To determine the cognitive impact of improving blood flow, we performed behavioral assays that assess cognitive and motor function after acute (3h and 24h after one dose) and chronic (after 4 weeks of injection) treatment with anti-Ly6G or isotype control antibodies (IP, 2mg/kg mouse weight) in ~11-month old AD and wildtype mice (n = 10 per group). To measure short-term memory we used object replacement (OR), Y-maze, and novel object recognition tests. In the OR task, we found that after a single treatment memory function was significantly improved in AD mice to near wildtype levels (see Fig; 2-way ANOVA and Wilcoxon post-hoc test; 3h AD_{Tx} vs. 3h AD_{Ctr} p= 0.02). These results were even more pronounced after chronic treatment for 4 weeks (see Fig; 4 weeks AD_{Tx} vs. 4 weeks AD_{Ctr} p= 0.01). We found similar improvement in the Y-maze and novel object tasks. In contrast, motor function/coordination deficits in AD mice were not improved by anti-Ly6G therapy (as assessed by open field and balance beam walk).

In conclusion, treatment with anti-Ly-6G antibodies significantly improved impaired cognitive function in AD mice, thus strengthening the hypothesis that stalled blood flow in capillaries contributes to cognitive decline in AD.



Disclosures: O. Bracko: None. **J.C. Cruz Hernández:** None. **L. Vinarcsik:** None. **N. Nishimura:** None. **C.B. Schaffer:** None.

Poster

038. Immune Responses in Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 38.14/G33

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Are parvalbumin interneurons selectively targeted by ineffective autophagy and mitophagy in aging brain?

Authors: *Q. TANG, B. KRAEMER, M. DIXIT, J. B. RUDEN, P. J. SPITZLER, L. L. DUGAN;
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Abstract: PV neurons are critically involved in information processing, memory and cognition, and dysfunction of these interneurons is observed in both aging and AD. We previously reported that aging produces a decrease in the number of PV neurons throughout hippocampus and frontal cortex, and demonstrated this was mediated by inflammatory induction of the innate immune enzyme, NADPH oxidase. However, the mechanism whereby Nox activation led to PV neuron is unclear. Nox has been shown to inhibit autophagy, and we recently observed ineffective autophagy in aging brain. Here we asked whether age-related PV neuron loss might reflect vulnerability to impaired autophagy. We in fact found a significant loss of PV-immunoreactive fibers in areas where autophagy protein aggregates were observed. These aggregates included p62 and lamp1, and often contained PV. We also noted loss of GAD67 immunoreactivity associated with the same autophagy aggregates. GAD67 is the inducible GAD found in PV interneurons, further support the idea that ineffective autophagy may be damaging PV interneurons. Other inhibitory and excitatory neuronal types appeared to be spared. We then asked why PV interneurons might be selectively targeted. PV interneurons are arguably the most metabolically active neurons in brain, are enriched in mitochondria to support their high metabolic demand, and are vulnerable to hypoxia and other metabolic stressors. Thus, PV interneurons likely recycle mitochondria at an enhanced rate, relying on mitophagy for this. We found that the mitophagy proteins parkin and pink1 colocalized with PV process, and further observed structures which contained partially degraded mitochondria, which were PV immunoreactive on the exterior. Based on our published data and the above results, we propose that inflammatory stress and Nox induction works in concert with the high metabolic load (metabolic stress) that PV interneurons experience, to impair mitophagic clearance of damaged mitochondria. Ineffective mitophagy then further impairs metabolism in these neurons, making them more susceptible to local autophagic injury, possibly from their own mitophagic cargo. This could provide a link between inflammation, impaired autophagy-mitophagy, and vulnerability of PV neurons to aging.

Disclosures: **Q. Tang:** None. **B. Kraemer:** None. **M. Dixit:** None. **J.B. Ruden:** None. **P.J. Spitzler:** None. **L.L. Dugan:** None.

Poster

038. Immune Responses in Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 38.15/G34

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG049952

NSF Fellowship

GRF Fellowship

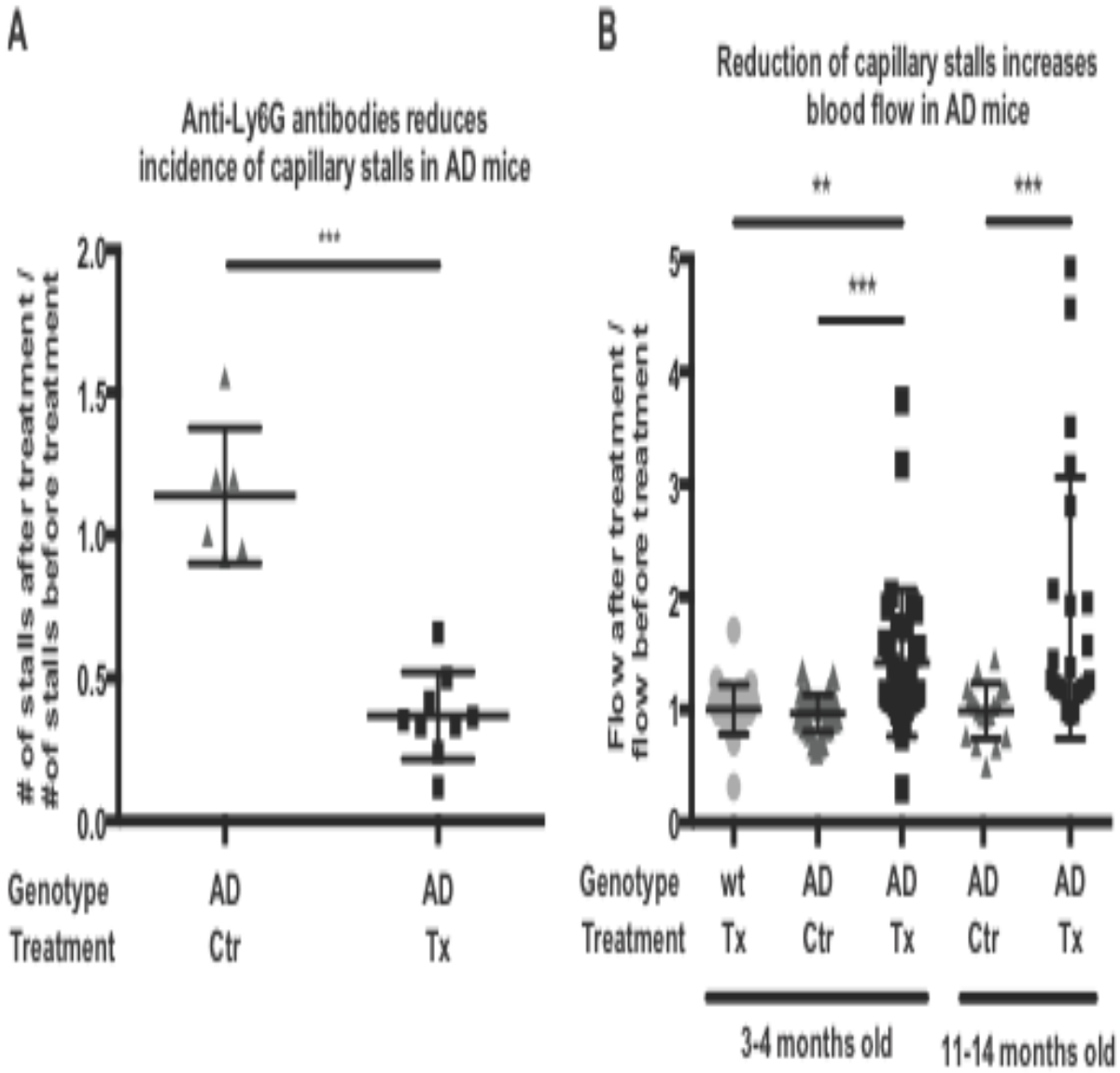
Title: Stalled blood flow in brain capillaries is responsible for reduced cortical perfusion in mouse models of Alzheimer's disease

Authors: ***J. CRUZ**[1], O. BRACKO[1], C. KERSBERGEN[1], V. MUSE[1], I. IVASYK[1], M. HAFT-JAVAHERIAN[1], J. ZHOU[1], J. D. BEVERLY[1], E. SLACK[1], G. OTTE[1], T. P. SANTISAKULTARM[1], C. IADECOLA[2], N. NISHIMURA[1], C. B. SCHAFFER[1]; [1]Cornell Univ., Ithaca, NY; [2]Weill Cornell Med. Col., New York, NY

Abstract: A ~30% reduction in cerebral blood flow is observed in patients and animal models of Alzheimer's disease (AD). Although this hypoperfusion likely contributes to cognitive impairment and disease progression, no physiological explanation for this phenomenon has emerged. We observed that leukocytes often plug capillaries in the brains of AD mouse models, effectively stopping blood flow in the smallest brain blood vessels. Here, we tested the hypothesis that these leukocytes plugs are the cause of the overall blood flow reduction. Using *in vivo* two-photon excited fluorescence imaging in transgenic AD mice (APP/PS1), we found that 1.6% of brain capillaries had stalled blood flow compared to 0.3% in age-matched, littermate controls (AD n=28 and wild-type n=12; $p<0.0001$, Mann-Whitney). This capillary stalling occurred early in disease progression, before deposition of amyloid plaques. Using in vivo labeling approaches, we determined that ~60% of the capillary stalls were caused by leukocytes. We found that administration of antibodies against the neutrophil receptor Ly-6G (1A8 clone) reduced the fraction of capillaries with stalled flow by 63%, while isotype control antibodies did not impact the stalls (AD_{Tx} n = 9, AD_{Ctr} n= 6; $p = 0.0004$, Mann-Whitney; **Fig 1A)**. Blood flow in penetrating arterioles increased by 40% after anti-Ly-6G treatment in both 3-4 month old ($p < 0.001$, Kruskal-Wallis test with Dunns multiple comparisons correction) and

11-14 month old AD mice ($p < 0.001$), while isotype antibodies led to no change in flow speed (**Fig 1B**). Anti-Ly-6G treatment had no impact on blood flow speed in 3-4 month old wildtype mice.

Brain hypoperfusion likely contributes to cognitive deficits in AD patients and, by impairing vessel-mediated clearance of amyloid-beta, may accelerate disease development. We have shown that neutrophils plug blood flow in a small fraction of brain capillaries and that blocking this adhesion substantially increases brain blood flow in mouse models of AD. Therapies that interfered with this capillary plugging could complement those aimed at reducing amyloid deposits.



Disclosures: **J. Cruz:** None. **O. Bracko:** None. **C. Kersbergen:** None. **V. Muse:** None. **I. Ivasyk:** None. **M. Haft-Javaherian:** None. **J. Zhou:** None. **J.D. Beverly:** None. **E. Slack:**

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Poster

038. Immune Responses in Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: JPB Foundation

Belfer Neurodegeneration Consortium

NIH RO1 AG047661

New York Stem Cell Foundation-Robertson Award

NIH 1R01EY023173

NIH 1DP1NS087724

Title: Gamma oscillations attenuate amyloid pathology and trigger a distinct microglia response in mouse models of Alzheimer’s disease

Authors: *H. F. IACCARINO, A. C. SINGER, A. J. MARTORELL, A. RUDENKO, F. GAO, T. Z. GILLINGHAM, R. G. CANTER, R. RUEDA, E. N. BROWN, E. S. BOYDEN, L.-H. TSAI;
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Abstract: Gamma oscillations (20-50 Hz), a common local field potential signature in many brain regions, are generated by a resonant circuit between fast-spiking (FS)-parvalbumin (PV)-interneurons and pyramidal cells. Changes in gamma have been observed in several neurological disorders. However, the relationship between gamma oscillations and cellular pathologies of these disorders is unclear. Here, we investigated this relationship using the 5XFAD mouse model of Alzheimer’s disease (AD) and found reduced behaviorally driven gamma activity before the onset of plaque formation or evidence of cognitive decline. Because of the early onset, we aimed to determine if exogenous gamma manipulations could influence the progression of disease pathology. We discovered that optogenetically driving FS-PV-interneurons at gamma frequency (40 Hz) reduced levels of amyloid-β $(Aβ)_{1-40}$ and $Aβ_{1-42}$ isoforms in the hippocampus of 5XFAD mice. Neither driving FS-PV-interneurons at other frequencies, nor driving excitatory neurons,

reduced Aβ levels. Furthermore, driving FS-PV-interneurons at 40 Hz reduced enlarged endosomes and amyloid precursor protein (APP) cleavage intermediates in hippocampus. Gene expression profiling revealed an induction of microglia specific genes associated with morphological transformation of and increased Aβ phagocytosis by microglia. Finally, we showed that 40 Hz activity alleviated tau pathology in the TauP301S mouse model. Overall, our findings uncover a previously unappreciated function of the brain's gamma rhythms in neuroprotection by recruiting both neuronal and glial responses to mitigate AD-associated pathology.

Disclosures: **H.F. Iaccarino:** None. **A.C. Singer:** None. **A.J. Martorell:** None. **A. Rudenko:** None. **F. Gao:** None. **T.Z. Gillingham:** None. **R.G. Canter:** None. **R. Rueda:** None. **E.N. Brown:** None. **E.S. Boyden:** None. **L. Tsai:** None.

Poster

038. Immune Responses in Alzheimer's Disease

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Program#/Poster#: 38.17/G36

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG048232

Title: Diminished mitochondrial activity in myeloid cells in response to $A\beta_{42}$ oligomer stimulation is associated with altered kynurenine pathway activity

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Abstract: Alzheimer's Disease (AD) is a debilitating condition affecting over 5 million Americans that is characterized by mitochondrial dysfunction and neuroinflammation. Some studies suggest that supplementation with nicotinamide adenine dinucleotide (NAD+) may ameliorate AD phenotypes. Interestingly, the kynurenine pathway (KP), which arises as a result of tryptophan metabolism to kynurenine (KYN), is the only *de novo* biosynthetic source of NAD+. Moreover, the KP has been implicated in models of Huntington's and AD, leading to the hypothesis that dysregulation of the KP may disrupt endogenous NAD+ synthesis and contribute to AD-associated mitochondrial dysfunction. We tested whether $A\beta_{42}$ oligomers, which are necessary for development of AD phenotypes and result in a vigorous innate immune response, affect endogenous NAD+ production in myeloid cells. Stimulating primary murine macrophages with $A\beta_{42}$ oligomers (1μM), we measured KP enzyme expression (qRT-PCR), mitochondrial

activity (XTT and seahorse analysis), NAD+/NADH, and ATP in the absence and presence of KYN supplementation. $A\beta_{42}$ stimulation resulted in significant decreases in XTT activity, NAD+, and ATP levels ($p < 0.0001$) after 20 hours and decreases mRNA levels of quinolinate phosphoribosyltransferase (QPRT) ($p < 0.04$), which encodes the enzyme that produces *de novo* NAD+ from the KP. Analysis of SIRT3, a sirtuin responsible for resolving mitochondrial stress, revealed a significant decrease in SIRT3 enzymatic activity ($p<0.001$) within mitochondria corresponding to decreases in *de novo* NAD+. No changes in cell death, as measured by propidium iodide staining, were observed between conditions. KYN supplementation rescued $A\beta_{42}$-mediated loss of XTT activity, NAD+, and ATP levels, suggesting that the KP contributes to *de novo* NAD+ production in the setting of immunogenic $A\beta_{42}$ stimulation. Our findings suggest a novel role for the KP as a potential therapeutic target for generating NAD+ and healthy mitochondrial activity in models of AD.

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Poster

038. Immune Responses in Alzheimer's Disease

Location: Halls B-H

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Program#/Poster#: 38.18/G37

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG043718

RX001450

Title: Role of RAGE in Alzheimer's Disease

Authors: *M. S. KINDY, J. YU, H. ZHU, S. TAHERI;
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Abstract: Alzheimer's disease (AD) is a common neurodegenerative disorder of mostly unknown etiology, with the exception of some genetically inherited cases. AD is an age-related chronic neurodegenerative disease and is the most common form of senile dementia, associated with memory loss and failure of executive function to the point of becoming highly dependent on expensive and intensive care. AD is characterized by the aggregation of amyloid-β (Aβ) peptide into neuritic plaques and hyperphosphorylated tau protein accumulating into neurofibrillary tangles (NFTs). Oxidative stress, microglial activation and inflammatory responses seem to contribute to the pathogenesis. The receptor for advanced glycation endproducts (RAGE) is a

multi-ligand receptor of the immunoglobulin superfamily of cell surface molecules. The formation of advanced glycation end products (AGEs), the first ligand of RAGE identified, requires a complex series of reactions including non-enzymatic glycation and free radical reactions involving superoxide-radicals and hydrogen peroxide. Binding of RAGE ligands results in activation of nuclear factor-kappaB (NF-κB). Previous studies have implicated RAGE in the pathogenesis of AD, but to date, the specific role has not been elucidated. We hypothesize that the detrimental actions of RAGE are triggered upon binding to its ligands, such as AGEs (advanced glycation end products), S100/calgranulin family members, and High Mobility Group Box-1 (HMGB1) proteins. We have determine the role of RAGE in the pathogenesis of AD in animal models of AD; determined the mechanism of action of RAGE engagement and activation in AD; and determined the potential role of RAGE in the pathogenesis of AD in patients and autopsy samples. From these studies, we have a better understanding of the role RAGE plays in AD and to design therapeutic strategies to treat the disease.

Disclosures: **M.S. Kindy:** None. **J. Yu:** None. **H. Zhu:** None. **S. Taheri:** None.

Poster

038. Immune Responses in Alzheimer's Disease

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Program#/Poster#: 38.19/G38

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Midwestern Alzheimer's Advisory Committee

Title: Region- and age-specific effects of apoe4 on expression of trem2 mRNA and microglial phenotype in young adult targeted replacement mice

Authors: ***T. JONES**, J. VALLEJO, B. CHAVIRA, C. DE VERA, M. CASTRO, G. JENTARRA;
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Abstract: Background: The APOE4 genotype is a risk factor for late-onset Alzheimer disease (AD). Altered neuroinflammatory signaling is consistently observed in the AD brain. Communication between neuronal and microglial cells occurs in part through triggering receptor expressed on myeloid cells 2 (TREM2). TREM2 regulates the balance between phagocytic and pro-inflammatory activity in microglia, and loss-of-function of this receptor is consistent with an enhanced inflammatory state, characteristic of AD brain. Mutations in TREM2 confer increased risk for AD, suggesting that this receptor is protective in the CNS. We evaluated the effects of APOE4 expression on microglial functional profile in APOE3 (E3) and APOE4 (E4) targeted

replacement (TR) mice. *We hypothesized that the cortex and hippocampus of E4 mice would exhibit a pro-inflammatory bias that would increase as the mice age consistent with progression of inflammation in AD brain.*
Methods: E4 and E3 mice (4 and 6 months old) were transcardially perfused with sterile PBS. The brain was removed and the cortex and hippocampus dissected and snap frozen for qRT-PCR analyses.
Results: At 4 months of age, cortical TREM2 mRNA was decreased in E4 mice compared E3 mice. Arginase 1 (Arg1) mRNA, associated with anti-inflammatory (M2) microglia, was significantly decreased in the cortex of APOE4-TR mice at 4 months, while NOS2 mRNA was not altered. In contrast, hippocampal TREM2 mRNA was increased in E4 mice, while Arg1 and NOS2 were unaltered, indicating regional heterogeneity to the inflammatory changes that may be relevant to AD. Interestingly, by 6 months of age, hippocampal TREM2 mRNA was decreased, while Arg1 and NOS2 mRNAs were increased in E4 mice. With respect to age, cortical TREM2 mRNA significantly decreased over time in both groups of mice, whereas hippocampal TREM2 mRNA significantly decreased in E4 but not E3 mice. Cortical Arg1 expression increased over time in both E3 and E4 mice while in the hippocampus only E4 mice had increased Arg1 mRNA. NOS2 mRNA was decreased in the cortex of both E3 and E4 mice, while in the hippocampus, there was a significant increase specific to E4 expression.
Conclusion: The purpose of this study was to determine whether two known risk alleles, APOE4 and TREM2, may be acting synergistically to enhance risk for developing late-onset AD. We show that the APOE4 genotype modifies inflammatory signaling in a distinct manner in the cortex vs the hippocampus and that the effects of APOE4 on microglial phenotype are more pronounced in the hippocampus as the mice age. The differing inflammatory profiles in these two regions could underlie the distinct temporal progression of symptoms known to occur in AD.

Disclosures: **T. Jones:** None. **J. Vallejo:** None. **B. Chavira:** None. **C. De Vera:** None. **M. Castro:** None. **G. Jentarra:** None.

Poster

038. Immune Responses in Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 38.20/G39

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Dimethyl fumarate modulates pro-inflammatory microglia activation via the nuclear-erythroid factor 2-independent and -dependent pathways

Authors: H. PARAISO[1], P.-C. KUO[2], J.-H. YEN[2], G. A. WEMHOFF[3], R. D. SWEAZEY[3], F.-L. CHANG[4], *I.-C. I. YU[5];
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Abstract: Microglia (MG), the brain resident immune cells, can be activated to produce pro-inflammatory responses towards various stimuli. Classically activated M1 MG may exacerbate neuronal death in ischemic stroke and Alzheimer's disease. Here, we investigated the suppression of the M1 MG using dimethyl fumarate (DMF), a fumaric acid ester approved as a multiple sclerosis therapy. DMF may confer neuroprotective effects via activation of the nuclear-erythroid factor 2 (Nrf2) pathway to regulate oxidative stress induced by M1 MG. We showed that DMF suppresses molecular and functional characteristics of M1 MG induced by lipopolysaccharide (LPS). DMF inhibited the expression of pro-inflammatory cytokines TNF-α, IL-1β, and iNOS and reduced the level of co-stimulatory surface molecules CD86, CD80, and CD40 in primary cultured MG activated by LPS. In vivo, C57BL/6 mice administered DMF for 3 days prior to a systemic LPS challenge decreased frontal cortex mRNA expression of TNF-α, IL-1β, iNOS, and IL-6. To see whether these observations were mediated through Nrf2, we examined the induction of Nrf2 target genes HO-1, NQO1, Gclc, and Gclm. We found that Nrf2 gene targets were upregulated in both cultured MG and the mouse frontal cortex, suggesting that DMF activates Nrf2. Interestingly, in MG cultured from Nrf2 knockout mice, we found that Nrf2 differentially mediated the effects of DMF regarding the expression of co-stimulatory surface molecules. Regulation of CD40 by DMF was Nrf2 independent, while modulation of CD86 and CD80 by DMF was Nrf2 dependent. To address whether DMF impacts neurotoxicity associated with M1 MG, we examined cell viability of hippocampal neurons exposed to conditioned media collected from LPS or LPS/DMF treated MG. We observed that neurons receiving the conditioned media from DMF treated MG had a significantly higher viability when compared to neurons receiving conditioned media without DMF, suggesting a potential neuroprotective effect of DMF. In summary, our findings demonstrate that DMF inhibits neuroinflammation through modulating M1 MG activation by Nrf2-independent and -dependent pathways.

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Poster

039. Memory and Cognition in Alzheimer's Disease and Other Neurodegenerative Disorders

Location: Halls B-H

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Program#/Poster#: 39.01/G40

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA Grant 5R37AG-006265-31

NIA Grant 3R37AG-006265-25S1

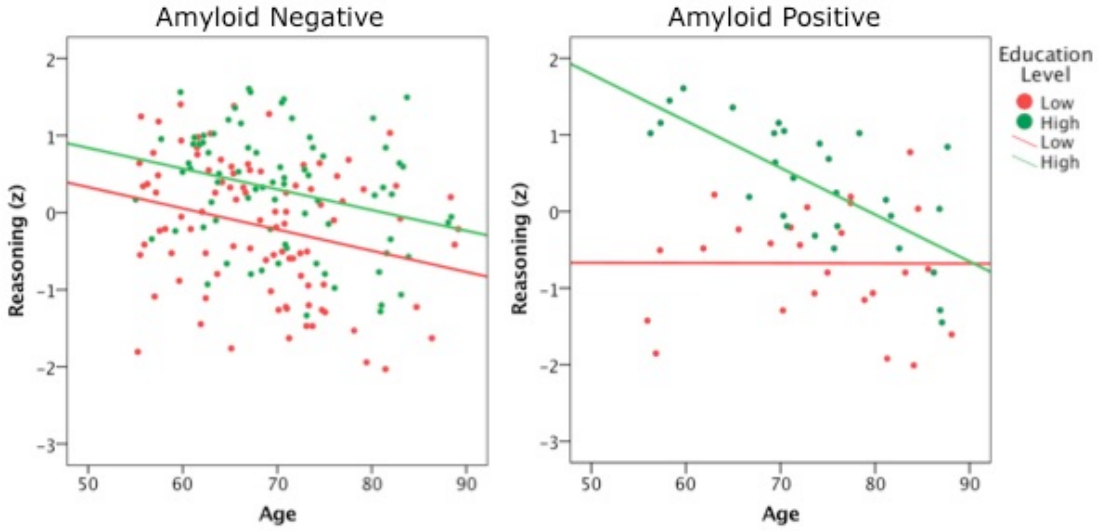
Avid Radiopharmaceuticals

Title: Educational attainment confers protection against amyloid deposition during early but not later old age on higher order cognition

Authors: M. E. FARRELL[1], G. N. BISCHOF[2], *D. C. PARK[1];
[1]Ctr. For Vital Longevity, Univ. of Texas At Dallas, Dallas, TX; [2]Dept. of Nuclear Med., Inst. of Neurosci. & Med. (INM-3) Res. Ctr., Juelich, Germany

Abstract: Background. Both normal aging and amyloid deposition, a biomarker for preclinical Alzheimer's disease, are associated with declines in cognition. Education has long been established as a protective factor against dementia onset, a phenomenon referred to as cognitive reserve. The present study sought to determine whether educational attainment mitigates the impact of amyloid burden on cognitive performance across multiple domains, and whether this relationship was modified by increasing age. Methods. 224 participants (age 55-89) from the Dallas Lifespan Brain Study were included. All had completed extensive cognitive testing and an F-18 Florbetapir PET scan. ANOVAs were conducted of the effect of Age, Amyloid Status (positive or negative) and Education Level (<bachelor's, bachelor's +) on reasoning, spatial working memory, verbal working memory, episodic memory and processing speed, while controlling for sex. Results. The analyses yielded a significant three-way interaction of age, education, and amyloid status for reasoning and spatial working memory, but not verbal working memory, episodic memory or processing speed. The High Education/Amyloid Positive Group experienced a protective effect of high educational attainment for reasoning and spatial working memory, performing similarly to High Education/Amyloid Negative at younger ages (55-69), but that benefit dissipated with increasing age. In contrast, Low education/ Amyloid Positive adults performed at a consistently poor level across age. Conclusion. These findings indicate that a high level of educational attainment confers resilience and protects individuals carrying amyloid from the negative effects of amyloid on reasoning and spatial working memory, though this protective effect is mitigated by increasing age. The benefits of education were differentially beneficial to reasoning and spatial working memory, suggesting that educational attainment is most protective for measures of higher order, abstract processing where experience and world knowledge offers little support for task performance.

Figure 1. Effects of Age, Educational Attainment and Amyloid Status on Reasoning



Disclosures: **M.E. Farrell:** None. **G.N. Bischof:** None. **D.C. Park:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Avid Radiopharmaceuticals.

Poster

039. Memory and Cognition in Alzheimer's Disease and Other Neurodegenerative Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 39.02/G41

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Electrophysiological markersof top-down control in younger and older participants.

Authors: *N. NAPIORKOWSKI[1], H. MÜLLER[1], T. TÖLLNER[1], A. PETERSEN[2], I. WIEGAND[2], K. FINKE[1];
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Abstract: Mild Cognitive Impairment (MCI) and more so, Alzheimer’s disease (AD) leads to reduced visual top-down control. Thus, decreasing efficiency in prioritizing relevant over irrelevant information might be a potential early cognitive marker of AD (Redel et al., 2012). It is less clear, however, whether such a gradual decline in top-down control also occurs in healthy aging. Furthermore, neural correlates, such as, e.g., neurophysiological markers, of individual

top-down control efficiency are not defined. In the current study, two groups of younger (n = 30; mean age = 27) and older participants (n = 33; mean age = 69)were assessed with partial report based on Bundesen's theory of visual attention (TVA; Bundesen, 1990). Report accuracy was used for computationally modelling and quantifying the individual efficiency of top-down control. Furthermore, EEG was assessed simultaneously in order to measure electrophysiological correlates on individual top-down control. In a second session, individual visual processing speed and visual short-term memory storage capacity were assessed based on a TVA-based whole report in order to control for effects of these variables. ERP analyses focused on ERP components that might be related to top-down control (Luck & Hillyard, 1994; Wiegand et al., 2015): the Posterior positive contralateral (Ppc) and the Posterior-Contralateral Negativity (PCN or N2pc). We asked whether top-down control efficiency changes with age and tested whether Ppc and PCN are related to individual top-down control in both age groups. Groups did not differ significantly in top-down control. When participants within both groups were split into efficient and inefficient ones with respect to top-down control, significant differences were found in the amplitude of the Ppc and the PCN in both age groups. These differences remained significant when controlling for visual processing speed and visual short-term storage capacity. Our findings suggest that the efficiency of top-down control is robust and reliably reflected in individual amplitudes of the Ppc and PCN in normally aging individuals. With respect to pathological neurodegeneration, we suggest that top-down control and its neurophysiological correlates might be early neuro-cognitive indicators of AD.

Disclosures: **N. Napiorkowski:** None. **H. Müller:** None. **T. Töllner:** None. **A. Petersen:** None. **I. Wiegand:** None. **K. Finke:** None.

Poster

039. Memory and Cognition in Alzheimer's Disease and Other Neurodegenerative Disorders

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Program#/Poster#: 39.03/G42

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ERC Grant 337822-TRANSMEM

Title: Memory for lifelike events in older adults with memory impairment

Authors: ***C. OEDEKOVEN**[1], J. L. KEIDEL[1], G. RACZEK[2], C. M. BIRD[1];
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Abstract: Memory for events is severely affected early on in the course of Alzheimer's disease (AD). This parallels neuroimaging investigations which show hypometabolism and atrophy in brain areas involved in memory retrieval. In this study we examined the memory retrieval network in older adults at risk of AD using a naturalistic memory task involving memory for short videos. Previous research in young adults has shown that retrieval practice of memories for events stabilizes them and thus improves later recall (Bird, Keidel et al. 2015). These study highlighted posterior cingulate as a key region involved in retrieval-mediated consolidation. This region is one of the areas affected earliest in AD. The aim of our fMRI study was to investigate the cognitive and neural processes underpinning memory for lifelike events in older adults and how they are impaired in the very early stages of AD.
As part of an ongoing study, we scanned a group of 54 older adults (aged 72.6 years, range 43-88, 21 female) recruited from Memory Assessment Service clinics. In the MRI scanner, participants watched and then silently retrieved 8 short video clips. Afterwards they described the videos to the experimenter. Univariate analyses were conducted using SPM8 and whole-brain searchlight representational similarity analyses (RSA) using the CosmoMVPA toolbox.
On average participants recalled 6.7 details for each video, but there was large variability in individuals' performance (range 1.2 - 14.8 details). In a regression analysis of memory retrieval taking into account the amount of details remembered, better performance was correlated with increased activity in bilateral posterior medial frontal lobe and bilateral inferior frontal gyrus (IFG). An RSA identified a network of regions where patterns of activity during encoding correlated with immediate recall of the same video, which included striatal as well as hippocampal regions.
The correlation of bilateral IFG activity with the amount of detail recalled is consistent with the proposal that these regions integrate episodic information with prior semantic knowledge (Binder et al., 2009) and might be an indicator of compensatory activity in accordance with the Posterior-Anterior Shift with Aging (Cabeza and Dennis 2012; Davis et al., 2008). In comparison to the earlier study in young adults, we did not see reinstatement effects in the posterior cingulate. This may reflect breakdown in neuronal function in this region and partially explain the generally low group performance on the memory task.

Disclosures: **C. Oedekoven:** None. **J.L. Keidel:** None. **G. Raczek:** None. **C.M. Bird:** None.

Poster

039. Memory and Cognition in Alzheimer's Disease and Other Neurodegenerative Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 39.04/G43

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: National Science Foundation

Title: Visual episodic memory does not depend on precuneus disease: evidence from posterior cortical atrophy

Authors: *K. WIN[1,2], P. YUSHKEVICH[3], D. WOLK[2], M. GROSSMAN[1,2];
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Abstract: Motivation: Posterior cortical atrophy (PCA) is characterized by a progressive decline in visuoperceptual processing. Predominant sites of atrophy are occipitotemporal and parietal cortices (OTPC), including precuneus, which has been shown to be involved in memory. However, it is unclear whether visual episodic memory (VsEM) deficits in PCA are directly due to precuneus atrophy. The underlying pathology of most PCA cases is Alzheimer's disease (AD), but episodic memory (EM) has rarely been assessed. Here, we comparatively examined VsEM and visuoperceptual function in PCA and AD patients, and related these measures to precuneus atrophy. Because hippocampus, besides precuneus, mediates EM, we also examined the contribution of hippocampus (HC) to the VsEM.
Method: 17 elderly controls (Ctl), 13 PCA and 26 AD were matched in age, sex, and education. PCA and AD were matched in disease severity. We used Rey Complex Figure (RCF) copy to assess visuoperceptual function, RCF recall to assess VsEM, and d-prime to assess recognition memory. All subjects underwent T1 and high-res T2 MRI. A multi atlas algorithm was applied to label HC subfields: Cornu Ammonis (CA1), dentate gyrus (DG), and subiculum.
Results: Compared to Ctl, PCA patients were significantly impaired on RCF copy and RCF recall but had intact d-prime; AD patients were impaired on RCF copy, recall, and d-prime. Compared to PCA, AD patients were more impaired on Rey Copy, and d-prime. Equivalent deficit on RCF recall was observed between PCA and AD. Multiple regression analysis showed that only RCF copy, not d-prime, significantly predicted RCF recall in PCA; in AD, both RCF copy and d-prime predicted RCF recall. Whole brain imaging showed that PCA had a significant atrophy in OTPC, including bilateral precuneus and right HC; AD had a widespread atrophy in OTPC, including bilateral precuneus and HC. Regression analyses showed that in PCA, RCF copy was related to left superior occipital gyri, left superior and inferior parietal gyri, including precuneus and angular gyrus (AG); in AD, to right precuneus and those same areas involved in PCA. RCF recall in PCA was related to left AG; in AD, to right HC, right superior and inferior parietal gyri, including AG and precuneus. Examination of HC subfields showed that right CA1 was differentially atrophied in PCA but this atrophy did not modulate VsEM. In AD, RCF recall was related to bilateral CA1 and left DG.
Conclusion: Despite precuneus atrophy in PCA, impaired VsEM function is more directly related to visuoperceptual impairments. In AD, VsEM is modulated in part by HC and precuneus. We conclude that precuneus disease in PCA does not necessarily compromise VsEM function.

Disclosures: K. Win: None. **P. Yushkevich:** None. **D. Wolk:** None. **M. Grossman:** None.

Poster

039. Memory and Cognition in Alzheimer's Disease and Other Neurodegenerative Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 39.05/G44

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Parsons- Quinn Endowment, University of Notre Dame

NPC Therapeutics LLC

Title: Chronic HDACi therapy to long term neurological disease

Authors: M. ALAM, M. GETZ, B. COMBS, *K. HALDAR;
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Abstract: Crossing the blood-brain barrier and safely harnessing molecular dysregulation through drugs like histone deacetylase inhibitors (HDACi) has been a major challenge in the treatment of neurological disorders. We earlier reported the development of a triple combination formulation (TCF) comprising the pan-HDACi vorinostat, the caging agent 2-hydroxypropyl-b-cyclodextrin (HPBCD), and polyethylene glycol (PEG) to treat Niemann-Pick type C (NPC) disease, a difficult- to- treat the cerebellar disorder. While vorinostat alone showed no benefit in a mouse model, TCF boost HDACi across the blood-brain barrier, to preserve neurites and Purkinje cells, delay symptoms of neurodegeneration, extend mouse lifespan from weaning well into adulthood without toxicity even when used long term. Ongoing mechanistic analyses of TCF action in the brain suggests chronic, low levels of transcriptional dysregulation can be tolerated by reduction of the inflammatory response to rescue function of NPC1 and as well as broad range of cellular targets (deficiencies in some of which also cause neurological disease). Together, the data suggest that the TCF application may provide therapeutic and mechanistic insight into a broad range of neurological diseases caused by both monogenetic and polygenetic defects. Preclinical studies currently in progress suggest an approved optimized formulation will enter Phase Ib trials for the first monogenetic neurological indication (NPC) within 12 months.

Disclosures: **M. Alam:** None. **M. Getz:** None. **B. Combs:** None. **K. Haldar:** None.

Poster

039. Memory and Cognition in Alzheimer's Disease and Other Neurodegenerative Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 39.06/G45

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: HBRP, KTIA_NAP_13-2-2015-0011

Title: Translational validity of the psychomotor vigilance paradigm in an acute pharmacological model of neurocognitive decline in primates

Authors: *I. HERNADI, V. OLAH, A. TRUNK;
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Abstract: Age-associated pathological decline in cognitive function have become a major demographic health threat worldwide. Until now there are no effective pharmacological interventions against neurocognitive disorders, such as Alzheimer's disease (AD). In order to develop a new pre-clinical disease model in non-human primates (NHP) we implemented the so called 'reverse translational' approach where we developed a unified version of the psychomotor vigilance task (PVT) in humans and NHPs. We collected reaction time (RT) data to target stimuli as task performance indices. Fixation times (FT) before target stimuli were distributed randomly from 1 to 10 sec (expectancy effect). Then we investigated the effects of pharmacologically induced cognitive impairment in NHPs. The main endpoints were to detect similarities of target expectancy between healthy humans and NHPs, then we tested the transient adverse effects of pharmacological amnestic agent, scopolamine (15 ug/kg b.w.) on task performance in NHPs. Finally we measured the reversing effects of cholinesterase inhibitor donepezil (100 and 200 ug/kg b.w.) on scopolamine induced impairments of target expectancy in NHPs. Results suggested that both humans and NHPs showed similar task performance with comparable RT distribution (expectancy effect). In NHP subjects the prior scopolamine treatment further increased the RT and abolished the previously observed target expectancy effects, and donepezil pre-treatment significantly reversed impairments of task performance. Based on the present results we conclude that the common primate PVT paradigm has high translational validity and it may be suitable for preclinical testing of novel therapeutic interventions against cognitive impairment.

Disclosures: I. Hernadi: None. **V. Olah:** None. **A. Trunk:** None.

Poster

039. Memory and Cognition in Alzheimer's Disease and Other Neurodegenerative Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 39.07/G46

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG0435043

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NIH Grant NS044266

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Dana Foundation

Wyncote Foundation

Title: Relative frontal and temporal contributions to social knowledge in bvFTD

Authors: *A. HALPIN, R. LANGEY, N. MIN, C. YORK, O. KOFMAN, K. RASCOVSKY, D. IRWIN, C. JESTER, C. MCMILLAN, M. GROSSMAN;
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Abstract: Behavioral variant frontotemporal dementia (bvFTD) is a form of frontotemporal degeneration (FTD) characterized by a progressive deterioration of social comportment and cognition. These social limitations may be due to a loss of social knowledge or faulty implementation of known social rules. Given that fMRI studies have associated social knowledge with right anterior temporal activation in healthy adults, we sought converging evidence for the neural underpinnings of social knowledge in bvFTD with predominant temporal or frontal disease. Social knowledge was assessed using the Social Norms Questionnaire (SNQ), which measures whether an individual can appreciate widely accepted social boundaries. We administered the SNQ to 49 bvFTD patients, 21 Alzheimer's disease patients (AD) and 22 healthy controls (HC). 16 temporal-bvFTD patients were identified based on the co-occurrence of semantic variant primary progressive aphasia (svPPA) and 33 patients were classified as frontal-bvFTD given their predominant frontal disease and associated executive deficits. Groups were matched for age, education, and disease duration. We report SNQ Total Scores (SNQ-T; overall measure of social norm interpretation) as well as SNQ Overadherence Scores (SNQ-O; rigid interpretation of social norms), and SNQ Break Scores (SNQ-B; difficulty identifying socially unacceptable behavior). We found that temporal-bvFTD have impaired SNQ-T scores relative to frontal-bvFTD ($U=91$; $p<0.01$), AD ($U=65$; $p<.01$) and HC ($U=16$; $p<.01$). Temporal-

bvFTD also had impaired SNQ-B scores relative to frontal-bvFTD (U=53; p<0.01), AD (U=50; p<.01) and HC (U=41; p<.01). In addition, temporal-bvFTD patients had impaired SNQ-O scores relative to HC (U=64; p.001). Frontal-bvFTD had impaired SNQ-T and SNQ-O scores relative to HC (U=235; p=.025) and (U=224; p=0.015) respectively. AD also had impaired SNQ-T scores and SNQ-O scores relative to HC (U=111, p=.003) and (U=114; p=.004) respectively. Frontal-bvFTD and AD did not differ from HC on the SNQ-B (all p>0.1). It is unlikely that social limitations are related to disease severity since AD had lower average MMSE scores and fewer social deficits than temporal-bvFTD. Together, we observed more overall social norm impairments in temporal-bvFTD than any other group, and only temporal-bvFTD had impaired break scores. These finding emphasize the role of temporal cortex in supporting social knowledge.

Disclosures: **A. Halpin:** None. **R. Langey:** None. **N. Min:** None. **C. York:** None. **O. Kofman:** None. **K. Rascovsky:** None. **D. Irwin:** None. **C. Jester:** None. **C. McMillan:** None. **M. Grossman:** None.

Poster

039. Memory and Cognition in Alzheimer's Disease and Other Neurodegenerative Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 39.08/G47

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Science, ICT & Future Planning(2015R1C1A1A02037513)

Title: Cumulative effect of systemic chemotherapy on cognitive function

Authors: ***S. JUNG**[1,2], J. MIN[3];
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Abstract: Introduction: Although more than a third of cancer survivors treated with chemotherapy suffer from chemotherapy-induced cognitive impairment, the underlying mechanism has been poorly understood. Moreover, most systemic chemotherapy is performed as a cyclic manner with multiple doses. There have been several studies which suggested that the mechanism of chronological effect of multiple doses of chemotherapy differ from the mechanism of the effect of single-dose chemotherapy. We aimed to demonstrate the cumulative effect on

cognitive function of systemically administered Adriamycin (DXR) in the rat models. Methods: Nine-to-ten-week-old male Sprague-Dawley rats were injected intravenously with a weekly dose of 2.5mg/kg DXR for 6 weeks (total 15mg/kg) or the same volume of saline as control. Rats were assessed with a battery of behavioral tests to assess global motor performance (rotarod test), cognitive function (object recognition test and radial arm maze test) and mood-related responses (tail suspension test and elevated plus maze test). Changes in the protein expression in the prefrontal cortex, the hippocampus and the cerebellar cortex in the DXR-treated and control group were analyzed by Western blotting. Results: The DXR-treated rats displayed persistent deterioration in the attention since day 7 and in the spatial working memory since day 1 following DXR injection as compared to controls. They showed depressive behavior since day 7 following DXR injection without anxious behavior. β-amyloid expression was increased in the prefrontal cortex and in the hippocampus since day 7. Conclusions: Taken together, these results indicate that cyclic chemotherapy can impair attention and working memory and may increase the risk of depression which is accompanied by alteration of β-amyloid expression in the brain.

Disclosures: **S. Jung:** None. **J. Min:** None.

Poster

039. Memory and Cognition in Alzheimer's Disease and Other Neurodegenerative Disorders

Location: Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The Wyncote Foundation

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NIH Grant AG017586

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NIH Grant AG043503

Title: Reversal learning deficits in behavioral variant frontotemporal degeneration

Authors: *K. TERNES[1], J. KABLE[2], J. MCGUIRE[3], K. RASCOVSKY[1], C. MCMILLAN[1], M. GROSSMAN[1];
[1]Univ. of Pennsylvania Dept. of Neurol., Philadelphia, PA; [2]Univ. of Pennsylvania Dept. of Psychology, Philadelphia, PA; [3]Boston Univ. Dept. of Psychological & Brain Sci., Boston, MA

Abstract: Behavioral variant frontotemporal degeneration (bvFTD) is clinically characterized by deficits in executive function and social behavior. One source of executive dysfunction involves a lack of cognitive flexibility, however the neuroanatomic source of this deficit is poorly understood. Reversal learning tasks evaluate simple strategy learning and whether an individual can flexibly adapt these strategies. We examined the neural basis of reversal learning in bvFTD (N=25), mild Alzheimer's disease (AD; N=12), and healthy controls (HC; N=17) who also had structural MRI. All patients and controls were matched for demographic factors including age, education and gender (all $p>0.1$). All patients and controls completed a computerized task in which subjects chose between two multicolored fractal stimuli containing different shape and color features. We used a probabilistic reinforcement paradigm to reinforce selection of one of the two fractals in the first "learning" block and implicitly reinforced the novel fractal in the second "reversal" block. We categorized subjects as "learners" if they endorsed the target stimulus in >50% of first block trials. Of the "learners", we categorized "reversers" as those that endorsed the novel stimulus in >50% of second block trials. Chi-square analyses evaluated the proportion of "learners" and "reversers" in patient groups. We observed an equal distribution of learners between each group: 76% HC, 76% bvFTD, 58% AD ($X2=1.51$; $p=0.68$). However, within-group analysis showed the rate of reversers was less in bvFTD (42%; $X2=5.23$, $p=0.02$) unlike AD (57%; $X2=0.003$; $p=0.96$) and HC (77%; $X2=0.001$; $p=0.98$) who were equally likely to reverse. The rate of reversal in bvFTD also differed from HC and AD ($X2=3.80$; $p=0.05$). Neuroimaging analyses using voxel-based morphometry showed gray matter atrophy in orbitofrontal cortex (BA 10, 47) and dorsolateral prefrontal cortex (BA 9) in bvFTD non-reversers relative to HC ($q<0.005$ TFCE FWE-corrected). Together, the behavioral and imaging findings suggest disease in DLPFC and OFC contribute to selective reversal learning deficits in bvFTD by limiting cognitive flexibility and decision-making especially when contingencies and rewards change.

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Poster

039. Memory and Cognition in Alzheimer's Disease and Other Neurodegenerative Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 39.10/G49

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01-MH56031

Title: Cerebral metabolic correlates of disorientation in alzheimer's disease.

Authors: *G. WEISSBERGER[1], R. MELROSE[2], T. NARVAEZ[2], D. HARWOOD[2], M. MANDELKERN[2], D. SULTZER[1];
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Abstract: Orientation to time, date, and place is commonly used clinically to stage and monitor progression of Alzheimer's disease (AD) but few studies have examined its neural substrates. The present study aimed to compare the neural substrates underlying performance on DRS and MMSE orientation items to the remaining items of the DRS Memory subtest and MMSE in patients with mild to moderate AD in order to identify neural structures unique to orientation. 90 patients (*M* age = 78.4, *SD* = 7.9; 80% male; *M* education = 13.9, *SD* = 3.5) with mild to moderate AD were recruited from the Los Angeles (LA) community and the Greater LA Veteran's Administration Healthcare System. Participants underwent Fluorodeoxyglucose Positron Emission Tomography (FDG-PET) and a comprehensive neuropsychological battery in which they were administered the DRS (*N*=87; *M* = 103.5, *SD* = 20.6) and MMSE (*M* = 19.4, *SD* = 5.6). Scores on DRS orientation, MMSE orientation, remaining items from DRS Memory (DRS Memory "other" items), and remaining items from the MMSE (MMSE "other" items) were separately summed and correlated with cerebral glucose metabolism (CGM). PET analyses were conducted in SPM8. Results maps were thresholded at the voxel level at $p<.001$. Findings were considered significant at the cluster level $p<.05$, corrected using the Family Wise Error procedure (FWE). The SPM analysis revealed multiple areas of significant positive association between cortical metabolism and total orientation score of the DRS. Specifically, hypometabolism related to worse performance on DRS orientation in 4 clusters spanning many regions associated with AD pathology including the posterior and middle cingulate gyri, bilateral inferior temporal lobe, left middle temporal lobe, and left middle occipital lobe ($ps\leq.05$). In contrast, no significant clusters arose in correlating DRS Memory "other" items to CGM. With regards to the MMSE, a positive association between CGM and orientation items arose across 3 significant clusters spanning the bilateral inferior temporal lobe and middle cingulate gyrus ($ps\leq.02$). MMSE "other" items were positively associated with CGM in the left middle and inferior temporal lobe and left inferior parietal lobe ($ps\leq.02$). Findings suggest that disorientation in AD is related to cerebral hypometabolism in a widespread network of structures commonly associated with AD pathology. Although orientation is commonly considered a proxy for memory, findings reveal distinct neurobiological correlates between orientation and measures of memory on the DRS, as well as other cognitive items on the MMSE.

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Poster

039. Memory and Cognition in Alzheimer's Disease and Other Neurodegenerative Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 39.11/G50

Topic: C.02. Alzheimer's Disease and Other Dementias

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NIH Grant NS066654

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Title: Brain activity, functional connectivity and auditory feedback processing in primary progressive aphasia variants

Authors: *K. RANASINGHE[1], L. B. HINKLEY[2], H. KOTHARE[2], A. J. BEAGLE[1], D. MIZUIRI[2], S. HONMA[2], I. HUBBARD[1], A. E. WELCH[1], M. MEYER[1], Z. MILLER[1], J. F. HOUDE[3], M. GORNO-TEMPINI[1], K. A. VOSSEL[1,4], S. S. NAGARAJAN[2];
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Abstract: Primary progressive aphasia (PPA) is a clinical syndrome characterized by progressive loss of language abilities with three phenotypic clinical presentations, including logopenic variant (lvPPA), nonfluent variant (nfvPPA), and semantic variant (svPPA). Structural and functional neuroimaging studies have implicated unique anatomic involvements within the language network in each variant. Resting state brain oscillations represent coordinated activity in large groups of neurons and hence provide a tool to quantify spontaneous neuronal activity and functional network integrity of neural circuits. We hypothesized that resting state brain oscillations will show unique deficits within the language network in each variant of PPA and these regional deficits will provide anatomical correlates for distinct alterations in speech-motor control. We examined lvPPA, nfvPPA and svPPA patients using magnetoencephalogrpahy, compared to an age-matched control group. Each patient underwent a complete clinical

evaluation including a comprehensive battery of language tests. We examined the spectral power and whole brain resting state functional connectivity patterns of alpha frequency oscillations (8-12Hz), and responses to auditory feedback perturbations of pitch, in each patient group, compared to age-matched controls. We found that lvPPA patients have significantly reduced alpha power over the posterior superior temporal, posterior parietal and occipital cortices, with a left predominant distribution. In contrast, nfvPPA patients had significantly reduced alpha power over inferior frontal cortex bilaterally. The two subgroups also showed unique deficits of functional connectivity patterns, where lvPPA patients showed reduced functional connectivity over the left posterior superior temporal and occipital regions while nfvPPA patients showed reduced functional connectivity of bilateral inferior frontal regions, compared to age-matched controls. svPPA patients showed minimal spectral differences compared to age-matched controls. We also found significantly altered auditory feedback responses in lvPPA and nfvPPA. These results demonstrate neural correlates of region specific abnormalities and unique spatiotemporal patterns of network dysfunction in distinct phenotypes of PPA, and provide evidence for neural substrates of speech motor behavioral deficits in PPA .

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Poster

039. Memory and Cognition in Alzheimer's Disease and Other Neurodegenerative Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 39.12/H1

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH-NIA K23AG039414

P01AG019724

P50AG23501

Consortium for Frontotemporal Dementia Research

Title: Structural deficits and altered intrinsic connectivity in presymptomatic progranulin mutation carriers

Authors: *S. LEE[1], A. C. SIAS[1], E. KOSIK[1], J. A. BROWN[1], J. DENG[1], A. A. VIDOVSZKY[1], A. M. KARYDAS[1], G. COPPOLA[3], D. H. GESCHWIND[3], R. RADEMAKERS[4], H. J. ROSEN[1], B. L. MILLER[1], W. W. SEELEY[2];
[1]Neurol., [2]Neurol. and Pathology, UCSF Memory and Aging, San Francisco, CA; [3]Neurol., UCLA Neurobehavior Div., Los Angeles, CA; [4]Neurosci., Mayo Clin., Jacksonville, FL

Abstract: Mutations in the progranulin gene (*GRN*) cause autosomal dominant frontotemporal lobar degeneration (FTLD), a common cause of early-onset dementia. Symptomatic *GRN* carriers develop heterogeneous clinical syndromes even within the same family. These syndromes include behavioral variant frontotemporal dementia (bvFTD), primary progressive aphasia (PPA), corticobasal syndrome (CBS) and Alzheimer's disease (AD). Previous studies have suggested that presymptomatic *GRN* carriers show similar gray matter volume compared to healthy controls, but reduced task-free fMRI connectivity in the salience network, known to be disrupted in bvFTD. To date, no studies have systematically explored whether presymptomatic *GRN* carriers show alterations in four intrinsic connectivity networks (ICNs) known to be disrupted in the four major *GRN* clinical syndromes. *GRN* carriers were deemed presymptomatic during a clinical consensus conference and underwent an extensive neuropsychological battery. We compared 17 presymptomatic *GRN* carriers (age 53.1 ± 11.6 years, 10 females) to demographically-matched healthy controls using voxel-based morphometry to delineate gray matter differences. Using seed-based task-free fMRI, we probed: (1) the salience network, which is atrophied in bvFTD, (2) the PPA network, (3) the CBS network, and (4) the default mode network, atrophied in AD. Although presymptomatic *GRN* carriers and controls showed similar performance on cognitive testing, carriers showed reduced gray matter in the posterior midcingulate cortex, dorsal midinsula, and small clusters throughout lateral frontotemporoparietal cortices ($p < 0.001$ uncorrected). ICN mapping showed robust regions of enhanced connectivity in key ICN hubs for all four networks. Interestingly, increased thalamic connectivity was a unifying feature across all ICNs studied. Only the PPA network revealed connectivity reductions emerging in the pons and cerebellum. While presymptomatic carriers showed areas of reduced gray matter in scattered foci, extensive regions of increased connectivity emerged in the four ICNs that degenerate during the symptomatic phase. Longitudinal studies will determine if such hyperconnectivity represents a compensatory response as carriers approach symptom onset, or whether it manifests throughout the presymptomatic phase.

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Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.01/H2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NRF-MSIP-R1A1A1008173

Title: Similarity-based cortical thickness analysis of mild cognitive impairment (MCI)

Authors: *C. E. HAN[1], H. KAM[2], J.-K. SEONG[3,4];
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Abstract: Mild Cognitive Impairment (MCI) patients may convert into Alzheimer's disease (AD), the most common cause of dementia; however does not all of them. The former is called 'MCI converters' while the latter is called 'MCI non-converters'. Comparing those two MCI types has a great value to understand disease progression and may help to design early interventions. The 'region-specific' differences of brain atrophy between them were previously reported. However, difference in *overall* atrophy patterns was less studied. Thus, we here provide a statistical framework of similarity-based analyses using a MRI dataset from the Alzheimer's Disease Neuroimaging Initiative (ADNI). The dataset consists of T1-weighted structural images of 101 MCI converters, 104 MCI non-converters, and 114 matched healthy subjects where each patient has 3 volumes at the baseline, 1 year and 2 year follow-ups. First, we constructed a similarity matrix between any pair of subjects in their cortical thickness of whole brain which is a surrogate of brain atrophy. We obtain the cortical thickness from T1-weighted MR images using FreeSurfer with visual validation. Due to inter-subject variability of the brain shape, we resample the brain surfaces and its corresponding cortical thickness with 81,924 vertices using our in-house software. The pairwise similarity between any pair of subjects is computed with both a correlation coefficient and the mutual information. Then, we performed following statistical analyses. If the mean inter-group similarity is lower than the mean intra-group similarity, there exists a group difference. To estimate the significance level, we used permutation testing. We observed the significant difference between MCI groups ($p<0.001$). For the correlation with non-imaging measures including the Mini Mental State Examination (MMSE), for each patient we average similarities with the healthy subjects. Since similarity is defined between a pair of subjects, it only captures relative locations of subjects to the others. The averaging anchors each subject to the healthy subjects and thus enables a correlation study. We observed a weak but significant positive correlation with MMSE ($r\sim 0.2$), and a moderate but significant negative correlation with age ($r\sim -0.37$). As a longitudinal analysis, we collected

similarity at 1 year and 2 year with respect to the baseline. We compared slopes of similarity decrease over time between groups expecting larger slopes in the MCI converters; however, it was insignificant. Our approach may provide a complementary view of disease progression in terms of cortical thickness. Further analyses are needed including investigation of shape manifolds.

Disclosures: **C.E. Han:** None. **H. Kam:** None. **J. Seong:** None.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

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L.K. Whittier Foundation

Title: A new biomarker of pericyte injury and blood-brain barrier dysfunction

Authors: ***A. P. SAGARE**[1], M. D. SWEENEY[1], A. MONTAGNE[1], J. MAKSHANOFF[1], D. LAZIC[1], M. G. HARRINGTON[2], B. V. ZLOKOVIC[1];
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Abstract: Increasing evidence supports that neurovascular dysfunction contributes to several neurodegerative disorders. Our recent study shows that blood-brain barrier (BBB) disruption and increased permeability, especially in the hippocampus, positively correlates with elevated levels of soluble platelet-derived growth factor receptor-β (sPDGFRβ) in cerebrospinal fluid (CSF) in patients with mild dementia. PDGFRβ is expressed in the brain by vascular mural cells - brain capillary pericytes and arterial vascular smooth muscle cells (VSMCs). To determine which vascular cell type(s) contributes to increased sPDGFRβ in CSF, we compared PDGFRβ expression and sPDGFRβ shedding in response to injury in early passage primary cultures of

human brain pericytes, brain arterial VSMCs, and brain endothelial cells. We developed quantitative Western blot and meso-scale discovery (MSD) assays to analyze the levels of sPDGFRβ. PDGFRβ protein was undetectable in endothelial cells, but was found both in pericytes and VSMCs. PDGFRβ relative protein abundance was higher by 4.2-fold ($p < 0.05$) in pericytes compared to VSMCs. Compared to the basal sPDGFRβ levels in the culture medium (1.43 ± 0.15 ng/ml), both hypoxia (1% O_2) or amyloid-β peptide (25 μM) increased shedding of sPDGFRβ into the culture media compared to normoxia (21% O_2) over 48 h. This was associated with the corresponding loss of cell-associated PDGFRβ from pericytes without any change in cellular levels of PDGFRβ in VSMCs. We further validated sPDGFRβ as a CSF marker of pericyte injury *in vivo* in 16-month-old pericyte deficient *Pdgfrβ*$^{+/-}$ mice and Alzheimer's Tg2576 mice which develop significant age-dependent pericyte loss. We found significant 289% and 58% increase in CSF levels of sPDGFRβ in *Pdgfrβ*$^{+/-}$ and Tg2576 mice, respectively. Thus, sPDGFRβ is a biomarker of pericyte injury, and elevated sPDGFRβ levels in cerebrospinal fluid in patients with dementia and/or other neurodegenerative disorders likely reflects pericyte injury. These results together support the potential for sPDGFRβ to be developed and validated as a biomarker of brain pericyte injury and BBB dysfunction.

Disclosures: A.P. Sagare: None. **M.D. Sweeney:** None. **A. Montagne:** None. **J. Makshanoff:** None. **D. Lazic:** None. **M.G. Harrington:** None. **B.V. Zlokovic:** None.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.03/H4

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Vascular risk factors, education and physical fitness are associated with differences in brain volumes

Authors: *C. PINTZKA[1], T. HANSEN[1], A. K. HÅBERG[1,2];
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Abstract: Vascular risk factors are associated with brain atrophy and are recognized as important risk factors for developing dementia, including Alzheimer's disease (AD). Education and physical fitness have been shown to reduce dementia risk and have been associated with larger brain volumes. The aim of the present study was to investigate the effects of different factors on brain volumes in a large general population sample. We included participants from the HUNT MRI study (n=1006, age 50-66 years), which is a representative sample from the Nord-

Trøndelag Health Study (The HUNT Study), a large multiphase, health study on the inhabitants in the county of Nord-Trøndelag. All participants were scanned with the same 1.5T MRI scanner. A T1 weighted MPRAGE ADNI volume was used to estimate brain volumes (cortical, white matter, hippocampal and ventricular) using FreeSurfer 5.3. Intracranial volume (ICV) was derived using SPM. Each volume was first standardized and corrected for differences in sex, age and ICV using separate ANCOVAs. A multivariate regression analysis was run using the following factors from the HUNT clinical databank: education, physical fitness (VO2-max), smoking, blood pressure (SBP), waist-to-hip-ratio (WHR), non-fasting glucose, resting heart rate, alcohol consumption, presence of diabetes, and serum levels of total and HDL-cholesterol, and triglycerides. 87 subjects were excluded due to processing failure and 20 were due to brain pathologies. Clinical information were lacking in 196 subjects, leaving a total of 703 available for analysis. Using the brain parenchymal fraction (volume of gray and white matter divided by ICV) as a measure of brain health, education (p=.015), SBP (p=.007), smoking history (p=.029), VO2-max (p<.001) and WHR (p=.011) were found to be significant predictors. Education and VO2-max were positively associated with brain parenchymal fraction whereas SBP, smoking history and WHR were negatively associated. These five factors predicted 11.2% of the variance in total cortical volume, 2.4% of the variance in white matter volume, 4.0% of the variance in hippocampal volume, and 3.4% of the variance in total ventricular volume. Education (4.3%) and VO2-max (2.3%) were the most influential factors for total cortical volume. The variance in total hippocampal volume was best explained by WHR (1.3%), education (1.1%) and VO2-max (1.1%). Education and VO2-max have a significant positive effect primarily on gray matter volumes (cortical and subcortical). In our sample, these effects outweigh the negative effects of known vascular risk factors and might provide a cheap and non-pharmacological way to improve the overall brain health.

Disclosures: C. Pintzka: None. **T. Hansen:** None. **A.K. Håberg:** None.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.04/H5

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Hippocampal and ventricular volumes of idiopathic normal-pressure hydrocephalus and the cerebrospinal fluid tap test

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of Med., Daegu, Korea, Republic of; [3]Dept. of Pharmacology, Kyungpook Natl. Univ. Sch. of Med., Daegu, Korea, Republic of

Abstract: We investigated differences in ventricular and hippocampal volumes between CSF tap test (CSFTT) responders and non-responders in idiopathic normal-pressure hydrocephalus (INPH) patients and compared these parameters in INPH patients with that of age- and gender-matched healthy controls. We also evaluated relationships between ventricular and hippocampal volumes and clinical profiles in INPH patients.
We enrolled 48 patients with INPH and 29 healthy controls. Ventricular and hippocampal volumes were measured on MRI, including 3-dimensional volumetric images.
INPH patients, when compared to healthy controls, had significantly larger ventricular and smaller hippocampal volumes ($p<0.01$). No difference in ventricular and hippocampal volumes was found between CSFTT responders and non-responders in INPH patients. And hippocampal volumes showed significant negative correlations with Clinical Dementia Rating Scale scores ($r=-0.443$, $p<0.01$) and Unified Parkinson's Disease Rating Scale motor scores ($r=-0.499$, $p<0.01$) in INPH patients.
Volumetric assessment of ventricular and hippocampal regions may have no predictive value in differentiating between CSFTT responders and non-responders in INPH patients. Our findings may help us understand the potential pathophysiology of unique symptoms associated with INPH.

Disclosures: K. Kang: None. **J. Kim:** None. **M. Lee:** None.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

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Program#/Poster#: 40.05/H6

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: VA-I21BX002215

Cure Alzheimer's Fund

Title: Proteomic analysis of blood, induced pluripotent stem cells, three dimensional neurons, and post-mortem brain tissue specimens from the same alzheimer patients for biomarker exploration

Authors: M. CHEN[1,2], H.-K. LEE[1,3], *C. VELAZQUEZ[4], P. MORIN[1,5], T. STEIN[1,6], W. XIA[1,7];

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Abstract: Two pathological hallmarks of Alzheimer's disease (AD) are Tau-containing neurofibrillary tangles and Aβ-containing neuritic plaques. Currently, a combination of measurements of the 42-residue of Aβ peptide (Aβ42), total Tau, and phosphorylated Tau (pTau) protein in human cerebrospinal fluid (CSF) is the best fluid predictor of AD progression and therapeutic efficacy. Due to the heterogeneity and multifactorial nature of AD, multiple biomarkers from peripheral and central nervous system need to be explored for their relevancy to disease onset, progression, and therapeutic prognosis. In this study, we have collected peripheral blood mononuclear cells (PBMC) and plasma from AD patients hospitalized in our Geriatric Research Education Clinical Center (GRECC)-managed hospice Dementia Special Care Unit. We have converted PBMC to induced pluripotent stem cell (iPSC) lines, and we have further differentiated iPSC into human three-dimensional (3D) neurons. At autopsy, postmortem brain tissue specimens from the same subjects were collected and processed for biochemical and Mass Spectrometry-based analysis. AD pathology was confirmed in patients from whom we derived blood, iPSC and 3D neurons. Quantitation of Aβ and Tau by ELISA illustrated much higher levels of Aβ40, Aβ42, and phosphorylated Tau at residues Thr 181 and Thr 231 in brain tissue from superior and inferior frontal cortex area, compared to those from cerebellum region. Liquid chromatography/mass spectrometry was used to analyze plasma, iPSC, 3D neurons and post-mortem brain tissue labelled with isobaric mass tags for relative protein quantification. Our study revealed compartmental segregation as well as association of differentially expressed proteins for biomarker exploration. Among them, several calcium binding proteins exhibited clear link to two AD pathological proteins, Aβ and Tau. We present a unique platform to discover and validate novel AD biomarkers that can be studied in a much large population.

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Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.06/H7

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MEXT/JSPS KAKENHI Grant Number 26350901

Title: Relations between cognitive decline, regional specificity of brain atrophy and cerebrospinal fluid biomarkers in elderly patients with Alzheimer's disease

Authors: *Y. OIWA[1], T. KITAGAWA[1], I. NAKANISHI[1], H. TANAKA[1], M. ARITA[2]; [1]Wakayama Med. Univ., Katsuragi-Cho, Ito, Wakayama, Japan; [2]Wakayama Med. Univ. Sch. of Hlth. and Nursing Sci., Wakayama, Japan

Abstract: Background and Objective. Biochemical changes in the cerebrum are responsible for pathophysiological changes in Alzheimer's disease (AD). However, it is possible that cognitive decline in elderly AD patients is attributable to both AD-related and additional age-related changes. Contributing factors specific for cognitive decline and brain atrophy in elderly AD are still unclear. To distinguish AD-specific changes from normal aging in the elderly AD, we examined correlations between cognitive functions, MRI morphometry and cerebrospinal fluid (CSF) biomarkers. Study design and subjects. A total of 115 subjects, 64-84 years old: 76 with AD, 24 with mild cognitive impairment, and 15 cognitively normal elderly, were included in this cross-sectional study. Severity of atrophy in the target volume of interest registered in the medial temporal structures and the whole brain were measured by using MRI with voxel-based specific regional analysis system for AD (VSRAD) program. Fifty-seven of them underwent lumbar puncture to measure CSF amyloid-β1-42 (Aβ42), total tau protein (T-tau) and phosphorylated tau (P-tau).Results. Cognitive decline was correlated with aging, severity of atrophy in the entorhinal cortex, extent of gray matter atrophy in the whole brain, increase in CSF T-tau and P-tau, and decrease in CSF Aβ42. These changes in the CSF biomarkers showed good correlation with cognitive decline, but not with aging. Severity of the entorhinal cortical atrophy was correlated with increase in ratios of T-tau/ Aβ42 and P-tau/ Aβ42. In multivariate analyses, elevation of P-tau/ Aβ42 ratio carried independent prognostic significance for the severity of AD.Conclusion. This study suggests that atrophy of the entorhinal cortex associated with higher CSF P-tau and lower CSF Aβ42 is a pathogenetic mechanism for cognitive decline in elderly patients with AD.

Disclosures: Y. Oiwa: None. **T. Kitagawa:** None. **I. Nakanishi:** None. **H. Tanaka:** None. **M. Arita:** None.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.07/H8

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: The Hippocampal Network Model - A neuroimaging approach for identifying dementia biomarkers in metabolic syndrome

Authors: *E. KOTKOWSKI, P. T. FOX;
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Abstract: Metabolic syndrome or systemic metabolic dysfunction (SMD) is one of the most common disease states disproportionately afflicting the Hispanic population in the United States. Evidence suggests that effects of SMD and neurocognitive dysfunction are bidirectional and complex, with genetic, environmental, and behavioral causes (Biessels et al., 2014). It has been recently shown that the underlying pathology does not resemble that of AD (Abner et al., 2016). Studies using contrast-enhanced MRI show age-dependent blood-brain barrier (BBB) breakdown in the hippocampus, worsening with mild cognitive impairment and correlating with injuries to the BBB pericytes (Montagne et al., 2015). Rodent models have also implicated the hippocampus in cognitive deficits resulting from T2DM (Stranahan et al., 2008). Consequently, we propose to create a hippocampal network model (HNM), using meta-analytic co-activation modeling (MACM) and structural equation modeling (SEM), to detect and quantify network abnormalities associated with neurocognitive decline in SMD. Our immediate research goal is to create a functional MACM of the hippocampi in order to develop the HNM that can be used to assess cognitive impairments in individuals with SMD. Our preliminary data has found 20 key nodes of functional connectivity with both hippocampi all of which address highly significant behavioral paradigms known to be affected in individuals with dementia (i.e. working and explicit memory, cognition, etc.).

Disclosures: E. Kotkowski: None. **P.T. Fox:** None.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.08/H9

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NPU II

Title: MR imaging of blood perfusion at the nucleus basalis of Meynert in modulation of attention and visual processing

Authors: *A. WOJNA PELCZAR[1], N. SZABO[2], P. FARAGO[1,2], A. KIRALY[1,2], T. Z. KINCES[2,3], I. REKTOR[1,3], B. TOMANEK[1];
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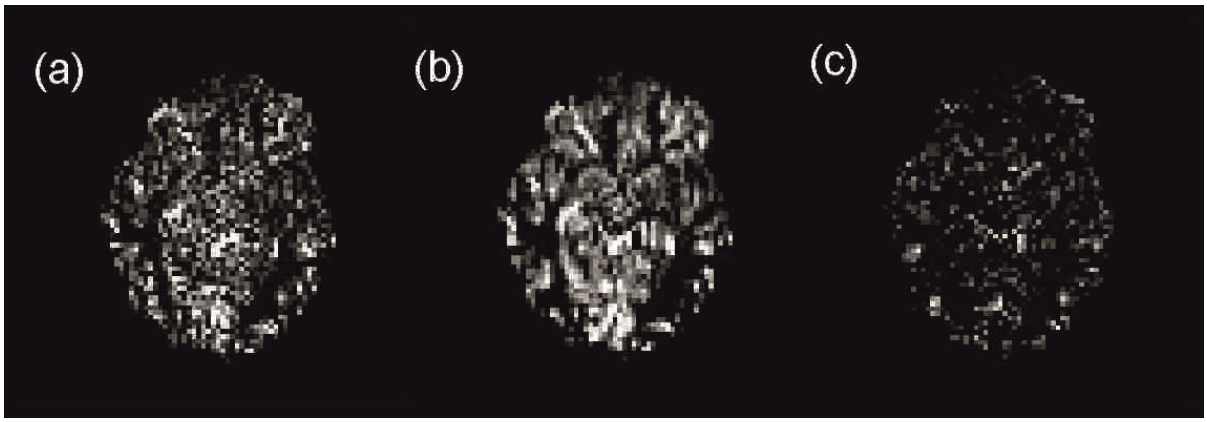
Abstract: Intoduction: Many pathological brain conditions are associated with abnormal cerebral blood flow (CBF). As nucleus basalis of Meynert (NMB) plays a major role in modulation of attention and visual processing, the aim of the study was to image CBF before and after a visual task to find changes in blood perfusion at the NMB level. **Methods:** 20 healthy individuals (age: 22-35) were enrolled to the study. MR experiments were performed using a clinical 3T scanner (Prisma). Arterial spin labelling (ASL) sequence was applied before and after visual stimuli (random dot kinematogram). The 2D EPI sequence was used: TE/TR = 15/2500 ms, flip 90°, matrix 256 × 256, 4 slices, 3 mm thick. For CBF evaluation the pulsed ASL (PASL) with 700 ms bolus duration and 1800 ms inversion time was applied. A single PASL measurement consisted of 45 volumes of the tag and 45 controlled images, allowing calculation of a perfusion map. Data processing was performed using the Bayesian Inference for ASL toolset. **Results:** The data showed difference between CBF before and after visual stimuli (Fig 1). It is possible to measure activity dependent CBF in NMB in healthy individuals. Potentially, this method could be used to detect abnormalities in dementing disorders such as Alzheimer's Disease (AD) as it is suggested, that NMB plays a role in the pathomechanism of AD. Although ASL provides low signal-to-noise ratio, it requires no intravenous contrast, is available on most clinical MRI scanners and it is fast (~2 min). This makes ASL particularly appealing for diagnostic purposes. **Figure 1:** Examples of the ASL images at the NMB level before (a) and after (b) visual stimulation for one subject. The difference between images is shown in (c). Changes in CBF within the NBM are visible in (c).



Disclosures: A. Wojna Pelczar: None. **N. Szabo:** None. **P. Farago:** None. **A. Kiraly:** None. **T.Z. Kinces:** None. **I. Rektor:** None. **B. Tomanek:** None.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.09/H10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: National Institutes of Health, Grant TL1TR00098

Title: Quantitative neuroimaging and biomarkers in Alzheimer's disease and Lewy body spectrum diseases

Authors: *K. TRINGALE[1], P. OOMEN[2], J. BREWER[1,3];
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Abstract: Dementia with Lewy bodies (DLB) is the second most common form of dementia after Alzheimer's disease (AD) and is characterized pathologically by alpha synuclein inclusions forming Lewy bodies. Dementia often presents when Lewy bodies reach the cortical and limbic areas of the brain as patients may experience cognitive decline, neuropsychiatric symptoms, and Parkinsonisms. Since DLB frequently occurs with concurrent AD-type pathology, characterized by neuritic plaques and neurofibrillary tangles, misdiagnoses are frequent. Patterns of cerebral atrophy in DLB have not been as well established as in AD. Accurate differentiation between the two types of dementia is important given the different etiologies, prognoses, and treatments. This work seeks to identify patterns of atrophy in patients with DLB compared to AD and cognitively normal controls (NC) to ultimately combine MRI with early structural biomarkers to improve diagnostic accuracy and early treatment. 101 patients aged 55 to 100 years old characterized as either NC, DLB, or AD underwent structural MRI scans with fully automated volumetric segmentation performed with the NeuroQuant software package. A subset of qualified patients also had CSF sample acquisition, analysis of B-amyloid 1-42 levels and APOE4 status, and brain autopsy. Analysis was completed via SPSS Statistics with gender, age, level of education, and intracranial volume as covariates. Groups did not significantly differ in age but did significantly differ in gender, level of education, MMSE score (29.2±1.0 for NC, 21.6±3.6 for DLB, 21.7±4.6 for AD), and level of AB42 (350.3±107.5 for NC, 437.7±50.3 for DLB, 174.2±70.7 for AD). Brain parenchymal fraction (BPF) indicated whole brain atrophy, which was most severe for AD (0.58) and intermediate for DLB (0.61), both which were significantly atrophied compared to NC (0.64). Regionally-specific volumetric imaging showed significant differences in NC and DLB, particularly the volumes of the hippocampus, amygdala, medial temporal cortex, fusiform cortex, parahippocampal gyrus, lateral ventricles, and total ventricles. Significantly different between NC and AD were the volumes of the hippocampus, amygdala, thalamus, entorhinal cortex, medial temporal cortex, fusiform cortex, inferior parietal cortex, inferior temporal cortex,

parahippocampal gyrus and the lateral ventricle and total ventricle. Significantly different between DLB and AD were the volumes of the amygdala, entorhinal cortex, medial temporal cortex, fusiform cortex, inferior parietal cortex, inferior temporal cortex. All mentioned brain regions were more severely affected in AD compared to DLB.

Disclosures: K. Tringale: None. **P. Oomen:** None. **J. Brewer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CorTechs Labs, Inc., Human Longevity, Inc.. F. Consulting Fees (e.g., advisory boards); Elan, Bristol-Myers Squibb, Avanir, Novartis, Genentech, Eli Lilly.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.10/H11

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Tau deposition and microglial activation in early stage Alzheimer disease

Authors: *T. TERADA[1,3], M. YOKOKURA[2], T. BUNAI[4], E. YOSHIKAWA[5], M. FUTATSUBASHI[5], T. MATSUDAIRA[3], T. OBI[3], Y. OUCHI[4];
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Abstract: Introduction Alzheimer disease (AD) is characterized by the deposits of amyloid-beta plaques and neurofibrillary tangles consisting of pathological tau. Several studies implicated pathological tau in mechanisms of neurodegeneration. Although it has been reported that the presence of amyloid-beta activates microglia, it remains unclear whether tau deposition and microglial activation are associated with each other. [^{11}C]PBB3 has been recently developed as a tau imaging positron emission tomography (PET) ligand. Here, we examined the degree and the relevance of these pathological markers by measuring the densities of tau and activated microglia in AD patients at an early stage using positron emission tomography (PET) with [^{11}C]PBB3 a newly-developed tracer of tau deposition and [^{11}C]DPA713 a second generation translocator protein (TSPO) tracer for activated microglia. **Methods** Seventeen AD patients (mean age 69.9±9.6) at the clinical dementia rating (CDR) being 0.5 or 1 underwent [^{11}C]PBB and [^{11}C]DPA PET measurements. The binding potential (BP_{ND}) was estimated on the simplified reference tissue model (SRTM) using PMOD 3.4 software. Statistical Parametric Mapping

(SPM) and region of interest (ROI) analysis were used to compare regional BP_{ND} levels between the AD and age matched control groups. **Results** Significant elevations of [^{11}C]PBB3 BP_{ND} were found focally in the temporal cortex in AD patients at the CDR0.5 level and broadly over the temporal, frontal, and occipital cortices in the patients with CDR1. TSPO imaging showed a significant increase of [^{11}C]-DPA713 BP_{ND} over the extensive brain regions especially in the temporal, frontal and occipital regions, which were found to be significantly overlapped to the region with tau deposition. **Discussion** The present in vivo findings were consistent with the pathological view of the spreading of tau pathology with AD progression. These results suggest that microglial activation seems to coexist with the progression of tau deposition, and that these two pathophysiological events contribute to the continuum of neuronal degeneration in AD.

Disclosures: T. Terada: None. **M. Yokokura:** None. **T. Bunai:** None. **E. Yoshikawa:** None. **M. Futatsubashi:** None. **T. Matsudaira:** None. **T. Obi:** None. **Y. Ouchi:** None.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.11/H12

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Estimation of age of onset in presymptomatic frontotemporal degeneration

Authors: *P. VADDI, E. MCCARTY WOOD, V. VAN DEERLIN, D. IRWIN, M. GROSSMAN, C. MCMILLAN;
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Abstract: Roughly 20% of frontotemporal degeneration (FTD) patients have a known genetic mutation with high penetrance. Presymptomatic mutation carriers are optimal candidates for neuroprotective clinical trials of disease-modifying agents. One challenge for trials is estimating when an individual is likely to become symptomatic. The current literature suggests that correlations between an individual's age of onset and their family members' age of onsets may help with this issue; however, the predictive accuracy of these studies have not been assessed. In this study, we investigate the predictive utility of the family's mean onset in *progranulin (GRN)* mutation carriers, an autosomal dominant mutation with very high penetrance (>95%) that results in FTD. Analyses were conducted on 25 pedigrees comprised of a total of 65 individuals. Among these, 32 have confirmed *GRN* mutations while 33 cases have clinical diagnoses compatible with an FTD phenotype (e.g., behavioral-variant or primary progressive aphasia) or related early-onset disorder. The latter 33 cases are also assumed to have GRN mutations because they are affected family members of the 32 cases confirmed to have GRN mutations. An

average of 3.79 individuals and an average of 2.49 generations are reported per family pedigree. Linear regression analyses reveal an association between an individual's onset and the family's mean onset (β=1.2015, $p<0.05$). However, a leave-one out cross-validation model suggests an individual's predicted onset was not associated with the family's mean onset (p=0.16). Together, these findings suggest that while individual and family onsets are correlated with one another, the predictive value of these associations should be interpreted cautiously. Future cross-validation studies are necessary in larger cohorts and other FTD mutations (*MAPT*, *C9orf72*) to evaluate the validity of age of onset estimates in presymptomatic FTD.

Disclosures: P. Vaddi: None. **E. McCarty Wood:** None. **V. Van Deerlin:** None. **D. Irwin:** None. **M. Grossman:** None. **C. McMillan:** None.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

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NIH Grant PO1 AG 017586

Title: Disease-specific relationship between longitudinal neuroimaging and cerebrospinal fluid

Authors: *C. A. JESTER, III[1], K. FIRN[1], K. TERNES[1], L. M. SHAW[2], D. IRWIN[1], D. WEINTRAUB[3], M. GROSSMAN[1], C. T. MCMILLAN[1];
[1]Neurol., [2]Ctr. for Neurodegenerative Dis. Res., Univerisity of Pennsylvania, Philadelphia, PA; [3]Psychiatry, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Progressive supranuclear palsy (PSP) and Parkinson disease (PD) are both characterized by parkinsonism. However, PSP is associated with elevated cerebrospinal fluid

(CSF) phosphorylated tau (ptau) and tau pathology while PD has alpha-synuclein (asyn) pathology. Neuropathological studies suggest that tau inclusions are abundant in white matter (WM), while asyn inclusions are relatively restricted to grey matter (GM) in PD. Thus multimodal neuroimaging provides a potential marker of pathology-specific longitudinal change. Here we investigate whether CSF markers of tau phosphorylated in the threonine 181 position (ptau) and total tau (ttau) can differentiate neuroimaging change in PSP (N=15) and PD (N=15). All patients had a baseline and follow-up T1 MRI of GM and DTI of WM a minimum of 6 months apart (mean difference=1.05 years ±0.45) and a lumbar puncture within 9 months (±14.6) of baseline scan. We used Advanced Normalization Tools (ANTs) to evaluate annualized GM atrophy in 112 cortical and subcortical ROIs and annualized WM change with fractional anisotropy (FA), in 48 WM ROIs. CSF was analyzed using ADNI standard operating procedures. Regression related baseline ptau and ttau to annualized change in PSP and PD (all $p<0.01$). Reduced ptau was uniquely associated with PSP WM change in cerebellar peduncle, bilateral cerebral peduncle, and fornix. Increased ttau, which is more broadly related to neuronal degeneration, was uniquely associated with PD GM atrophy in right pallidum, putamen, frontal pole, planum temporale and left thalamus. Thus, we report a double dissociation for baseline CSF and longitudinal neuroanatomic change, with WM decline in PSP related to ptau and GM decline in PD related to ttau. CSF may be a useful prognostic marker to stratify patients in protein-specific disease-modifying treatment trials.

Disclosures: **C.A. Jester:** None. **K. Firn:** None. **K. Ternes:** None. **L.M. Shaw:** None. **D. Irwin:** None. **D. Weintraub:** None. **M. Grossman:** None. **C.T. McMillan:** None.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.13/H14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Medical Research Council

Addenbrooke's Charitable Trust

Alzheimer's Research United Kingdom - Cambridge Network

Health Foundation

Marmaduke Sheild Fund

Title: Using event related potentials to investigate aging and Alzheimer's disease in adults with Down's Syndrome

Authors: *S. JENNINGS[1], S. CHENNU[2], T. BEKINSCHTEIN[3], V. NOREIKA[4], A. HOLLANAD[1], H. RING[1];
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Abstract: Down's Syndrome (DS) is a genetic disorder attributed to the triplication of chromosome 21, and is associated with premature aging and an increased risk of developing Alzheimer's disease (AD). AD typically presents with memory decline however some of the earliest clinical indicators of AD in DS are compromised frontal lobe functions, such as inhibitory control. This research project aims to test the potential value of event related potentials (ERPs), for identifying age-related changes to cognitive functions underpinned by the frontal lobes. ERPs are averaged responses generated from electroencephalographic (EEG) recordings of bio-electrical activity generated by cortical neurons. The method is non-invasive and inexpensive so could present a viable, widespread screening tool. The ERPs investigated in this project are MMN and P3a, which are maximal over fronto-central sites and thus reflect activity in a brain region of interest for DS-AD; and P3b which has been repeatedly suggested in the literature to be perturbed in AD. In this study, the 36 participants with DS had significantly smaller MMN and P3b waveforms than the 39 age- and gender- matched controls. However, participants with DS had significantly larger P3a waveforms. The ERPs will be correlated with age and cognitive decline measures, with a view to identifying potential markers of AD. There is great interest in developing markers for preclinical stages of AD, so that therapeutic interventions, when they become available, can be administered when there is still functionality to be preserved rather than the more challenging task of restoring lost functions.

Disclosures: S. Jennings: None. **S. Chennu:** None. **T. Bekinschtein:** None. **V. Noreika:** None. **A. Hollanad:** None. **H. Ring:** None.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.14/H15

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NHRI-PP-06

Title: Developing neuroimaging biomarkers for classification of Alzheimer's disease: A correlative study between brain network centrality, cognitive functions and biochemical measurements in clinical settings

Authors: *C.-P. LIN[1], S.-Y. LIN[1], T.-J. HSIEH[2], C.-C. HSU[2], L.-W. KUO[1];
[1]Inst. of Biomed. Engin. and Nanomedicine, [2]Inst. of Population Hlth. Sci., Natl. Hlth. Res. Inst., Miaoli County, Taiwan

Abstract: Patients suffering from Alzheimer's disease (AD) are often diagnosed after progressively altered behavior or life style, which are difficult to distinguish from the symptoms of mild cognitive impairment (MCI). The lack of early diagnosis and prognostic markers may delay patients from seeking medical care. This study aimed to characterize the brain network topology and develop neuroimaging biomarkers by employing graphical network analysis on functional MRI connectivity data. Additionally, a correlative study between neuroimaging biomarkers, cognitive functions and biochemical measurements was performed. A total of 47 subjects, including 13 AD patients, 13 MCI patients and 21 healthy controls (HC) were recruited in this study. MR protocols included resting-state functional MRI (rs-fMRI) and anatomical T1 scans. The cognitive function was assessed by mini-mental state examination (MMSE) and the biochemical measurements included albumin (ALB) and blood urea nitrogen (BUN). Region-wise group comparisons were performed using one-way ANOVA and 2-sample t-test. The correlation analysis was also performed on network centrality measures, MMSE scores and biochemical measures. Multiple comparisons were corrected with false discovery rate controlling procedure. Our preliminary results show significant differences of network centrality in left anterior cingulate, paracingulate gyri and heschl gyrus among three groups. Compared with HC group, the nodal centrality of those regions is decreased in AD group. Specifically, the nodal centrality in left anterior cingulate is positively correlated with MMSE score. The biochemical measurements, ALB and BUN, are generally correlated with network centrality. Our findings suggest that the network centrality in specific cortical regions may potentially reflect the altered cognitive functions or biochemical measures in AD patients. By increasing the subject numbers, a classification model based on brain network centrality could be established by utilizing novel machine learning algorithm and may improve the diagnostic power to identify AD in future clinical routine.

Disclosures: C. Lin: None. **S. Lin:** None. **T. Hsieh:** None. **C. Hsu:** None. **L. Kuo:** None.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.15/H16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: JSPS Grant-in-Aid for Young Scientists (B)

Title: *In vivo* assessment of synaptic properties in rTg4510 tauopathy transgenic mouse model by positron emission tomography

Authors: *M. SHIMOJO, M. TOKUNAGA, T. MINAMIHISAMATSU, S. UCHIDA, H. TAKUWA, Y. TAKADO, I. MATSUMOTO, M.-R. ZHANG, T. SUHARA, M. HIGUCHI, N. SAHARA;
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Abstract: Progressive synaptic degeneration with intracellular tau protein inclusion represented as neurofibrillary tangles (NFTs) and neuronal loss is a common feature of tauopathy including Alzheimer's disease (AD). Cumulative evidence has indicated that synaptic loss is well correlated with cognitive decline and memory impairment of AD patients. In fact, synaptic dysfunction and disrupted neuronal activity caused by toxic tau aggregates have been hypothesized as a central component of disease-related abnormalities in animal models. It is therefore crucial to assess in vivo alteration of synaptic properties in living subject for understanding complicated pathophysiological cascade. Especially, establishment of biomarkers and/or methodologies which are non-invasively applicable to clinical diagnosis is greatly appreciated. [^{11}C]ABP688, a highly selective radioactive ligand binding to allosteric site of the metabotropic glutamate receptor subtype 5 (mGluR5), is a useful probe to monitor glutamatergic excitatory synaptic status by positron emission tomography (PET) imaging. In the present study, we investigated age-dependent change of [^{11}C]ABP688 PET image using rTg4510 tauopathy mouse model which typically develops NFTs and forebrain atrophy by 6 months of age. Intravenous bolus administration of [^{11}C]ABP688 followed by PET scan demonstrated that region specific and age dependent decline of [^{11}C]ABP688 binding potential (BPnd) in rTg4510 mouse model. [^{11}C]ABP688 BPnd began to reduce in striatum at 2-3 month of age before obvious pathological findings. Subsequently, more drastic reduction was observed in broader regions of forebrain including both cortex and hippocampus at 5-6 months of age. Interestingly, reduction level of [^{11}C]ABP688 BPnd reached to plateau range by 5-6 months of age, though MRI volumetric analysis revealed progressive atrophy in forebrain until 8-9 months of age. Our findings suggest that [^{11}C]ABP688 PET imaging is a potent biomarker to assess excitatory synaptic abnormality in animal model, and further investigation in combination with pharmacology will potentially dissect more complicated synaptic status during neurodegenerative process.

Disclosures: **M. Shimojo:** None. **M. Tokunaga:** None. **T. Minamihisamatsu:** None. **S. Uchida:** None. **H. Takuwa:** None. **Y. Takado:** None. **I. Matsumoto:** None. **M. Zhang:** None. **T. Suhara:** None. **M. Higuchi:** None. **N. Sahara:** None.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.16/H17

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIHR Cambridge Dementia Biomedical Research

Title: Components and neural correlates of apathy and impulsivity in frontotemporal lobar degeneration syndromes.

Authors: *C. LANSDALL[1], I. COYLE-GILCHRIST[1], P. VÁZQUEZ RODRÍGUEZ[1], E. WEHMANN[2], A. WILCOX[1], K. DICK[3], P. JONES[1], K. PATTERSON[1], J. ROWE[1]; [1]Univ. of Cambridge, Cambridge, United Kingdom; [2]Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg-Eppendorf, Germany; [3]Univ. Col. London, London, United Kingdom

Abstract: Apathy and impulsivity are common and disabling consequences of frontotemporal lobar degeneration (FTLD) associated disorders. They are recognized as multifaceted constructs that often coexist, although their neural mechanisms are unclear. Treating apathy and impulsivity will become increasingly important as disease modifying treatments are developed to slow progression. We aimed to determine the link between apathy and impulsivity and identify their underlying dimensions and neural correlates in FTLD. The PiPPIN study recruited 204 patients and 50 controls. The assessment battery combined patient, carer and physician questionnaires and behavioural tasks. We derived dimensions of apathy and impulsivity using principal component analysis (PCA) on a subset of 199 patients and controls. Voxel based morphometry (VBM) analysis of T1-weighted MRI images was conducted on a subset of 100 FTLD patients and controls to identify the neural correlates of the extracted components. Eight principal components were identified, separating patient-rated questionnaires, carer-rated questionnaires and behavioural tasks. Apathy and impulsivity measures frequently loaded onto the same components, suggesting they overlap in their underlying mechanisms. Behavioural tasks and questionnaires correlated poorly, with direct implications for translational studies. Furthermore, carer and patient ratings loaded onto separate components and revealed distinct neural correlates, highlighting differences in recognition of behavioural change. VBM found corticospinal tract changes in proportion to the patient rated apathy/impulsivity component, likely reflecting preserved patient awareness of disease-related motor deficits, while insight into cognitive decline is limited. In contrast, carer ratings were associated with changes in frontostriatal circuits and brain stem systems. We propose that these associations reflect underlying changes in social, affective and motivational functions. This dimensional approach provides new insights into the neural basis of apathy and impulsivity in FTLD. Our data indicate common overlapping components related to corticospinal, thalamic and frontostriatal systems, which are differentially

sensitive to objective tests, patient and carer ratings. We propose that evaluating dimensions observed across neurodegenerative disorders may identify novel treatment targets, synergistic with the National Institute of Mental Health's Research Domain Criteria ('RDoC') approach to psychiatric symptomatology. Therapies will offer a broader impact if they are relevant for several different diagnoses.

Disclosures: C. Lansdall: None. **I. Coyle-Gilchrist:** None. **P. Vázquez Rodríguez:** None. **E. Wehmann:** None. **A. Wilcox:** None. **K. Dick:** None. **P. Jones:** None. **K. Patterson:** None. **J. Rowe:** 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; NIHR Cambridge Dementia Biomedical Research.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.17/H18

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant P41 EB015909

NSF Grant ACI-1053575 (XSEDE computational resourses)

NIH Grant U01 AG024904 (ADNI image resourses)

DOD Award W81XWh-12-2-0012 (ADNI image resourses)

Title: Local atrophy of entorhinal and trans-entorhinal cortex in mild cognitive impairment measured via diffeomorphometry

Authors: D. J. TWARD[1], C. C. SICAT[8], T. BROWN[8], E. A. MILLER[8], *J. T. RATNANATHER[2], L. YOUNES[3], A. BAKKER[4], M. ALBERT[5], M. GALLAGHER[6], S. MORI[7], M. I. MILLER[1];
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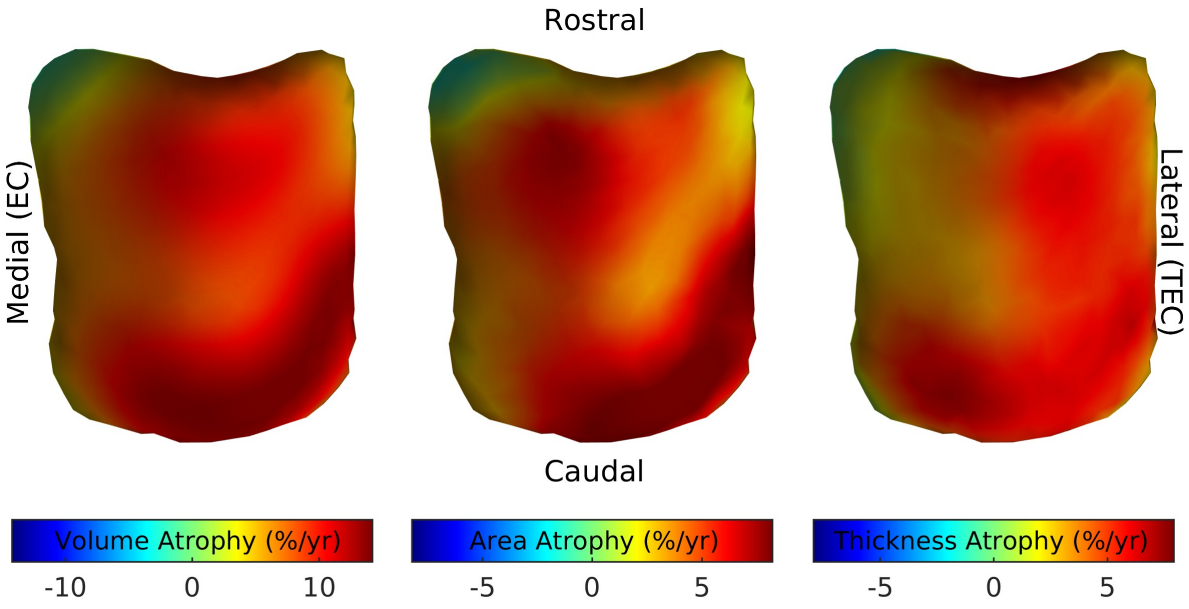
Abstract: Objective:
The entorhinal cortex (EC) is the site of the earliest pathological changes in Alzheimer's disease (AD). This study aimed to quantify the spatial distribution of tissue loss over time in the EC and

the immediately lateral trans-EC (TEC) in participants with mild cognitive impairment.
Methods:
Structural MRI scans from 40 subjects who completed at least 3 scans over 2 years and have a continuous left collateral sulcus were selected from the AD Neuroimaging Initiative database. For these subjects the EC and TEC were manually segmented on T1 structural scans.
After rigid alignment a template triangulated surface was estimated characterizing the population average of the structure. The template was then mapped to each scan in the time-series, minimizing a sum of square error between segmentations, using two geodesic trajectories through diffeomorphism space: 1) template to baseline, and 2) baseline to follow ups. Mapping to each segmentation in the time-series simultaneously reduces within subject variability due to inconsistencies in anatomical definitions.
Local measures of tissue loss were chosen based on the Jacobian of each mapping including a determinant for volume, a determinant of the 2x2 Jacobian tangent to the template for surface area, and a 1x1 Jacobian normal to the template for thickness. Their logarithm was fitted by least squares to a linear model at each template vertex, with a subject specific mean and a population average atrophy rate.
Results:
Participants were 50% female, with a mean age of 71.1 and a mean education of 16.8 years. Results are summarized in the figure showing the estimated population average atrophy rate in an inferior view expressed as percent per year for volume (left), surface area (center), and thickness (right).
Conclusions:
The pattern of tissue loss detected is consistent with early stage pathology identified in autopsied brains by Braak and Braak, with most loss in the sulcal EC. More tissue loss is seen laterally (TEC) than medially (EC proper), suggesting that this region may be a neuroimaging biomarker sensitive to early changes in AD.



Disclosures: **D.J. Tward:** None. **C.C. Sicat:** None. **T. Brown:** None. **E.A. Miller:** None. **J.T. Ratnanather:** None. **L. Younes:** None. **A. Bakker:** None. **M. Albert:** None. **M. Gallagher:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Agenebio. **S. Mori:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AnatomyWorks. F. Consulting Fees (e.g., advisory boards); AnatomyWorks. **M.I. Miller:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); AnatomyWorks. F. Consulting Fees (e.g., advisory boards); AnatomyWorks.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.18/H19

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG034570

Title: Relationship between performance on a famous faces test and AD biomarkers in a group of normal older adults

Authors: ***R. K. BELL**, T. J. MELLINGER, K. N. SWINNERTON, W. J. JAGUST;
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Abstract: Difficulty recalling names is a frustrating phenomenon that becomes more common as we age (Cohen & Faulkner, 1986; Finley & Sharp, 1989). This study aimed to examine the relationship between the Northwestern University Famous Faces test (NUFFACE), standard neuropsychological tests, and Alzheimer's Disease biomarkers in a group of healthy older adults. Forty cognitively normal adults (mean age 77.3 ±6.4, MMSE 28.3 ±1.5, years of education 16.2 ±2.8) participated in a neuropsychological testing session. Twenty-one also had a 1.5T structural MRI, PIB PET scan, and APOE4 genotyping. NUFFACE scores included the percent of faces the participant could name within 5 seconds (ID score) and the percent they correctly recognized (REC score). Partial correlations controlling for age, years of education, and sex assessed the relationship between NUFFACE scores, standard neuropsychological test scores, and hippocampal volume. A voxel-based morphometry analyses was performed with SPM8 using the same covariates. PIB and APOE4 status were explored as grouping variables. The ID score (r=.47, p=0.003) and REC score (r=.46, p=0.003) were significantly correlated with the California Verbal Learning Test and ID score had a trend to correlation with Category Fluency (r = .39 p = .07). Increasing age was significantly associated with lower ID and REC score (r = -.33

p = .04). PIB status (positive/negative), APOE4 carrier status, and hippocampal volume were not significant predictors of NUFFACE scores. The VBM analyses (p = .01 k = 250) revealed the left superior frontal gyrus, bilateral (left predominant) inferior precentral gyrus, and right superior parietal lobe as regions positively correlated with ID and REC score. Although face naming scores decline with age in cognitively normal adults, they are unrelated to typical AD biomarkers. The pattern in the VBM analysis suggests the association between diminished face-naming ability with age could be a reflection of age-related grey matter loss in frontal and parietal regions (Kalpouzos et al., 2007; Giussani et al. 2009) and not a pathological process. Longitudinal data will help determine if the rate of change in test performance is more likely to be associated with pathological decline.

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Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.19/H20

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: *In vivo* wide-field imaging and quantitative analysis reveals decrease of perivascular drainage in Alzheimer's disease

Authors: *S. KIM[1], P. LEE[2], J. KIM[2], Y. JEONG[1,2];
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Abstract: Alzheimer’s disease (AD) is the most common type of dementia and the hall marker of disease is the excessive accumulation of amyloid beta plaque and hyperphosphorylated tau protein. Recently researchers have doubted that excessive amyloid plaque could be a result of an imbalance between production and clearance, not just overproduction of amyloid beta. Growing evidences also support that only the clearance rate of an amyloid beta was impaired in the sporadic form of AD. Although the interest about brain clearance system is increased, the clearance happening within the cortex is not fully understood. Perivascular drainage (PVD) is one of the clearance mechanisms in the cortex referring drainage of solutes along the basement membrane of capillaries and arterial walls into subarachnoid space. Small molecules such as amyloid beta are cleared through this pathway while the contribution of PVD on AD is not well investigated.
In this study, we hypothesized that small molecules movement in the cortex is mainly dependent

both on PVD and diffusion. The PVD would give additional force macromolecules to move further toward vessels having the driving power. Unlike diffusion, this power induces unbalanced movement and distribution of small molecules in the cortex. We used *in vivo* two-photon microscopy and wide-field CCD camera to observe the PVD through fluorescent tagged dextran injected into brain parenchyma, and quantify asymmetry movement and decay rate of this substance in the brain. To assess the amount of the PVD, centering on the injection site, the analyzed area was divided into artery or vein-dominant, and the difference between those areas was used as the indices, the uniformity index (UI) and delta area above curve (ΔAAC). Injected FITC-dextran moves more and faster into artery-dominant area. The UI index illustrating the amount of unbalanced fluorescent distribution was higher than simple diffusion. The ΔAAC index that describes the difference of clearance rate between those area types was statistically different from diffusion. In aged and AD mice, the UI index were relatively steady and the ΔAAC was significantly decreased compared with normal mouse. These results demonstrates that the PVD is impaired in AD and suggest this decreased drainage causes the accumulation of amyloid beta around arteries like cerebral amyloid angiopathy. In addition, we can examine the possibility of these indices as the biomarker of brain clearance functioning.

Disclosures: **S. Kim:** None. **P. Lee:** None. **J. Kim:** None. **Y. Jeong:** None.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.20/H21

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Characterization of the tau radioligand [^{3}H]THK-5351 binding in Alzheimer brain tissue

Authors: C. WINTMOLDERS, A. BOTTELBERGS, J. MARÏEN, D. MOECHARS, *X. LANGLOIS;
Janssen Res. and Develop., Beerse, Belgium

Abstract: Abnormal tau aggregation and beta-amyloid plaque deposition are essential pathological hallmarks of Alzheimer’s disease (AD). Whereas initial drug discovery efforts have focused on the beta-amyloid cascade, targeting tau is now a predominant strategy for halting AD progression since it has been shown that the density of aggregated tau correlates more closely with neuronal dysfunction and cell death, unlike beta-amyloid. Recent efforts to develop PET radioligands binding selectively to aggregated tau have allowed the visualization of tau aggregates *in vivo*. Several Tau PET ligands, including [^{18}F]AV1451(T807), [^{11}C]PBB3 and [^{18}F]THK5351, are currently evaluated in AD patients. Not only will these radioligands help to

determine the pathophysiology of tau aggregation in AD but they will also allow monitoring the therapeutic effect of new treatments in development. Therefore, characterizing the tau selectivity of these radioligands is of prime importance. The present study was initiated to look at tau selectivity of THK5351 in AD brain tissue. In competition binding experiments using the tau radioligand [^{3}H]T808 in paired helical filament (PHF) extracts from AD brain and the beta-amyloid radioligand [^{3}H]AV-45 in human beta-amyloid extracts, THK5351 was showing a moderate predicted affinity (Ki=182 nM) for PHF tau and a poor selectivity versus beta-amyloid (Ki = 348 nM). Tested in parallel, T807 displayed a much higher affinity for PHF tau (Ki = 1 nM) and selectivity versus beta-amyloid (Ki = 246 nM). When testing [^{3}H]THK5351 directly in beta- amyloid extracts, its Kd derived from saturation experiments was equal to 443 nM. When tested on PHF tau, [^{3}H]THK5351 bound to a similar site as [^{3}H]T808 (Kd = 7 nM) but with a lower affinity (Kd = 600 nM) and also to another site. In autoradiography experiments, [^{3}H]THK5351(10 and 30 nM) was compared to [^{3}H]AV-45 (3 nM) and [^{3}H]T808 (10 nM) on AD brain sections. For comparison, immunohistochemistry with the anti-tau antibody AT8 and the anti-amyloid antibody 4G8 was performed on adjacent sections. Whereas [^{3}H]T808 showed a typical tau laminar pattern and [^{3}H]AV-45 a typical amyloid granular pattern, [^{3}H]THK5351 displayed a combination of both. When a high concentration of cold T807 was co-incubated with [^{3}H]THK5351, only the granular pattern remained. Moreover, cold THK5351 could fully inhibit [^{3}H]AV-45 binding whereas cold T807 was ineffective. Altogether, our data indicate that TH5351 binds to both tau and beta-amyloid in the AD brain tissue available to us. These results should be taken in consideration when interpreting recently available human PET data with [^{18}F]THK5351.

Disclosures: **C. Wintmolders:** A. Employment/Salary (full or part-time): Janssen. **A. Bottelbergs:** A. Employment/Salary (full or part-time): Janssen. **J. Marïen:** A. Employment/Salary (full or part-time): Janssen. **D. Moechars:** A. Employment/Salary (full or part-time): Janssen. **X. Langlois:** A. Employment/Salary (full or part-time): Janssen.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.21/H22

Topic: C.02. Alzheimer's Disease and Other Dementias

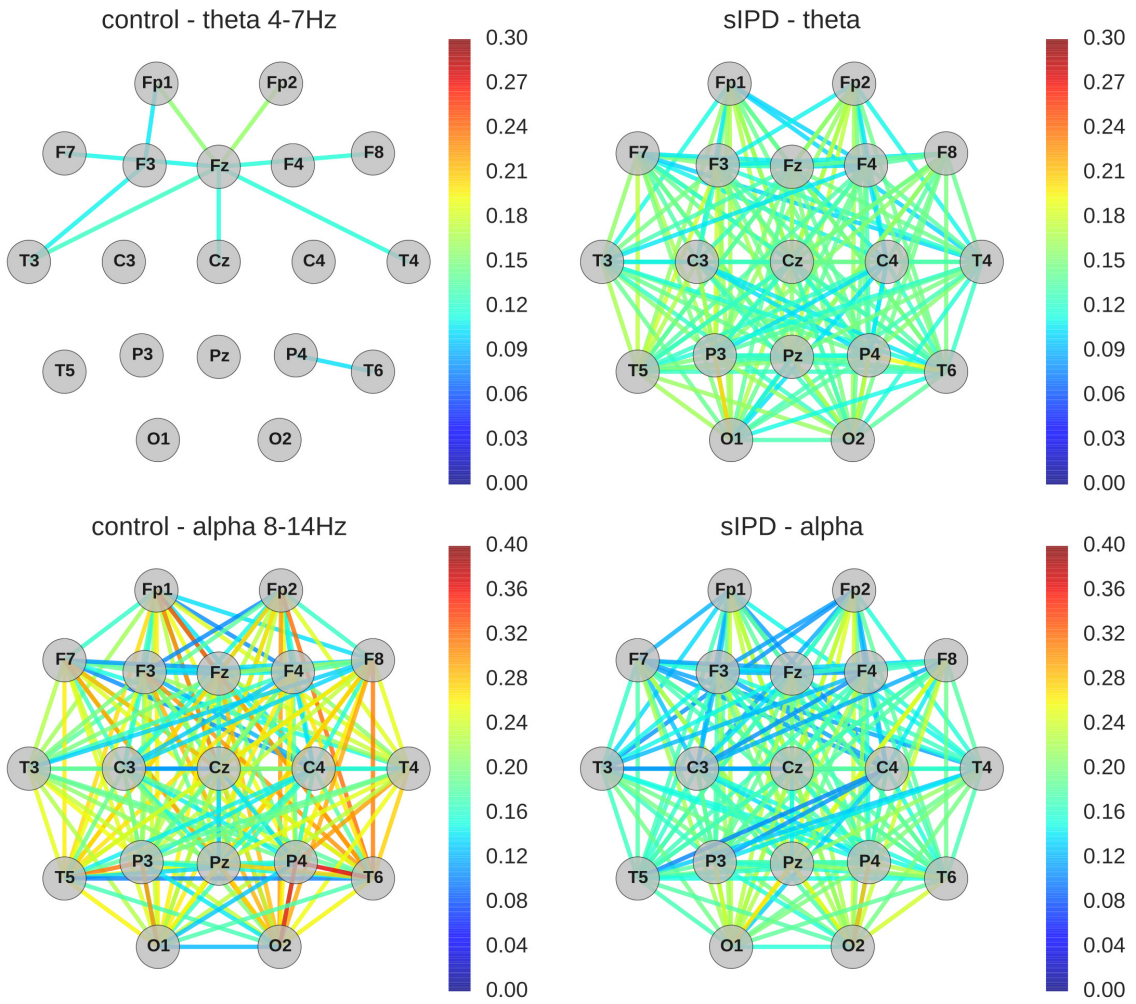
Support: NIHR-BRC

MRC grant

Title: Electroencephalogram-based functional connectivity changes in patients with prion diseases

Authors: *E. FRANKO[1], O. JOLY[2], T. WEHNER[3], S. MEAD[4];
[1]Nuffield Dept. of Clin. Neurosciences, Univ. of Oxford, Oxford, United Kingdom; [2]MRC Cognition and Brain Sci. Unit,, Cambridge, United Kingdom; [3]Natl. Hosp. for Neurol. and Neurosurg., London, United Kingdom; [4]NHS Natl. Prion Clinic, Natl. Hosp. for Neurol. and Neurosurgery, UCL, London, United Kingdom

Abstract: Prion diseases are universally fatal and often rapidly progressive neurodegenerative diseases. Electroencephalography (EEG) has long been used in the diagnosis of sporadic Creutzfeldt-Jakob disease, however, not much is known about the changes in connectivity. Here, we characterise the EEG-based connectivity in different types of human prion disease.
In the National Prion Monitoring Cohort study we recorded encephalography on 301 occasions in 29 healthy controls and 67 patients with prion disease. The patients had either inherited prion disease or sporadic Creutzfeldt-Jakob disease. In the sensor space, we computed the de-biased squared weighted Phase Lag Index (Vinck et al., 2011) as a measure of connectivity in three frequency bands (theta, alpha, beta) for patients with asymptomatic, symptomatic inherited prion disease, sporadic Creutzfeldt-Jakob disease and for healthy controls.
We found reduced connectivity in the alpha and beta bands in symptomatic patients compared to healthy controls. In contrast, in the theta band the connectivity was stronger in the symptomatic patient group than in controls (Figure 1). Moreover, similar differences were found between patients with asymptomatic and symptomatic inherited prion disease.
To the best of our knowledge, this is the first study that reports changes in EEG-based connectivity in different types of prion diseases. Our results suggest that this quantitative EEG-based functional connectivity separates the symptomatic patients from controls and from asymptomatic PRNP gene mutation carrier.



Disclosures: **E. Franko:** None. **O. Joly:** None. **T. Wehner:** None. **S. Mead:** None.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.22/H23

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: C5R Grant 365116

Title: Inhibitory control in alzheimer's disease, mild cognitive impairment and healthy aging: an fmri study

Authors: *A. C. LUEDKE[1], J. FERNANDEZ-RUIZ[2], A. GARCIA[1], D. P. MUNOZ[1];
[1]Ctr. for Neurosci. Studies, Queen's Univ., Kingston, ON, Canada; [2]Physiol., Natl. Autonomous Univ. of Mexico, Mexico City, Mexico

Abstract: Alzheimer's disease (AD) and amnestic mild cognitive impairment (aMCI) are associated with cognitive changes including response inhibition; a capacity commonly measured using the Stroop task. Although compensatory increases in neural activity during the Stroop task have been reported across aging, it is still uncertain whether AD and aMCI lead to a further increase in activity changes. The goal of this work is to elucidate the neural correlates of inhibitory control, as measured by Stroop interference, using fMRI, in AD, aMCI, and healthy older adults. 16 mild AD participants (mean age 74.6 ± 7.6, 8 female), 16 age-matched controls (mean age 74.5 ± 7.6, 8 female), and 12 participants with aMCI (mean age 64.4 ± 9.3, 6 female) completed a rapid event-related version of the Stroop task. We contrasted incongruent minus congruent conditions at stimulus onset to investigate neural activity related to Stroop interference within each group, i.e. conflict between stimulus word and colour (e.g. green written in yellow). Verbal responses were recorded, and Stroop interference (incongruent reaction time - congruent reaction time), and number of errors were calculated. There were significant group differences on Stroop interference and number of incongruent errors. The AD group had a significantly greater Stroop effect, and made more incongruent errors, compared to the aMCI and control groups. The imaging analyses showed that controls had more activity in brain areas relating to the incongruent condition (during inhibition) compared to AD, including the inferior frontal gyrus, precuneus, anterior cingulate, and dorsolateral prefrontal (DLPF), and orbitofrontal cortices. aMCI participants showed similar activity to the control group, with the exception of less activity in the DLPF and orbitofrontal cortices. In conclusion, when compared to healthy older adults the AD group showed hypoactivations in brain areas involved in inhibitory control, suggesting the ability to compensate when faced with interference is altered in AD. This is in line with the behavioural data, which revealed a significantly greater Stroop interference and more errors in AD. Despite having similar behavioural performance on the Stroop task as controls, the aMCI group showed neural changes related to inhibitory control, less activity in the DLPF and orbitofrontal cortices, which may suggest early changes not yet detectable behaviourally in the Stroop task.

Disclosures: **A.C. Luedke:** None. **J. Fernandez-Ruiz:** None. **A. Garcia:** None. **D.P. Munoz:** None.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.23/H24

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIRG-14-317353

NR015452

Title: Longitudinal alteration of intrinsic neural activity in the striatum in mild cognitive impairment

Authors: *P. REN[1], R. LO[3], B. CHAPMAN[1], M. MAPSTONE[4], A. PORSTEINSSON, 14623[2], F. LIN[1];
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Abstract: Objective: To identify neural targets for early detection and prevention of Alzheimer's disease (AD) associated neurodegeneration, the current study investigated the longitudinal change of the striatum, and the relationship between striatal dysfunction and AD pathology in mild cognitive impairment (MCI). **Methods:** Two-year longitudinal resting-state fMRI data from 15 healthy control (HC) and 20 MCI participants in the Alzheimer's Disease Neuroimaging Initiative (ADNI) database were obtained. We analyzed the amplitude of low-frequency fluctuations (ALFF) (0.01-0.08 Hz) and further decomposed two frequency bands (slow-4: 0.027-0.073 Hz; and slow-5: 0.01-0.027 Hz) in the caudate and putamen, two main components of the striatum. Measures of cerebrospinal fluid pTau and beta-amyloid$_{1-42}$ from baseline were used to calculate Aβ/pTau ratio. **Results:** Compared to the HC group, the MCI group showed significantly greater decline in putaminal ALFF, including the slow-4 band. Controlling for age and group (MCI vs. HC), lower baseline Aβ/pTau ratio was significantly associated with greater decline of ALFF in the right putamen in the overall sample. The slow-4 band, relative to slow-5 band, showed a stronger correlation between Aβ/pTau ratio and decline of ALFF in the right putamen. **Conclusions:** The function of the putamen declines early in the AD-associated neurodegenerative process. The abnormality of ALFF in the putamen, particularly the slow-4 frequency band, may be a sensitive measure of evolving AD pathology regardless of the current clinical MCI diagnosis.

Disclosures: P. Ren: None. **R. Lo:** None. **B. Chapman:** None. **M. Mapstone:** None. **A. Porsteinsson:** None. **F. Lin:** None.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.24/H25

Topic: C.02. Alzheimer's Disease and Other Dementias

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NIH Grant AG043503

Wyncote Foundation

Arking Family Foundation

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Title: Structural MRI shows accelerated reduction in gray matter density and white matter integrity in asymptomatic progranulin mutation carriers

Authors: *C. A. OLM[1], C. T. MCMILLAN[1], D. J. IRWIN[2], V. VAN DEERLIN[3], P. A. COOK[4], J. C. GEE[4], M. GROSSMAN[1];
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Abstract: Objective: Mutations in the progranulin (*GRN*) gene are associated with frontotemporal degeneration (FTD) spectrum disorders due to TDP-43 inclusions. Potential treatments are likely to halt or slow disease progression, but not reverse degeneration. Therefore, it is essential to identify biomarkers preferably before the onset of symptoms, if possible. We use longitudinal structural and diffusion MRI to identify regions displaying early degeneration in asymptomatic *GRN* mutation carriers (aGRN+), relative to family members who are non-carriers (aGRN-). Methods: Cognitively normal relatives of clinically diagnosed GRN+ FTD patients were identified as either aGRN+ (N=11, mean age=46.5) or aGRN- (N=6, mean age=39.7). For inclusion in this study, each participant completed two MRI sessions (mean intervals: aGRN+=3.2 years, aGRN-=4.0 years) with T1-weighted and diffusion tensor imaging (DTI) sequences. Voxel-wise difference images were created for each participant by subtracting the follow-up cortical gray matter probability (GMP) image from the baseline GMP image. Similarly, difference images were created using radial diffusivity (RD) to examine white matter (WM). We expect small differences to represent normal aging related changes in both groups, with larger values representing degeneration in the aGRN+ participants. We performed voxel-wise whole-brain comparisons of the difference images to identify regions of greater change in

aGRN+ participants relative to aGRN- participants ($p<0.05$, cluster extent>600mm^3). Finally, we used DTI tractography to find WM tracts connected to any affected GM region. Results: aGRN+ participants had greater decrease in GMP than aGRN- in left inferior temporal and supramarginal cortices. In white matter, aGRN+ participants had greater increase in RD in bilateral superior longitudinal fasciculi (SLF) and corona radiata. These results indicate accelerated declines in both GM density and white matter integrity in aGRN+ participants. The WM tracts connected to the identified GM regions overlapped the clusters of worsening WM in the left SLF. Conclusions: Longitudinal MRI provides evidence of accelerated GM and WM structural changes in aGRN+ participants relative to aGRN- family members. The affected regions have previously been shown to exhibit disease in clinically diagnosed GRN+ FTD patients. Thus, a potential biomarker for treatment trials may be structural changes in these regions in aGRN+ participants.

Disclosures: **C.A. Olm:** None. **C.T. McMillan:** None. **D.J. Irwin:** None. **V. Van Deerlin:** None. **P.A. Cook:** None. **J.C. Gee:** None. **M. Grossman:** None.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.25/H26

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: FWO to postdoc AVdJ

IWT to P.hD. student DS

Title: The olfactory system and its relevance for behavioral screening and diagnostic imaging in neurological disorders.

Authors: *A. VAN DER JEUGD[1], D. SHAH[2], I. BLOEMEN[1], L. VAN DEN BROECK[1], P. HANSQUINE[1], A. VAN DER LINDEN[2], R. D'HOOGE[1];
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Abstract: There is considerable variation in the prevalence and magnitude of olfactory dysfunction among neurodegenerative diseases. For example, the average olfactory dysfunction of Alzheimer's, Parkinson's, Huntington's and Down syndrome is severe (clinical Smell Identification Test scores ~20). Such findings have led to the suggestion that olfactory testing may aid in the diagnosis of several neurodegenerative diseases. Moreover, the Down syndrome-related dysfunction is unlikely secondary to the Alzheimer's-like neuropathology (amyloid beta

plaques and tau tangles) associated with this disorder because it occurs at an age before this pathology is manifest.
These deficits in odor identification, detection, discrimination, and memory manifest themselves before the time the classic phenotypic elements of the disorders appear, although it is unknown how far in advance the olfactory loss precedes the phenotypic expression. It is noteworthy that patients with multiple sclerosis exhibit olfactory dysfunction proportional to plaque burden in subfrontal lobes. It is also of interest that some patients with Creutzfeldt-Jacob disease present with olfactory dysfunction associated olfactory tract involvement of the prion protein, lending some credence to the concept that the olfactory pathway may represent a route of infection and possible means of spreading the infection.
We are using transgenic Alzheimer's mice as a preclinical model platform for developing ethological behavioral screening tests and imaging biomarkers for pathology, and for evaluating treatments of relevant symptoms. Resting-state fMRI has emerged as a way to probe the brain network in disorders. Furthermore, with the establishment of functional connectivity fingerprints in Alzheimer's brain, and correlating this to behavioral phenotypes related to olfactory dysfunction, we try to improve our understanding of the etiology and progression of Alzheimer's disease and use these tools to track disease progression and treatment response in preclinical trials.

Disclosures: **A. Van Der Jeugd:** None. **D. Shah:** None. **I. Bloemen:** None. **L. Van den Broeck:** None. **P. Hansquine:** None. **A. Van der Linden:** None. **R. D'Hooge:** None.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer Forschung Initiative e.V.

KKF-Grant B15-14

Studienstiftung des deutschen Volkes e.V.

Title: Confirming the network degeneration hypothesis for intrinsic brain networks and for distinct neurodegenerative syndromes

Authors: ***J. NEITZEL**[1,2], J. DIEHL-SCHMID[3], M. ORTNER[3], T. GRIMMER[3], I. YAKUSHEV[4], P. BUBLAK[5], C. PREUL[5], K. FINKE[1], C. SORG[2];

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Abstract: The network degeneration hypothesis suggests that for a given neurodegenerative syndrome, onset and spread of neurodegeneration is associated with a specific brain network and its dysfunction. Here, we tested this assumption for intrinsic brain networks (IBN) in different syndromes of frontotemporal lobar degeneration (FTLD) and two variants of Alzheimer's disease (AD). We expected that each syndrome is associated with reduced intrinsic functional connectivity (iFC) in one core network and potentially in additional networks.
37 biomarker-confirmed FTLD subjects, of which 19 presented with behavioral variant frontotemporal dementia (bvFTD) and 18 with primary progressive aphasia (PPA), and 36 biomarker-confirmed AD subjects, of which 23 presented with the amnestic variant (aAD) and 13 with posterior cortical atrophy (PCA) were assessed by resting-state functional MRI. The following network connectivity measures were determined for canonical IBNs such as the default mode network: (i) network iFC derived from independent component analysis and (ii) degree centrality (DC iFC) derived from graph theory analysis within IBN boundaries. Compared to 18 healthy controls, all four patient groups showed changed network iFC in syndrome-associated core networks. Specifically, in aAD patients we found reduced iFC in the default-mode network and additionally in the right and left attention networks. In PCA patients, iFC was reduced in the dorsal attention network and additionally in the default-mode network. For bvFTD patients, changed iFC was found in the saliency network as well as in the default-mode, right and left attention networks. PPA patients showed changed iFC in the left attention network and additionally in the saliency network. Furthermore, areas of reduced DC iFC overlapped with standard IBN templates taken from the literature.
The present findings suggest that the assumptions made by the network degeneration hypothesis applies to large-scale IBN. It seems that changes of iFC are tied to the brain's functional network architecture.

Disclosures: J. Neitzel: None. **J. Diehl-Schmid:** None. **M. Ortner:** None. **T. Grimmer:** None. **I. Yakushev:** None. **P. Bublak:** None. **C. Preul:** None. **K. Finke:** None. **C. Sorg:** None.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.27/DP02 (Dynamic Poster)

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Research UK

BRACE Charity

Title: Memory and hippocampal volume in behavioural variant Frontotemporal Dementia.

Authors: *C. PENNINGTON[1,3], D. TSIVOS[3], B. WOOD[2], M. KNIGHT[2], S. DILLON[3], R. KAUPPINEN[2], E. COULTHARD[1,3];
[1]ReMemBr Group, [2]Exptl. Psychology, Univ. of Bristol, Bristol, United Kingdom; [3]North Bristol NHS Trust, Bristol, United Kingdom

Abstract: Background: Behavioural variant frontotemporal dementia (bvFTD) is typified by profound alterations to personality and behaviour. Historically, memory was considered to be relatively preserved, but there is now increasing recognition of the impact bvFTD has upon memory, even in the early stages of the disease. The underlying neural substrates for amnesia are thought to differ between bvFTD and Alzheimer's disease (AD): hippocampal atrophy is primarily implicated in AD, whilst frontal network dysfunction is hypothesised to contribute to deficits in bvFTD. We used cutting-edge MRI techniques to investigate hippocampal sub-region atrophy in bvFTD and AD cohorts and healthy older adults, alongside ratings of anterograde visual and verbal memory. We hypothesise that AD and bvFTD affect distinct hippocampal subfields.
Methods: Patients with a clinical diagnosis of bvFTD or AD and age matched cognitively healthy control subjects underwent MR brain imaging, and completed the Montreal Cognitive Assessment (MoCA), the Hopkin's Verbal Learning Test Revised (HVLT-R) and Brief Visual Memory Task (BVMT).The hippocampus was segmented into 6 regions: Cornu ammonis 1, 2 and 3, Dentate Gyrus, Strata radiatum/lacunosum/moleculare and Subiculum, with separate volumes measured for the right and left hemispheres. Ethical approval for this study was obtained from the Frenchay NHS Research Ethics Committee.
Results: Preliminary data in 12 AD, 7 bvFTD and 49 healthy older people demonstrates significant impairment in HVLT-R and BVMT total recall scores in both patient groups. AD and bvFTD patients showed significant impairment on the total recall score of the BVMT and HVLT-R ($p<0.0001$, unpaired t test). No differences could be detected between the AD and bvFTD groups. Not all patients were able to undergo MR scanning: preliminary analysis showed equivalent degrees of atrophy of all hippocampal sub-regions in the bvFTD (N=5) and AD groups (N=6).
Discussion: This study found significant memory impairment in bvFTD, with affected individuals performing at a level equivalent to AD on tests of both visual and verbal memory. Intriguingly, we found no differences in the degree of hippocampal sub-region atrophy present in bvFTD and AD. Although sample sizes are currently small, this raises the possibility that episodic memory deficits seen in AD and bvFTD could both be due to hippocampal atrophy, rather than having separate neural substrates. Further testing aims to increase sample size and seek correlations between cognitive performance and specific hippocampal sub-regions.

Disclosures: C. Pennington: None. **D. Tsivos:** None. **B. Wood:** None. **M. Knight:** None. **S. Dillon:** None. **R. Kauppinen:** None. **E. Coulthard:** None.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

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Program#/Poster#: 40.28/I2

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Mary S. Easton Center for Alzheimer's Disease Researc recruitment fund for PI

R01 AG051386-01

Title: Novel extracellular vesicle-based approach for alzheimer's disease diagnosis and monitoring effects of the treatment

Authors: *T. V. BILOUSOVA, J. CAMPAGNA, M. ALAM, P. HEINZELMAN, D. BAI, P. SPILMAN, V. JOHN;
UCLA, Los Angeles, CA

Abstract: Alzheimer's disease (AD) is the most prevalent age-related form of dementia and currently no cure exists. Many potential therapeutics have been tested in the clinic and have failed. Two main reasons why clinical trials of potential new AD therapeutics have not been successful include: the late stage of intervention and the absence of sensitive, noninvasive methods to follow the effect of treatment on pathology-related biomarkers to enable optimization of the drug dose. We are attempting to address this problem by using an extracellular vesicle-based approach. The ability to detect brain-derived extracellular vesicles (EVs) in blood opens a window into the brain and creates the possibility of development of brain-specific liquid biopsy for AD and other neurological conditions. Our new method comprises immunopanning-magnetic isolation of brain-derived EVs from plasma with consecutive analysis of EV surface markers using a recently developed "ExoScreen" method (Yoshioka et al., 2014) based on Perkin-Elmer's AlphaLISA (a bead-based luminescent oxygen channeling immunoassay) technology, and downstream analysis of the disease stage and treatment-specific biochemical changes in EV compositions. We are currently testing the method using AD animal models being treated with selective BACE inhibitors and SIRT1 enhancers. We are also performing longitudinal analysis of human plasma samples from patients with known brain imaging and mini-mental state examination (MMSE) status to correlate brain biochemical and functional changes with the brain-derived EV composition in blood. The method is aimed to overcome heterogeneity in brain-derived EV quantity within plasma samples and detect small scale changes in the disease-specific and treatment-related biomarkers. When fully developed, our method may create a non-invasive blood-based early diagnostic test for AD, a way to monitor drug treatment effects, and to identify novel biomarkers and targets for new AD therapeutic development.

Disclosures: **T.V. Bilousova:** None. **J. Campagna:** None. **M. Alam:** None. **P. Heinzelman:** None. **D. Bai:** None. **P. Spilman:** None. **V. John:** None.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 40.29/I3

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Severity score a predictive tool for Alzheimer's disease

Authors: *F. V. CHIRILA[1], D. L. ALKON[2];
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Abstract: The value of diagnostic biomarkers derives from their ability to monitor disease progression and remission, as well as their predictive accuracy before the onset of the disease. Detection of the disease even before its onset, could provide important opportunities for prevention and/or planning therapeutic strategies. In the early stages of Alzheimer's disease (AD), within four years from the dementia onset, clinical diagnosis has a limited rate of success. Furthermore, clinical diagnostic accuracy before dementia onset has not been previously validated. Here, we present evidence for the potential predictive value of three peripheral human biomarkers for Alzheimer's disease (AD): Morphometric Imaging, PKCε and AD Index. The Biomarker Severity Score as described herein may be a continuous logistic fit function on the normalized values, between 0 and 100% of the output signal of the biomarkers for the Age-matched control (AC) and Alzheimer's disease (AD) patients. The gap in the severity score between the AD patients and AC patients is greater than 40% ($P<0.0023$, two tailed t-test, two samples, unequal variance) for each biomarker and indicates that each of these three biomarkers have the potential to detect the signature of Alzheimer's disease several years before the dementia onset($P<0.012$, two tailed t-test, two samples, unequal variance). Strategies for detecting AD before dementia onset with these biomarkers are presented here.

Disclosures: **F.V. Chirila:** None. **D.L. Alkon:** None.

Poster

040. Diagnostic Biomarkers for Alzheimer's Disease

Location: Halls B-H

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Program#/Poster#: 40.30/I4

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: AG12101

AG022374

HL111724

HL118624.

AG 08051

AG 13616

Title: High burden of vascular risk factors is associated with greater longitudinal increase in CSF total Tau.

Authors: *L. GLODZIK[1], T. BUTLER[2], C. RANDALL[2], E. TANZI[2], R. OSORIO[2], H. RUSINEK[2], A. DESHPANDE[2], Y. LI[2], M. DE LEON[2];
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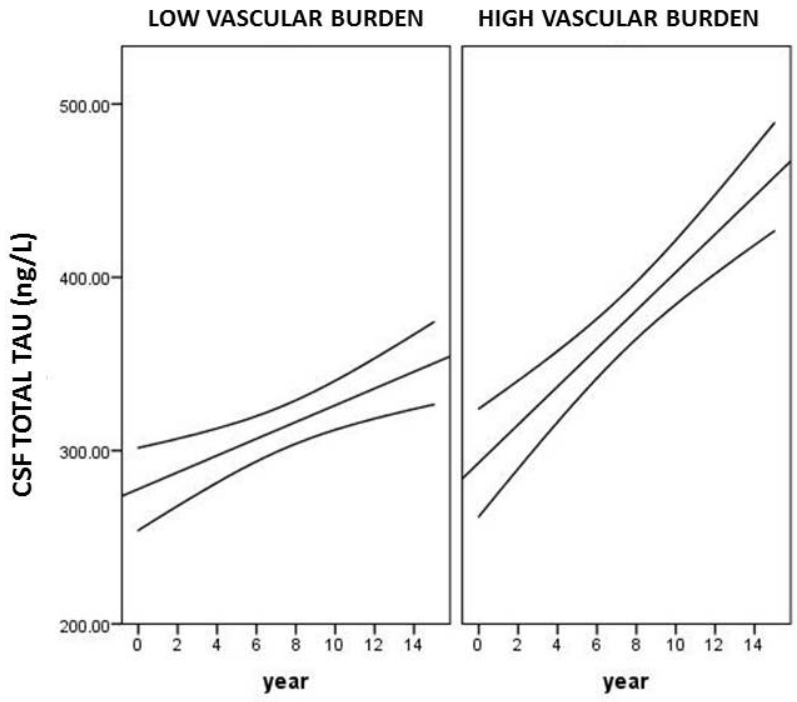
Abstract: Background: Although the contribution of vascular risk to Alzheimer's diseases (AD) widely acknowledged, little is known how vascular conditions affect cerebrospinal fluid (CSF) biomarkers of AD. We investigated both cross-sectional levels and longitudinal trajectories of phosphorylated tau (p-tau), total tau (t-tau) and amyloid beta 42 in cognitively healthy subject with high and low vascular risk burden. Methods: Data was collected from 307 (mean age 64.1 ± 9.8 years, 63% female) subjects enrolled in NIH funded studies of aging and cognitive decline at the Center for Brain Health. All subjects underwent medical, neuropsychological, neurological and MRI examinations, and a lumbar puncture. CSF p-tau, t-tau and amyloid beta 42 were assayed. Baseline vascular risk burden was defined based on the Framingham Cardiovascular Risk Profile: as high ≥ 10% (n=91) or low <10% (n=216). Longitudinal data was available for 143 subjects; mean follow-up time was 3.9 ± 2.8 years. Results: At baseline high and low burden groups did not differ in any CSF biomarker. However, subjects with high vascular burden had greater increase in total tau longitudinally: Random effects mixed model: group*time interaction at p=.04, with age and baseline tau levels as covariates (Figure 1). These effects were not found for p-tau or amyloid beta 42. Conclusion: Our findings support the notion that vascular risk factors are related to increases in CSF marker of neuronal damage. We did not find evidence that more specific AD markers: ptau or amyloid beta 42 were affected.



Disclosures: **L. Glodzik:** None. **T. Butler:** None. **C. Randall:** None. **E. Tanzi:** None. **R. Osorio:** None. **H. Rusinek:** None. **A. Deshpande:** None. **Y. Li:** None. **M. de Leon:** None.

Poster

041. Postural Instability, FOG, and Kinematics in Parkinson's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 41.01/I5

Topic: C.03. Parkinson’s Disease

Support: MnDrive

Title: Avoiding virtual obstacles during treadmill gait in Parkinson's disease

Authors: ***C. LU**[1,2], M. MCCABE[1,2], E. TWEDELL[1,2], S. E. COOPER[1,2];
[1]Dept. of Neurol., [2]Motor Neurophysiol. Lab., Univ. of Minnesota, Minneapolis, MN

Abstract: Gait disorders are one of the most disabling symptoms of Parkinson's disease (PD) and one of the most refractory to treatment. We evaluated people with PD and healthy controls using a virtual obstacle avoidance task during visually cued treadmill walking.
We compared healthy controls to PD patients in the off-medication state. Participants were instructed to step on virtual "stepping stones" (blue squares projected onto the treadmill) during treadmill walking. Unpredictably, a stepping stone would change to an obstacle (red striped square). The participants were coached to step short to avoid the obstacles. Treadmill speed and the spacing of the stepping stones were set to match the walking speed and step length determined from self-paced overground walking. We measured the time of obstacle appearance relative to ipsilateral toe-off, and classified the following step as successful if the participant avoided stepping on the obstacle, failed otherwise. Probability of success was strongly associated with the time of obstacle appearance, with earlier-appearing obstacles more easily avoided. Logistic regression analysis demonstrated more obstacles were successfully avoided when appearing earlier. An independent *t* test showed systematic differences between groups, with PD patients requiring more time than controls to achieve equivalent obstacle-avoidance success rates.
The results demonstrate that in order for PD patients to successfully avoid an obstacle, they require a longer time to respond compared to healthy individuals. This could contribute to high fall risk in PD in daily activities. Further studies are warranted to investigate the mechanisms underlying the need for more response time. Possible mechanisms may include, but not be limited to, disturbances in motor planning (i.e., deficits in sensory and time perception), movement execution (i.e., unable to properly generate forces due to bradykinesia or delayed movement due to freezing of gait) or disordered response inhibition.

Disclosures: **C. Lu:** None. **M. McCabe:** None. **E. Twedell:** None. **S.E. Cooper:** None.

Poster

041. Postural Instability, FOG, and Kinematics in Parkinson's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 41.02/I6

Topic: C.03. Parkinson's Disease

Support: Michael J Fox Foundation

Title: Towards responsive deep brain stimulation for medically refractory freezing of gait in parkinson's disease.

Authors: *R. MOLINA[1], J. SHUTE[1], E. OPRI[1], D. MARTINEZ-RAMIREZ[2], K. FOOTE[2], A. GUNDUZ[1], M. OKUN[2];

[1]J. Crayton Pruitt Family Dept. of Biomed. Engin., [2]Ctr. for Movement Disorders and Neuroregeneration, Univ. of Florida, Gainesville, FL

Abstract: Freezing of gait (FoG) is a poorly understood symptom of Parkinson's Disease (PD) affecting ~53% of patients regardless of disease progression or medical therapy. Common PD treatments, such as medication or deep brain stimulation (DBS), do not consistently provide adequate management of FoG. There is an unmet and pressing need to develop novel therapeutic strategies to treat medically refractory FoG. Therefore, we seek to describe the role of the pedunculopontine nucleus (PPN), a brain stem structure found to excite spinal central pattern generators, in walking to outline the underlying neural mechanisms and pathogenesis of FoG. We will identify electrophysiological features of FoG for real-time detection of freezing. DBS paradigms will be designed using the extracted features and its effectiveness will be assessed using clinical scoring standards. Algorithms will be used concurrently to a neurologist's assessment of onset and cessation of FoG episodes.

All participants must meet preset criterion on the FoG-Q and a minimum of 5 freezing episodes incited by provocation protocols. Five patients will receive bilateral globus pallidus interna (GPi) and PPN DBS electrode implantation with two Activa PC+S Neurostimulation system, (Medtronic, Minneapolis, MN), one for each region. These novel devices allow simultaneous stimulation and recording from the depth electrodes using the Medtronic Nexus-D system, an external device enabling real-time control and data. Neural data is concurrently collected from multiple EMG+acceleration sensors (Delsys, Inc., Natick, MA), an 8-camera motion capture system (Vicon Peak, Oxford, UK), and ground reaction forces (Bertec, Newton, MA) over two-day monthly visits.

We have observed mu-low beta activity in GPi suppressed with medication and with increased task intensity. Similarly, low frequency activity (<10Hz) in the PPN increases with medication and with the intensity of the task. Using the clinical labels of FoG, low frequency activity has been identified to correlate to FoG episodes, a threshold detector was implemented and compared to a neurologist's time-aligned labeling of onset and cessation of FoG episodes during a continuous walking task. The subjects were asked to preform different tasks including freezing inducing tasks. Our initial results are promising and consistent across 3 patients who have come back for post-surgical recordings. There is current work in determining optimal detection and stimulation parameters.

Disclosures: R. Molina: None. **J. Shute:** None. **E. Opri:** None. **D. Martinez-Ramirez:** None. **K. Foote:** None. **A. Gunduz:** None. **M. Okun:** None.

Poster

041. Postural Instability, FOG, and Kinematics in Parkinson's Disease

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Program#/Poster#: 41.03/I7

Topic: C.03. Parkinson's Disease

Support: NIH NINDS R01 NS070265

NIH NCATS UL1TR000114

MnDRIVE Postdoctoral Fellowship

NSF IGERT 0903622

Title: Biomechanical and electromyographic events preceding and accompanying freezing episodes during gait initiation in people with Parkinson's disease

Authors: *C. D. MACKINNON[1,2], M. N. PETRUCCI[4,5], S. L. AMUNDSEN HUFFMASTER[3,2], E. T. HSIAO-WECKSLER[4], P. J. TUITE[3], C. LU[3,2];
[1]Neurol., Univ. of Minnesota Dept. of Neurol., Minneapolis, MN; [2]Movement Disorders Lab., [3]Neurol., Univ. of Minnesota, Minneapolis, MN; [4]Mechanical Sci. and Engin., [5]Neurosci. Program, Univ. of Illinois Urbana-Champaign, Urbana-Champaign, IL

Abstract: Approximately one half of people with Parkinson's disease (PD) will develop a debilitating symptom termed freezing of gait (FOG). FOG is characterized by sudden episodes of inability to produce effective stepping during which the individual feels that their feet are "glued to the floor". These episodes are usually accompanied by a characteristic festination ("trembling of the knees") and are often triggered during gait initiation, turning, navigating through narrow doorways or during obstacle avoidance. Currently, the mechanisms causing FOG are poorly understood. Studying the pathophysiology and biomechanics of FOG is greatly hampered by the difficulty with capturing FOG episodes in the laboratory environment. Here we report data from rare incidents in which multiple FOG episodes were captured with quantitative methods. Specifically, we examined if FOG episodes were preceded by a common sequence of events (e.g. reduced or absent anticipatory postural adjustments) that precipitate the failure to achieve toe-off and trigger festination. The data were captured during studies examining the effects of external cueing and/or mechanical assistance on gait initiation compared to self-initiated gait (Lu et.al., 2015; Petrucci et al., 2015). Whole-body 3D kinematics, ground reaction forces (GRFs) and center of pressure (CoP) from two force platforms, surface EMG bilaterally from 5 lower limb muscles, and accelerometry (3-axes) of the lower leg and thigh were collected. Freezing episodes were defined based on: failure to achieve toe-off, and the presence of repetitive oscillations of the lower limb (festination) and accompanying EMG bursts. There were two main findings from

the analyses of these trials. First, reduced or absent anticipatory postural adjustments were not a prerequisite for the triggering of an FOG episode. Second, FOG episodes were preceded by a failed unloading (no swing phase) followed by rapid loading of the intended step leg and an unloading of the intended stance leg (reciprocal APAs). This unexpected reloading of the intended step leg was accompanied by a paradoxical rise of the heel (sometimes simultaneously on both sides) and flexion of the knee and hip, akin to the kinematic pattern of another push-off phase. Rise of the heel(s) appeared to trigger reciprocal right-left oscillations (approx. 10 Hz) in forces and EMG that characterize festination. These findings suggest that FOG is triggered by failed execution of stepping leg unloading and an inappropriately-timed second push-off phase of the intended stepping leg.

Disclosures: C.D. MacKinnon: None. **M.N. Petrucci:** None. **S.L. Amundsen Huffmaster:** None. **E.T. Hsiao-Wecksler:** None. **P.J. Tuite:** None. **C. Lu:** None.

Poster

041. Postural Instability, FOG, and Kinematics in Parkinson's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 41.04/I8

Topic: C.03. Parkinson’s Disease

Support: PSC

FRSQ

Title: Proprioceptive training improves reaching movement accuracy and postural stability limits in Parkinson’s disease

Authors: *S. BERGERON[1,2], P. BLANCHET[1,3], D. MONGEON[1], M. BLANCHET[1], M. JEAN-DÉSILETS[1], J. TREMBLAY[1], F. PRINCE[1], J. MESSIER[1,2];
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Abstract: Evidence suggests impaired proprioceptive processing in Parkinson’s disease (PD). The aim of this study was to examine the impact of a proprioceptive-based exercise program on the performance of PD patients in two complex motor tasks that critically depend on proprioceptive processing. We assessed the performance of PD patients and aged-matched healthy controls (HC) in a three-dimensional (3D) reaching movements (n=10 PD, n=20 HC) and a postural stability limits tasks (n=11 PD, n=15 HC). In the reaching task, subjects

performed arm movements aimed at 3D targets defined by either vision or proprioception. The positions of the tip of the index finger and arm segments were recorded using an Optotrak motion analysis system (NDI inc.). In the postural task, subjects stood on a force platform (AMTI, inc.) with bare feet at comfortable stance width and arms crossed on the chest. They were instructed to lean as far as possible in four directions (forward, backward, rightward and leftward) without lifting their feet or flexing their hips and to maintain this maximal leaning position for 10 sec. Subjects were tested with and without vision. Accuracy and variability of reaching movements and center of pressure displacements during the stabilization phase of leaning movements were analyzed. PD patients were tested twice, before (pre-test) and after a 12 week proprioceptive training program (post-test). In the pre-test, PD patients displayed both a greater level of 3D absolute and variable reaching errors as well as smaller limits of stability relative to HC in the visual and proprioceptive conditions ($p<0.05$). Also, the between group difference in the magnitude of stability limits was larger along the medial-lateral axis than along the anterior-posterior axis ($p<0.05$). The proprioceptive training program significantly increased the spatial accuracy and decreased the variability of reaching movements ($p<0.05$). Furthermore, the program significantly increased the postural stability limits of PD patients, especially along the medial-lateral axis ($p<0.05$). Notably, the exercise program normalized the reaching accuracy and the stability limits in both the visual and proprioceptive conditions ($p>0.05$). These findings suggest that a proprioception-based training program improves the proprioceptive control of reaching and postural stability limits in PD.

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Poster

041. Postural Instability, FOG, and Kinematics in Parkinson's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 41.05/I9

Topic: C.03. Parkinson’s Disease

Title: A sensorimotor training that improves proprioception and transfers to untrained movements in Parkinson's disease

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Abstract: BACKGROUND AND PURPOSE: Recent research shows that sensorimotor training that challenges the proprioceptive system improves proprioceptive acuity and translates to improved motor function. It is well established that people with Parkinson’s disease (PD)

experience proprioceptive impairments along with motor deficits. It is unknown whether proprioceptive function can be enhanced in PD and to what extent improved proprioceptive function translates to improved motor performance. Here, we evaluate whether proprioceptive function in PD can be enhanced by specialized visuomotor training that emphasizes precise movements and determine if such proprioceptive improvements lead to improved motor performance. We administered a sensorimotor training to PD patients using a wrist robotic device coupled with a real-time virtual visual environment. **METHOD**: 12 participants (Mean age = 61.8 yrs; mean disease duration = 2.5 yrs) diagnosed with PD were tested in their ON medication state. Training involved tilting a virtual table to position a virtual ball on a target by making precise small amplitude wrist flexion/extension movements. With increasing proficiency, task difficulty increased by adjusting the responsiveness of the virtual ball. Wrist position sense acuity and the spatial precision of an untrained goal-directed wrist reaching movements were assessed without vision before and after training. Wrist position sense discrimination thresholds were obtained using controlled robotic motion to passively rotate the wrist joint. Mean movement precision error was determined using the absolute difference between passively presented target of 15° wrist flexion and subsequent active movement to the target by the participant. **RESULTS**: All 12 participants showed improvements in wrist proprioceptive thresholds (mean: pre/post = 1.6° / 1.1°). Wrist movement precision in the untrained reaching improved in 9/12 participants (mean: pre/post = 2.6° / 1.9°). **CONCLUSION**: Wrist proprioceptive function improves after brief specialized visuomotor training in PD patients. Movement precision in an untrained motor task also improved on average by 27% in most participants, indicating that such sensory-based training directly benefits motor function. These initial findings are promising and suggest that somatosensory-based training may enhance sensorimotor function in PD.

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Poster

041. Postural Instability, FOG, and Kinematics in Parkinson's Disease

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Topic: C.03. Parkinson’s Disease

Support: Michael J Fox Foundation for Parkinson's Research

The Halpern Foundation

John E Cahill Family Foundation

Title: Subthalamic neural and kinematic features of freezing of gait on and off DBS in freely moving Parkinson's subjects

Authors: *J. SYRKIN-NIKOLAU, Z. BLUMENFELD, T. PRIETO, A. VELISAR, M. TRAGER, T. MARTIN, A. SRIVATSAN, E. QUINN, H. BRONTE-STEWART;
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Abstract: Objective: Freezing of gait (FOG) is a common and debilitating symptom of Parkinson's disease (PD). Previous literature suggests that subthalamic nucleus (STN) resting state high beta band power is greater in self-reported PD freezers versus non-freezers. We report synchronized STN and kinematic data at rest and during movement including freezing episodes (FEs) in PD subjects on and off high frequency (HF) STN deep brain stimulation (DBS). Methods: 13 PD subjects (26 STNs), off-medication, performed a forward walking (FW) task, a stepping in place (SIP) task on dual force plates, and a novel FW turning and barrier course (TBC). STN LFPs were recorded from electrodes 0 - 2 or 1 - 3 of the DBS lead (model 3389, Medtronic, Inc.) via telemetry from an investigational sensing neurostimulator (Activa® PC+S, Medtronic Inc., FDA-, IDE-, IRB-, and CA Medicare-approved) before and while DBS was administered through electrode 1 or 2, respectively. Kinematic data was recorded using wireless Opal® inertial measurement unit (IMU) sensors (APDM, Inc.). Angular velocity signals from IMUs on the lower legs were used to determine gait cycle duration, swing phase duration, and swing angular range. Angular velocity signals from the lumbar IMU were used to determine forward direction and delineate periods of straight walking and turning. Kinematic analysis was performed in LabVIEW (National Instruments, Inc.) and MATLAB (The MathWorks, Inc.). Results: 13 subjects completed FW, SIP, and TBC, 6 of whom had FEs in at least one of the tasks. Preliminary results demonstrated that low beta band power was attenuated during walking when compared to the resting state (standing), which was more prominent in patients who had FEs. In some patients, there was also an attenuation of high beta power during periods of walking with FEs, in comparison to walking without freezing. STN DBS improved FOG in all freezers and attenuated beta band power. Conclusions: In some patients with FOG, neural signals during periods of walking with FEs show a decrease in high beta band power when compared to periods of walking without FEs. Analysis of the synchronized neural and kinematic signals during different walking tasks allows for a more nuanced understanding of the neural features associated with FOG.

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Poster

041. Postural Instability, FOG, and Kinematics in Parkinson's Disease

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NSF EEC-1028725

Title: Kinematic and neural triggered adaptive deep brain stimulation for tremor dominant Parkinson's disease

Authors: *A. VELISAR[1], J. A. HERRON[2], J. SYRKIN-NIKOLAU[1], Z. BLUMENFELD[1], M. TRAGER[1], T. MARTIN[1], H. J. CHIZECK[2], H. BRONTE-STEWART[1];
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Abstract: Objective: determine safety and tolerability of adaptive or closed loop Deep Brain Stimulation (aDBS) in the subthalamic nucleus (STN) in Parkinson's disease (PD), using a neural control variable to drive aDBS and to investigate the effect on resting tremor of aDBS using either a neural or kinematic variable.Methods: Safety and tolerability of neural triggered aDBS (NaDBS) was evaluated in13 PD subjects (16 STNs) implanted with an investigational sensing neurostimulator plus a firmware upgrade (Activa® PC+S-NexusD3 system, Medtronic Inc., FDA-, IDE-, IRB-, and CA Medicare-approved) to enable aDBS using neural or kinematic control variable. The Nexus-D system is a conduit that allows bi-directional communication between the Activa® PC+S and a portable computing device (PC). The PC is an external platform that informs the Activa® PC+S to adjust stimulation using preset safe parameters. STN LFPs were recorded from electrodes 0 - 2 or 1 - 3 of the DBS lead (model 3389, Medtronic, Inc.) and 140 Hz DBS was administered through electrode 1 or 2, respectively. The percentage of stimulation time with tremor less than 0.15 rad/s and the total electrical energy delivered during aDBS as a percentage of TEED during continuous DBS ($TEED_{cDBS}$), was investigated using NaDBS or kinematic triggered aDBS (KaDBS) for > 20 minutes, in a subset of tremor dominant (TD) PD subjects. KaDBS used limb tremor power, recorded from a Bluetooth enabled smart watch, as the control variable.Results: Stimulation changes (ramps and voltage limits) were safe and tolerated in all 13 subjects. The stimulation delivered was in the safe limits imposed. In one TD PD subject during NaDBS, resting tremor was <0.15 rad/s for 33% of stimulated time and

$TEED_{NaDBS}$ = 15% of $TEED_{cDBS}$. During KaDBS, resting tremor was <0.15 rad/s for 62% of stimulated time and $TEED_{KaDBS}$ = 13% of $TEED_{cDBS}$. In another PD subject, NaDBS resulted in no tremor for 95% of stimulated time and $TEED_{NaDBS}$ = 57% of $TEED_{cDBS}$, while KaDBS resulted in no tremor for 53% of stimulated time and $TEED_{KaDBS}$ = 11% of $TEED_{cDBS}$.Conclusions: 1) NaDBS was safe and tolerable in all patients tested; 2) Two TD PD subjects tested to date responded differently to the two aDBS strategies. This suggests that aDBS strategies need to be customized to each PD subject in the TD phenotype.

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Poster

041. Postural Instability, FOG, and Kinematics in Parkinson's Disease

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Program#/Poster#: 41.08/I12

Topic: C.03. Parkinson's Disease

Support: NIH Grant 1R15NS087447

Title: How tremor propagates throughout the upper limb

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Abstract: Tremor in all of its forms (Essential Tremor, Parkinsonian Tremor, Dystonia, etc.) is arguably the most common movement disorder, estimated to affect approximately 10 million people in the US. Medication and surgical interventions have significantly reduced patient suffering, but medication is generally only partially effective, and surgical procedures are highly invasive and reserved for severe drug-resistant tremor, leaving many tremor patients without satisfactory treatment options. Surprisingly, tremor-suppressing devices are underexplored despite great potential promise. However, we do not know where in the upper limb the tremor originates (mechanically), how it propagates, and where manifests most severely, greatly limiting our ability to create effective tremor-suppressing systems. The purpose of this study is to use simulation to understand how tremor propagates through the upper limb, and to determine the feasibility of determining the mechanical origin of tremor from measurements of tremor throughout the upper limb. We have developed a mathematical model of the dynamics of the upper limb suitable for studying tremor propagation. This model focuses on the 7 major degrees

of freedom (DOF) from the shoulder to the wrist. It takes as input the muscle activity in the 14 major muscles acting on these DOF and simulates the resulting force in each muscle, torque in each DOF, and displacement (tremor) in each DOF. This model includes realistic parameter values taken from the literature, including values for muscle time constants and moment arms as well as joint inertia, damping, and stiffness. Because the displacements in each DOF are relatively small, our linear model can capture the dynamics of this non-linear system and allow us to take advantage of existing tools for analyzing the frequency response of linear systems. We have begun to simulate the propagation of tremor by "injecting" tremor in various muscles or DOF and observing patterns in the resulting tremor in each DOF. We will inject oscillatory torque in a single DOF and then move on to combinations of multiple DOF to understand propagation patterns and determine if it is possible to identify from the tremor in each DOF where the tremor originated. Finally, we will determine the effect of changes in model parameters on the propagation pattern and the ability to determine the origin of the tremor. Establishing principles of propagation will help us move closer to our end goal, which is to measure a patient's tremor, determine the origin and propagation of their tremor, and design a system to suppress their tremor in an optimal manner.

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Poster

041. Postural Instability, FOG, and Kinematics in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: Coppenrath-Foundation, Germany

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Title: Postural control and freezing of gait in Parkinson's disease

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Abstract: BACKGROUND: The relationship between freezing of gait (FOG) and postural instability in Parkinson's disease (PD) is not clear. We analyzed the impact of FOG on postural control.
METHODS: 31 PD patients with FOG (PD+FOG), 27 PD patients without FOG (PD-FOG) and 22 healthy control (HC) were included and assessed in the ON state of medication. Postural control was measured with the Fullerton Advanced Balance (FAB) scale and with center of pressure (COP) analysis during quiet stance and maximal voluntary forward and backward leaning.
RESULTS: The groups were well matched concerning age, disease duration and disease severity. PD+FOG had significantly worse scores at the FAB scale (21.8 points, SD ± 5.8) in comparison to PD-FOG (25.6 points, SD ± 5.0) and HC (34.9 points, ± SD 2.4) (p<0.01). During quiet stance the average anterior-posterior COP position was significantly displaced towards posterior in PD+FOG in comparison to PD-FOG and HC (p<0.05). The COP position in the anterior-posterior orientation correlated with the severity of FOG (p<0.01). PD+FOG and PD-FOG did not differ in average COP sway excursion, sway velocity, sway regularity and postural control asymmetry.
CONCLUSIONS: Our results show that PD+FOG have reduced postural control in comparison to PD-FOG and HC. The COP shift towards posterior during quiet stance in PD+FOG leads to a restricted precondition to generate forward progression during gait initiation. The difference in COP position in PD+FOG patients may contribute to the occurrence of FOG or might be an altered stance position as a compensatory strategy to avoid forward falls.

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Poster

041. Postural Instability, FOG, and Kinematics in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

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MnDRIVE Postdoctoral Fellowship

Title: The effects of anodal tDCS over the supplementary motor area on anticipatory postural adjustments during gait initiation in people with Parkinson's disease with freezing of gait

Authors: *S. L. AMUNDSEN HUFFMASTER[1,2], C. LU[1,2], P. J. TUITE[1], C. D. MACKINNON[1,2];
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Abstract: People with Parkinson's disease (PD) who experience freezing of gait (FOG) often show reduced or absent anticipatory postural adjustments (APAs) that are required for balance and forward propulsion during self-initiated gait. Impaired self-initiated movements in PD may result from reduced activity in the supplementary motor area (SMA). Anodal transcranial direct current stimulation (A-tDCS) can be used to facilitate activity in cortical regions underlying the stimulating electrode. We investigated if A-tDCS over the SMA can transiently improve APA magnitudes and timings in people with PD and FOG.

In our double-blinded, cross-over study, 9 PD subjects with FOG (3 females, 68 ± 9 years, off medication) underwent 2 sessions of testing (sham or A-tDCS at 1 mA for 10 min, separated by >1 week). Structural MRI scans and Brainsight software were used to target stimulation to the scalp surface over the SMA. Eight gait initiation blocks (5-6 trials/block) were collected: (1) pre-tDCS, externally cued; (2) pre-tDCS, baseline, self-initiated; and (blocks 3-8) post-tDCS, every 12 min., self-initiated. For self-initiated trials, subjects stood for 3-5 seconds before walking forward. For cued trials, subjects were given acoustic "warning" and "go" tones. Ground reaction forces were collected from force platforms beneath the feet. APA magnitudes and timing were quantified using Matlab (Mathworks, MA). Outcome measures included the magnitudes and times from APA onset to: step foot peak loading force, stance foot peak unloading and loading, peak lateral shift of the center of pressure (COP), and two peak posterior COP shifts, as well as the time to first and second toe-off. Two-way repeated measures ANOVAs (factors: stimulation type and trial block) were performed.

External cueing significantly increased nearly all of the APA measures magnitudes relative to baseline self-initiated trials ($p<0.001$) and decreased most of the time measures ($p<0.01$). There was no effect of stimulation type on APA magnitudes or timings ($p > 0.13$). With A-tDCS, some APA times significantly improved for 1 person, but worsened in 2 subjects. APA magnitudes significantly improved for 2 subjects and worsened in 2 subjects. There was no effect of visit order.

A-tDCS applied over the SMA did not have a consistent effect on people with PD with FOG. Results appear to be subject dependent, although this is confounded by symptom fluctuations and trial-to-trial variability. The lack of significance could be related to A-tDCS dosing and location. Alternatively, our results may suggest that the SMA has a limited role in self-initiated gait impairment in people with FOG.

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Poster

041. Postural Instability, FOG, and Kinematics in Parkinson's Disease

Location: Halls B-H

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Program#/Poster#: 41.11/J3

Topic: C.03. Parkinson's Disease

Title: Impact of the α4β2 partial agonists on gait and motor coordination alterations in a rat model of Parkinson's disease

Authors: *W. M. HOWE[1], K. DLUGOLENSKI[2], A. ROSSI[2], D. VOLFSON[2], P. TIERNEY[2], R. KOZAK[2];
[1]Pfizer Inc, Cambridge, MA; [2]Pfizer, Inc, Cambridge, MA

Abstract: Disruptions in limb coordination, gait, and increased fall propensity are hallmarks of neurodegenerative disorders such as Parkinson's disease that are not ameliorated by standard dopamine replacement therapies. Alterations of gait and fall propensity are hypothesized to arise as top-down, cognitive control of motor output declines as a result of dual dysfunction of striatal dopaminergic and forebrain cholinergic systems. α4β2 nAChR selective nicotinic receptor (nAChR) agonists may mimic increased cholinergic modulation of top-down control, and therefore could represent a promising option for correcting the specific symptoms due to loss of such modulation. In the present set of experiments, we sought to test the capacity of α4β2 nAChR agonists to ameliorate gait disruptions associated with striatal dopaminergic depletion in rats. As a first step, two cohorts of animals (sham lesion; n=6, and unilateral 6-OHDA striatal lesion; n=5) were trained to run on a treadmill. Parametric variations of treadmill speed and angle were employed to provide a dynamic estimate of limb coordination and potentiate the delineation of specific alterations following loss of dopaminergic innervation of the striatum. Results, so far, indicate that there are no gross group differences in the capacity of animals to learn to run on the treadmill or achieve sustained bouts (>6 s) of running at different velocities and angles. Regarding gait symmetry and forepaw/hind paw coordination during running, dopaminergic depletion was associated with alterations in stride length, duration, paw angle, and unilateral inter-leg coordination. On-going experiments directly compare the capacity of α4β2 nAChR agonists to normalize these alterations in lesioned animals relative to l-dopa. The combined results of the present studies seek to extend our knowledge of the neural mechanisms contributing to the alterations in gait that accompany neurodegenerative diseases like Parkinson's, and illustrate the potential benefit of novel, non-dopaminergic treatment strategies such as α4β2 nAChR agonists.

Disclosures: **W.M. Howe:** A. Employment/Salary (full or part-time): Employee of Pfizer, Inc. **K. Dlugolenski:** A. Employment/Salary (full or part-time): Pfizer, Inc. **A. Rossi:** A. Employment/Salary (full or part-time): Pfizer, Inc. **D. Volfson:** A. Employment/Salary (full or

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Poster

041. Postural Instability, FOG, and Kinematics in Parkinson's Disease

Location: Halls B-H

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Center for Mind Brain Restoration

The Bachman-Strauss Dystonia & Parkinson Foundation

Title: Normal and parkinsonian gait signatures in mice and human: a translational approach

Authors: A. WORLEY, C. ASHTON, L. BROOM, C. TOCCI, L. C. SHIH, *V. G. VANDERHORST;
Dept of Neurol., BIDMC, Boston, MA

Abstract: Impairment of gait can manifest itself in various ways and due to pathology at any CNS level. This implicates independent involvement of multiple CNS pathways, but these circuitries remain poorly understood. Powerful functional-anatomical approaches are available to unravel these circuitries in mouse models. However, a major hurdle that stands in the way of translation involves differences between quadruped gait in mice and biped gait in humans. We present an approach that facilitates the comparison of spatial and temporal gait parameters between these models. We will use this approach to study parkinsonian gait.
To capture mouse data we used 9 cohorts of 8-18 C57Bl6J, VGaT-ires-cre, VGluT2-ires-cre, VGluT3-ires-cre, SERT-cre, $VGaT^{fl/fl}$ or $VGluT2^{fl/fl}$ mice. In each group, we manipulated a distinct CNS target. This included 6OHDA microinjections into the substantia nigra or systemic MPTP to induce parkinsonism, and selective modulation or loss of function of forebrain or pontomedullary cell groups using chemogenetic approaches or deletions. Gait data was captured on a runway using high speed video analysis (MATlab). Each cohort served as its own control. For human data, we recruited subjects with Parkinson's Disease (PD) and controls, which walked across a walkway (Protokinetics) at varying speeds. Step by step gait measures were plotted as a function of velocity, and best fit regression models were determined prior to comparing data sets.

In mice, each gait parameter behaves differently as a function of velocity. At walking speeds this can be captured using a simple linear or non-linear regression model. Human data shows remarkably similar relationships. Regression curves of gait parameters in the experimental mouse cohorts shifted differentially, indicating region and cell-type specific control of individual gait parameters. In PD subjects compared to control, curves for stride length and cadence shifted significantly, resembling the MPTP mouse model and two pontomedullary models. This raises the question of whether the pontomedullary reticular formation mediates part of the gait abnormalities seen in PD.
This approach provides a powerful tool to analyze gait in both mouse models and human gait disorders. It opens up translational opportunities to close the gap between mouse and human gait models.

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Poster

041. Postural Instability, FOG, and Kinematics in Parkinson's Disease

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Topic: C.03. Parkinson’s Disease

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ARL

Title: Gait analysis of unilateral 6-OHDA Parkinson’s rat model

Authors: *B. K. HARVEY[1], H. A. BALDWIN[2], P. P. KOIVULA[2], J. C. NECARSULMER[2], K. W. WHITAKER[3,2];
[1]NPR Section, [2]NIDA - NIH, Baltimore, MD; [3]US Army Res. Lab., Aberdeen Proving Grounds, MD

Abstract: Gait analysis is a method for determining locomotor defects related to movement or neurological disorders. It is a particularly valuable method for assessing the progression and severity of a disease phenotype in humans and animal models of conditions associated with movement disorder. Parkinson’s disease (PD) is a progressive neurological disorder, marked by the loss of dopaminergic neurons in the nigrostriatal pathway, that is involved in the production of voluntary movement. Some of the major clinical manifestations of PD are abnormal gait, rigidity, slowness of movement, and shaking. The ability to recapitulate and measure such the

neurological sequelae in rodent models of PD are important for studying and evaluating potential therapeutics. Here we describe the use of a gait analysis system, the CatWalk, to identify objective metrics altered by lesioning of the nigrostriatal pathway in the rat brain. Previously, studies have used CatWalk to analyze gait abnormalities in the 6-hydroxydopamine (6-OHDA) model of PD, but these studies did not account for the effects of speed on reported gait parameters. Here, we account for the effects of speed on CatWalk gait analysis parameters in a unilateral 6-OHDA model of Parkinson's Disease and show that hind paw step cycle parameters, hind paw print area, and step sequence are significantly altered, when compared to non-lesioned animals.

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Poster

041. Postural Instability, FOG, and Kinematics in Parkinson's Disease

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Topic: C.03. Parkinson's Disease

Support: EU FP& 604063 HealthPAC

Title: Sensorimotor integration in Parkinson's disease: optimal or not?

Authors: *S. SENGUPTA[1,2], L. P. J. SELEN[3], W. P. MEDENDORP[3], P. PRAAMSTRA[3]; [1]Donders Ctr. for Neurosci., Donders Inst., Nijmegen, Netherlands; [3]Donders Inst. for Brain, Cognition and Behaviour, [2]Radboud Univ., Nijmegen, Netherlands

Abstract: While slowness of movement is an obligatory characteristic of Parkinson's disease (PD), there are conditions where patients move uncharacteristically fast, attributed to deficient motor inhibition or impulsivity. Here we investigate deficient inhibition in the optimal sensorimotor integration framework, using a virtual ball catching paradigm (Faisal and Wolpert, 2008). In this time restricted task, in which time spend for perception is at the expense of time available for execution, healthy young subjects have been reported to optimally trade-off time for perception and time for action. An adapted version of this virtual ball catching paradigm affords an opportunity to investigate deficient inhibition by requiring participants (PD and controls) to withhold movement till the combined minimum uncertainty has been reached. Participants (7 PD patients and 7 age matched healthy controls) were instructed to catch a ball falling in a parabolic trajectory from the top of the screen. The ball disappeared as soon as movement was initiated to catch the ball, separating the task into distinct perception and action phases. The time at which

the participant initiates movement, switching from perception phase to action phase, is called the "switching time''. In order to compute the optimal switching time we also collected data to quantify sensory uncertainty and motor uncertainty independently. We determined sensory uncertainty as a function of viewing duration and horizontal velocity of the ball and motor uncertainty as a function of movement amplitude and movement time.
The mean switching time (+/- 1SD) for the elderly healthy controls was 620ms +/- 66 ms and that of the Parkinson's disease patients 592ms+/- 56ms. However, in comparison to the age matched healthy controls, PD patients initiated movements qualitatively closer to their statistical optimal switching time, although the performance of PD patients (47% correct) was poorer than the elderly healthy controls' performance (62% correct). Therefore, optimal integration of sensory and motor uncertainty and quantitative switching times suggest contradicting results on impulsivity in Parkinson's disease.

Disclosures: S. Sengupta: None. **L.P.J. Selen:** None. **W.P. Medendorp:** None. **P. Praamstra:** None.

Poster

041. Postural Instability, FOG, and Kinematics in Parkinson's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 41.15/J7

Topic: C.03. Parkinson's Disease

Support: CIHR-DMRF fellowship

Title: Altered modulation of long-latency afferent inhibition by rubber hand illusion in patients with parkinson's disease

Authors: *R. ISAYAMA[1,3,2], G. JEGATHEESWARAN[3,2], M. VESIA[2], K. UDUPA[2], B. ELAHI[3], C. A. GUNRAJ[2], L. CARDINALI[4,5], A. FARNE[5], R. CHEN[3,2];
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Abstract: Objective: To examine multi-modal sensory integration in patients with Parkinson's disease (PD) using Rubber Hand Illusion (RHI) and its influence on sensorimotor integration. **Background:** To perform goal-directed actions, the brain must integrate sensory information about the limb position from multiple sources and transform this information to appropriate motor commands. This process is altered in PD patients as shown in reduced long-latency afferent inhibition (LAI), a neurophysiological parameter for sensorimotor integration that

presumably involves cortical association areas. However, the underlying mechanisms of impaired sensorimotor integration in PD remain unclear. We hypothesized that LAI is altered during the integration of multi-modal sensory inputs. **Methods:** Eleven patients with PD and twelve age-matched healthy controls (HC) were tested. In order to manipulate visuo-tactile-proprioceptive integration, we used RHI paradigm, in which subjects viewed a rubber hand being stroked by a brush during the application of synchronous (test condition) or asynchronous (control condition) brush strokes on their own unseen hand. LAI was elicited by an electrical digital nerve stimulation (DNS) to the participants' index finger followed by transcranial magnetic stimulation (TMS) to the primary motor cortex. The amplitudes of the conditioned (with DNS) motor evoked potentials (MEPs) to the unconditioned (without DNS) MEPs were measured before and immediately after the synchronous or asynchronous condition. PD patients were evaluated either on (PD-ON) or off (PD-OFF) dopaminergic medications on two separate days. **Results:** Behavioral data indicated that both PD and HC groups showed a greater degree of the RHI in the synchronous than the asynchronous condition. Overall, LAI was significantly reduced in PD-OFF compared to PD-ON and HC. Comparison between PD-ON and HC showed significant interaction between condition and group. In the synchronous condition compared to baseline, LAI decreased in HC but increased in PD-ON, suggesting altered LAI response to RHI in PD-ON. **Conclusions:** In PD-OFF, LAI was reduced and was not modulated by RHI indicating the deficit of sensorimotor integration. In the on medication condition, reduced LAI at baseline was improved during RHI by strengthening the inhibitory effect of DNS to M1 suggesting that dopaminergic medications modulate the interaction between visuo-tactile-proprioceptive integration and LAI.

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Poster

041. Postural Instability, FOG, and Kinematics in Parkinson's Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 41.16/J8

Topic: C.03. Parkinson's Disease

Title: Impact of enhancing expectations on self-efficacy and motor learning in individuals with Parkinson's disease

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Abstract: Reduced self-efficacy has been demonstrated as an independent predictor of postural instability and gait deficits for individuals with Parkinson's disease (PD), leading to the possibility that self-efficacy may be a potential target to improve motor performance in PD. Studies in non-disabled adults have shown that boosting self-efficacy via increasing an individual's expectations for future success can enhance motor performance and learning. However, this positive effect remains to be determined in PD. This study aims to investigate the impact of enhancing expectation on self-efficacy and motor learning when individuals with PD practice a novel balance task. Twenty participants with PD practiced balancing on a stability platform by keeping the platform level for as long as possible. Participants were assigned to an enhanced expectancy (EE) group or a control group. At early practice, the EE group received a statement designed to enhance expectation of future task success while the control group received no statement. Feedback was provided to both groups after each 30-s trial in the form of time in balance, calculated as accumulated time when the platform position was within +/-5 degrees from horizontal. Task performance, as measured by time in balance and the number of bouts when the platform was within +/-5 degrees, was assessed during practice and at a retention test conducted 24 hours later. Self-efficacy for 3 levels of task performance (balancing for 10, 15 and 20 seconds) was assessed at 4 time points, while a motivational questionnaire was completed by each participant following practice and retention. Self-efficacy of the EE group significantly increased after the statement for the medium performance level only (i.e. balancing for 15s) (p= .04). Enhanced self-efficacy did not appear to affect time in balance. No group difference was observed in time in balance during practice (p= .88) and at retention (p= .08). However, there was a trend that the EE group achieved similar time in balance with fewer bouts than the control group at retention (p= .09). In addition, the questionnaire showed that the EE group reported greater nervousness than the control group during practice (p= .03) and at retention (p= .02), an unexpected finding that may have counteracted the effect of enhanced self-efficacy. The preliminary results suggest that enhancing expectation appears to boost self-efficacy and result in a modification of motor control that may reflect longer durations of balance stability in PD. Further analysis will explore the impact of nervousness, which may reveal to clinicians the potential importance of improving self-efficacy without inducing nervousness.

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Poster

041. Postural Instability, FOG, and Kinematics in Parkinson's Disease

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: NINDS Grant 1R01 NS089972

DOD Award # SCI140238

Miami Project to Cure Paralysis

Title: MRI guided targeting and stimulation of the mesencephalic locomotor region in the Yucatan minipig

Authors: F. D. BENAVIDES[1], I. OPRIS[1], A. J. SANTAMARIA[1], F. J. SANCHEZ[1], L. M. VILLAMIL[1], Y. NUNEZ-GOMEZ[2], J. P. SOLANO[2], J. D. GUEST[1], *B. R. NOGA[1];
[1]Miami Project To Cure Paralysis, [2]Pediatric Critical Care, Univ. of Miami Sch. of Med., Miami, FL

Abstract: **Objective:** The mesencephalic locomotor region (MLR) is a target of interest to generate locomotion with deep brain stimulation (DBS) after spinal cord injury (SCI). Testing efficacy of DBS in a large animal model is an important translational step. We developed stereotaxic techniques to target the pedunculopontine (PPN) and cuneiform nuclei (CnF) in the MLR of Yucatan minipigs based on magnetic resonance imaging (MRI). A targeting protocol was tested using an MRI compatible frame and accuracy assessed in a gelatin phantom model. Subsequently, the refined protocol was assessed in animals. **Methods:** An MRI compatible frame was designed around the pig's dimensions. Contrast-filled markers permitted 3-dimensional trajectory planning. Gelatin filled porcine skull phantoms were used to test equipment, calculation procedures, and accuracy before animal experiments. The phantoms contained 24 MRI visible cylindrical plastic (PEEK) targets (1mm OD, 2mm length). The software used for trajectory and distance calculations to enter targets inside the gel phantom included Amide and ImageJ. We used a porcine neuro atlas (Felix et al, 1999) to estimate the position of PPN/CnF (medial to the lateral lemniscus below the inferior colliculus 4-6 mm lateral to the aqueduct). At surgery, a second MRI confirmed the trajectory after advancing a MRI compatible cannula. Physiological targeting was initiated under chloralose anesthesia by stimulating the electrode and assessing patterns of locomotor activation in agonist/antagonist muscles using 4 limb electromyography. This activity was assessed in response to variation of the intensity and frequency of the stimulation parameters. **Results:** Locomotor-like patterns were elicited in all animals by stimulation and changes in blood pressure and heart rate were recorded. Phantom testing proved essential to refine the stereotactic methods, resulting in an accuracy

close to 99%. The apparatus and methods allowed successful placement of an MRI-visible cannula and DBS electrode at the intended target. Physiologic testing confirmed activation of patterned locomotor activity. **Conclusion:** We developed accurate, practical methods to target the MLR in minipigs using high resolution MRI and confirmatory neurophysiology. This methodology will support further studies of the impact of graded SCI MLR-evoked locomotor stimulation.

Disclosures: F.D. Benavides: None. **I. Opris:** None. **A.J. Santamaria:** None. **F.J. Sanchez:** None. **L.M. Villamil:** None. **Y. Nunez-Gomez:** None. **J.P. Solano:** None. **J.D. Guest:** None. **B.R. Noga:** None.

Poster

041. Postural Instability, FOG, and Kinematics in Parkinson's Disease

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: NINDS Grant 1R01 NS089972

DOD Award # SCI140238

Title: Deep brain stimulation of the mesencephalic locomotor region improves emg correlation with kinematics in the yucatan minipig

Authors: *I. OPRIS, F. D. BENAVIDES, F. J. SANCHEZ, L. M. VILLAMIL, J. D. GUEST, B. R. NOGA;
Miami Project, Univ. of Miami Miller Sch. of Med., Miami, FL

Abstract: Objective: Deep brain stimulation (DBS) of the pedunculopontine (PPN) and cuneiform nuclei (Cnf) in the mescencephalic locomotor region or MLR (mlrDBS) of the Yucatan minipig is a promising model for assessing therapies aimed at improving locomotion after spinal cord injury (SCI) or Parkinson Disease (PD). However, a detailed quantitative assessment of the electromyographic (EMG) activity with respect to the locomotion kinematics, heart rate (HR), and respiratory rate (RR), in Yucatan minipig is lacking. **Methods**: DBS was applied to PPN and Cnf of the Yucatan minipig assisted with magnetic resonance image based targeting. Locomotion patterns were obtained from EMG activity recorded in agonist/antagonist muscles in all 4 limbs. The EMG activity was tuned by changing stimulation parameters (intensity and frequency) of the targeted region. Physiological and behavioral parameters were collected during stimulation. The coupling of EMG patterns with locomotor kinematic variables

(limbs position, speed and acceleration) and HR/ RR, was characterized using a multi-variate regression model. The coefficient of determination (R^2), defined as the proportion of variance accounted for by the EMG activity-to-behavior multi-variate regression model, allowed us to assess efficacy of stimulation by comparing the effect of DBS of Cnf and PPN nuclei with the control values observed during voluntary locomotion (without DBS). To characterize the phase change during locomotion (stance and swing phases of each limb) we evaluated the phase difference in EMG activity corresponding to locomotion limb phases before and after stimulation. **Results**: DBS elicited locomotor patterns in animals and changes in RR and HR. The regression analysis demonstrated a significant increase in the coefficient of determination with DBS stimulation. Also, the locomotion phase difference decreased following DBS. When the stimulation current increased from 20uA to 40uA and up to 200uA there was a significant change in the speed of locomotion. **Conclusion**: mlrDBS modulation of locomotion may play a key role in the therapeutic improvement of gait disorders associated with SCI and PD. This approach may also be instrumental in the design of brain machine interface (BMI) and neuroprosthetics for SCI or PD patients.

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Poster

041. Postural Instability, FOG, and Kinematics in Parkinson's Disease

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QOLER Medical Group

Sasson Hospital

Title: Posture-gait control by the lateral part of the mesopontine tegmentum

Authors: *K. TAKAKUSAKI[1], M. TAKAHASHI[2], R. CHIBA[2];
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Abstract: Recently the lateral part of the mesopontine tegmentum is one of targets of the deep brain stimulation (DBS) as a therapeutic strategy for treating posture-gait deficiency in Parkinsonian patients. The lateral part of the mesopontine tegmentum contains functionally important structures involved in the control of posture and gait. Specifically, the mesencephalic locomotor region (MLR), which may consist of the cuneiform nucleus (CNF) and pedunculopontine tegmental nucleus (PPN), occupies the interest with respect to the pathophysiology of posture-gait disorders. The purpose of this study is to understand the mechanisms involved in the control of postural muscle tone and locomotion by the mesopontine tegmentum. For this purpose, experiments were performed in decerebrate cat preparations. Our investigations revealed the presence of functional topographical organizations with respect the regulation of postural muscle tone and locomotion in the mesopontine tegmentum, i.e., MLR, are mostly located in the ventral part of the CNF and the vicinity in the dorsal part of the PPN. Moreover, the dorsal part of the CNF and the ventral of the PPN may contribute to the increase and decrease in the level of postural muscle tone, respectively. Therefore, the MLR is, functionally, surrounded by the areas involved in the augmentation and suppression of postural muscle tone. The atonia induction zone well corresponds to PPN pars compacta where abundant cholinergic neurons are located. The organization was modified by neurotransmitter systems, particularly the cholinergic PPN projection to the pontine reticular formation, corresponding to the nucleus reticularis pontis oralis. Bilateral pontine atropine injections reduced the number of atonia-evoking sites while that of the locomotor-evoking region was increased. Because efferents from the forebrain structures as well as the cerebellum converge to mesencephalic and pontomedullary reticular formation, changes in the functional organizations may be involved in the appropriate regulation of posture-gait synergy depending on the behavioral context. On the other hand, abnormal signals from the higher motor centers may produce dysfunction of the mesencephalic-reticulospinal system. Particularly, efferents from the basal ganglia output to the CNF/PPN area may strongly alter the activity of this system in Parkinson's disease. Here we highlight the significance of elucidating the mechanisms of the mesencephalic control of posture and locomotion so that thorough understanding of the pathophysiological mechanisms of posture-gait disorders can be made.

Disclosures: K. Takakusaki: None. **M. Takahashi:** None. **R. Chiba:** None.

Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

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Title: A physiological role for monomeric α-synuclein.

Authors: *M. LUDTMANN[1], P. ANGELOVA[1], N. NINKINA[2], S. GANDHI[1], V. BUCHMAN[2], A. ABRAMOV[1];
[1]Mol. Neurosci., Inst. of Neurol., London, United Kingdom; [2]Sch. of Biosciences, Cardiff Univ., Cardiff, United Kingdom

Abstract: Despite the wealth of publications about misfolded alpha-synuclein in Parkinson's disease, very little has been reported about this protein's physiological function in health. This current study established an important function for unfolded monomeric alpha-synuclein in the regulation of ATP synthase activity. Using mice lacking alpha, beta and gamma-synuclein, we report that extracellular monomeric alpha-synuclein enters the cell and localises to mitochondria, interacts with the ATP synthase and modulates its function. Using a combination of biochemical assays, live cell imaging and mitochondrial respiration analysis we found that mitochondria of alpha, beta, gamma-synuclein knock-out mice are uncoupled as characterized by increased mitochondrial respiration and reduced mitochondrial membrane potential. Furthermore, synuclein deficiency results in reduced ATP synthase efficiency and lower ATP levels. Exogenous application of monomeric alpha-synuclein is able to increase the ATP synthase activity and rescue the mitochondrial phenotypes observed in synuclein-deficiency. Overall, the data suggest that monomeric alpha-synuclein is a previously unrecognised physiological regulator of mitochondrial bioenergetics through its ability to interact with the ATP synthase and increase its efficiency.

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Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

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Program#/Poster#: 42.02/J13

Topic: C.03. Parkinson's Disease

Title: Extracellular and exosomal alpha-synuclein modulate innate immunity and possibly contribute to inflammation in Parkinson's disease

Authors: *V. GROZDANOV[1], N. VERHAGEN[1], C. MEIER[1], L. BOUSSET[2], A. C. LUDOLPH[1], R. MELKI[2], J. H. WEISHAUPT[1], K. M. DANZER[1];
[1]Univ. of Ulm, Ulm, Germany; [2]Paris-Saclay Inst. of Neurosci., Gif-sur-Yvette, France

Abstract: Immune dysregulation and inflammation have been previously linked to Parkinson's disease (PD), but it remains unclear whether inflammation is a result of the disease or if it is an important factor contributing to neurodegeneration. Genes involved in familial inheritance of PD (SNCA, LRRK2) are widely expressed in the innate immune system and polymorphisms in genes involved in the immune response are associated with increased risk for PD. Furthermore, PD is characterized by pathological aggregation and accumulation of alpha-synuclein. We have previously shown that the inflammatory response by peripheral blood monocytes is dysregulated in PD patients and that extracellular alpha-synuclein oligomers induce a robust cytokine response in blood monocytes. However, the relevance of these findings for the disease remained unclear. Here, we studied the immune response of monocyte-derived macrophages from PD patients and healthy controls, as well as murine microglia to extracellular alpha-synuclein oligomers and higher molecular aggregates, which are possibly involved in different stages of synucleinopathy. We demonstrate that different alpha-synuclein species exhibit a different potency for immune activation and likely induce inflammation during early processes of the disease. We show that exosomes, which have been previously implicated in spreading of alpha-synuclein pathology, also contribute to the immune response by monocytes, macrophages and microglia. Furthermore, we characterized exosomes from PD patients and healthy controls as well as from a transgenic α-syn oligomer mouse model and assessed their ability to induce the innate immune response, paralleling our findings with exosomes from cell lines with induced alpha-synuclein pathology. We show that extracellular alpha-synuclein skews innate immune cells to a pro-inflammatory phenotype, while increased levels of alpha-synuclein expression in immune cells do not directly induce inflammatory hyperactivity. Our findings strengthen the evidence that extracellular alpha-synuclein-induced inflammation may be an important disease mechanism contributing to the death of dopaminergic neurons and suggest inflammatory response and alpha-synuclein clearance as important targets for therapies in PD.

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Poster

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Topic: C.03. Parkinson's Disease

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Title: Uptake and sequestration of prion-like alpha-synuclein in a cellular model

Authors: ***M. MARANO**[1], S. SRI RENGANATHAN[1], P. E. FRASER[2], A. TANDON[1]; [1]Inst. of Med. Sci., [2]Med. Biophysics, Univ. of Toronto, Toronto, ON, Canada

Abstract: This study aims to investigate the mechanisms underlying the intercellular propagation of alpha-synuclein (a-syn) involved in Parkinson's disease (PD). In PD, patients develop neuronal protein inclusions, composed primarily of a-syn, called Lewy bodies (LB). These LBs are considered an intrinsic component of the PD neuropathology and a post-mortem diagnostic criterion. LBs are thought to originate in olfactory and enteric nerves prior to their entrance in the central nervous system; this distinctive progression of LBs from the periphery to the brain, first described by Braak and colleagues, is indicative of disease stage. Additionally, the presence of LBs in otherwise healthy neuronal grafts implanted in PD patients suggests a-syn propagates in a prion-like manner where misfolded a-syn migrates and recruits healthy a-syn within neighbouring cells.

Our goal is to develop a two-stage fluorescent assay assessing the propagation of a-syn pathology. In the first step recombinant a-syn, in a predominantly oligomeric state, was used to treat immortalized human embryonic kidney cells (HEK 293) stably expressing the A53T a-syn mutant conjugated to YFP, resulting in the formation of punctate structures. My work will characterize these punctate structures by looking for markers of aggregation and determining their subcellular location. For the second stage, we intend to treat a "donor" population of HEK 293 cells, transiently transfected with a-syn, with recombinant a-syn and subsequently co-seed these cells with a "recipient" A53T-YFP cell population to assess cell-to-cell a-syn transmission.

This project will allow us to expand our understanding of how toxic a-syn is transmitted between cells and to investigate how the cell processes aggregated a-syn. This information is important to understanding the molecular basis for the initiation and progression of synucleinopathies such as PD or Dementia with Lewy bodies. My work will solidify our understanding of the intercellular exchange of a-syn. In addition, by characterizing the systems at work under disease conditions, therapies can be directed towards augmenting cellular pathways to cope with pathological protein accumulation.

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Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

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Topic: C.03. Parkinson’s Disease

Support: NIH Grant RO1AG0145264

Title: 27 hydroxycholesterol induces alpha-synuclein expression in dopaminergic neurons-implications in synucleinopathies.

Authors: *J. SCHOMMER, M. KLEINJAN, G. MARWARHA, O. GHRIBI;
Univ. of North Dakota, Grand Forks, ND

Abstract: Accumulation of α-synuclein protein is a common hallmark of a group of brain disorders collectively known as synucleinopathies. These disorders include Parkinson’s disease, dementia with Lewy bodies, multiple system atrophy and Alzheimer’s disease. The role of α-synuclein in the pathogenesis of synucleinopathies is not understood, but experimental studies point to a potential neurotoxic role of high levels of this protein in its soluble or aggregated forms. The causes of synucleinopathies are likely multi-factorial with several factors including genetic susceptibility and environmental agents (dietary and chemicals) potentially participating in the pathogenesis of these diseases. The objective of our study was to test the hypothesis that the cholesterol oxidation product 27-Hydroxycholesterol (27-OHC) increases α-synuclein transcription through over-activation of its cognate receptor, liver X receptor (LXR). Reducing over-activation of LXR can potentially prevent α-synuclein overproduction and slow or reverse synucleinopathy progression. 27-OHC is the most abundant endogenous oxysterol and increased levels of this oxysterol may cause deleterious effects. We incubated human neuroblastoma (SHSY5Y) cells, mouse dopaminergic neurons differentiated from embryonic stem cells, and

human dopaminergic neurons differentiated from human normal dopaminergic neuronal precursor cells with 27-OHC and examined the effects of increased 27-OHC on the expression levels of α-synuclein. Our results show that 27-OHC dose-dependently regulates the transcription of α-synuclein through modulation of LXR in the three different cell types. Despite extensive research, no disease-modifying therapy is currently available for synucleinopathies and the search for diagnostic tests and biomarkers are still under development. The search for disease-modifying therapies or diagnostic markers would benefit from identification of factors that promote over-production of α-synuclein protein as well as the elucidation of new cellular mechanisms that regulate the transcription of α-synuclein. Identification of the oxysterol 27-OHC and the LXR as the underlying cellular mechanisms by which 27-OHC increases α-synuclein levels may help in designing therapeutic agents that can prevent, reverse, or stop the over-production of α-synuclein and ultimately may protect against synucleinopathies.

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Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

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Program#/Poster#: 42.05/J16

Topic: C.03. Parkinson’s Disease

Title: Modulation of brain mitochondrial function by aggregated proteoforms of human α-synuclein

Authors: *J. B. WATSON[1], A. KUNZ[1], G. SEROBYAN[1], A. YACOUB[1], J. WHITELLEGE[1], M. CILLUFFO[2], T. SARAFIAN[1];
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Abstract: The α-synuclein protein exists in a variety of aggregated forms associated with Parkinson’s disease (PD) pathology, but the specific proteoform structures that cause PD toxicity are not clearly defined. Our previous studies showed that the membrane potential was decreased while reactive oxygen species (ROS) were elevated in brain mitochondria from a pre-manifest mouse model for PD over-expressing the full-length, monomeric form of NH_2-terminally acetylated human α-synuclein (ASOTg)[Sarafian TA et al, 2013, PLoS One 8(5):e63557]. Since α-synuclein oligomers or fibrils were not detected with amyloid conformational antibodies in mitochondrial fractions from ASOTg mice, presumably excess amounts of the monomeric form were sufficient to disrupt brain mitochondria function. Preliminary studies in synapse-enriched

synaptoneurosomes (SNs) from the same overexpressing mice also detected lower mitochondrial membrane potential, but ROS and glutathione (reduced form, GSH) were diminished as well. Overall ASOTg studies suggested that there may be paradoxical effects of excess α-synuclein on non-synaptic and synaptic mitochondria. Additional immuno-gold/transmission electron microscopy detected gold labelling within numerous SN membranous structures, including mitochondria, incubated with exogenous fibrillated forms of α-synuclein. Experiments are now in progress to examine further the functions of both brain and SN mitochondria reconstituted with aggregated recombinant proteoforms (oligomers, fibrils) relative to monomeric and COOH-truncated forms of human α-synuclein.

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Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

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Department of Neuroscience, Oberlin College

Title: A gene-environment interaction study to examine the role of human wild-type alpha-synuclein in cadmium-induced neurotoxicity

Authors: ***J. A. JIMENEZ**[1], W. CHONG[1], G. KWAKYE[1], M. SAITO[2];
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Abstract: Alpha-synuclein (α-syn) is a ubiquitous protein whose native function is unknown. α-syn comprises 60% of Lewy bodies that are aggregates present in the brains of postmortem Parkinson’s disease (PD) patients and implicated in the pathogenesis of dopaminergic neurons. 5-10% of PD cases are familial with the remaining being idiopathic. Long-term exposure to

pesticides or metals such as cadmium (Cd) has been proposed to represent a risk factor for PD. Cd is an industrial and environmental pollutant that causes severe damage to a variety of organs, including the brain, and impairs cellular mechanisms implicated in PD. Here we investigated the role of wild-type α-syn in Cd-induced neurotoxicity in an established α-syn dopaminergic cell model of PD that overexpresses human wild-type α-syn (N27-syn) or empty vector (N27-vec). We demonstrate that N27-syn expressing cells exhibit a significant dose-dependent susceptibility to Cd neurotoxicity compared to N27-vec following a 24h exposure. Furthermore, we show that N27-syn cells exhibit reduced levels of the antioxidants glutathione and superoxide dismutase, and increased production of reactive species compared to N27-vec, suggesting the contribution of oxidative stress pathways in the cell death mechanism. We further hypothesized that wild-type α-syn may modulate Cd homeostasis. Thus, we quantified intracellular Cd levels in N27 cells following a 6h and 24h Cd exposure with inductively-coupled plasma mass spectrometry (ICP-MS) and report that N27-syn cells accumulate significantly more Cd in a time-dependent manner compared to N27-vec cells. Together these findings suggest that wild-type α-syn enhances Cd-induced neurotoxicity resulting in oxidative stress mediated cell death.

Disclosures: **J.A. Jimenez:** None. **W. Chong:** None. **G. Kwakye:** None. **M. Saito:** None.

Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 42.07/J18

Topic: C.03. Parkinson’s Disease

Support: NIH-5P20GM103653 (YHK)

Title: The SUMO conjugase Ubc9 regulates the stability of alpha-synuclein and Dopamine Transporter in Parkinson’s disease models.

Authors: E. CARTIER[1], J. GARCIA-OLIVARES[2], E. JANEZIC[1], M. L. LIN[3], *Y.-H. KIM[1]; [1]Biol. Sci., Delaware State Univ., Dover, DE; [2]Natl. Inst. of Mental Hlth., Bethesda, MD; [3]Univ. of Florida, Gainsville, FL

Abstract: Post-translational modification (PTM) has been addressed as a key regulatory mechanism for modulating protein aggregation/degradation in neurodegeneration. However, a form of PTM, Small Ubiquitin Modifier (SUMO) has not been well studied in Parkinson’s disease (PD) pathology. Although SUMOylation may increase the solubility of alpha-synuclein, SUMOylated proteins including alpha-synuclein have been detected in the halo of Lewy bodies. Thus it is still unclear in understanding the role of SUMOylation in dopaminergic neurons. Here,

we assess the role of SUMO conjugase, Ubc9 as a critical post-translational modifier to regulate the solubility, stability, and function of dopamine transporter (DAT) and alpha-synuclein in dopaminergic neurons *in vitro* and *in vivo*. The objective of this work is to assess SUMOylation as a novel regulatory target for preventing alpha-synuclein mediated protein aggregation and regulating dopamine uptake via DAT in dopaminergic neurons. This implies that pathological changes in the SUMOylation of DAT and/or alpha-synuclein may lead to alteration in dopamine reuptake and acute regulation of protein (mis)folding or aggregation, which is related to the neuropathology of PD. We identified that both DAT and alpha-synuclein are constitutively SUMOylated in mouse striatum and midbrain. Our *in vitro* preliminary results demonstrated that Ubc9 over-expression protects rat N27 dopaminergic cells against MPP+ induced oxidative stress and prevents DAT and alpha-synuclein degradation via inhibition of proteasome and lysosome. Moreover, Ubc9-mediated SUMOylation increases the surface level of DAT in the plasma membrane and further its action enhances DAT functional expression in the plasma membrane, triggering an increase in dopamine uptake capacity. In the MPTP-lesioned mice, the chronic treatment substantially reduces the level of SUMO1 conjugated to alpha-synuclein in the mouse striatum. This implies that pathological changes in the SUMOylation of DAT and alpha-synuclein may lead to alteration in acute regulation of dopamine clearance/recycling and protein (mis)folding or aggregation, respectively. Therefore, SUMOylation of DAT and alpha-synuclein can be a potential therapeutic target for neurological disorders such as ADHD, depression, and PD.

Disclosures: E. Cartier: None. **J. Garcia-Olivares:** None. **E. Janezic:** None. **M.L. Lin:** None. **Y. Kim:** None.

Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

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Program#/Poster#: 42.08/K1

Topic: C.03. Parkinson’s Disease

Title: Function of TMEM175 in synucleinopathy & parkinson’s disease.

Authors: *S. JINN[1], H. WONG[3], D. TOOLAN[4], S. GRETZULA[4], B. VOLETI[4], P. CRAMER[4], R. E. DROLET[4], M. TADIN-STRAPPS[3], D. STONE[2];
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Abstract: Parkinson's disease (PD) is a neurodegenerative disorder with clinical symptoms of tremor, rigidity, cognitive impairment and depression. It is characterized by the loss of dopaminergic neurons that develop Lewi bodies (LB), depositions of α-synuclein and other protein aggregates that cause downstream cellular toxicity and death. Impaired lysosomal and mitochondrial function has been shown to be critical for the cellular proteolytic capacity. Recently, a variation in human transmembrane protein 175 (TMEM175), a K^+ channel localizing to lysosome was identified by GWAS as a candidate risk factor for PD. Mediating potassium conductance on lysosomal and endosomal membrane, TMEM175 was shown to regulate lysosomal membrane potential, pH stability, and organelle fusion, suggesting the possibility that it may play a causal role in the development of PD. We have shownthat deficiency of TMEM175 in rat primary neurons leads to an increase in hyperphosphorylated and detergent insoluble a-synuclein aggregates in the preformed fibril seeding model of α-synuclein aggregation, supporting the genetics data linking TMEM175, α-synuclein aggregation, and PD. We also confirmed that absence of TMEM175 results in unstable lysosomal pH in SH-SY5Y neuroblastoma cell leading to altered fusion of autophagosome to lysosome. Moreover, maturation of lysosomal proteins, lysosomal enzyme activity, and mitochondrial oxygen consumption capacity were compromised in cells lacking TMEM175, suggesting the importance of TMEM175 in lysosomal function. Together, our data suggest that TMEM175 might be a key therapeutic target of Parkinson's disease as a critical player that modulates proteolytic capacity of lysosome.

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Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 42.09/K2

Topic: C.03. Parkinson's Disease

Title: Phenotyping of human of ipsc-derived dopaminergic neurons containing the engineered a53t alpha-synuclein mutation

Authors: *B. W. JARECKI[1], K. MANGAN[2], K. KIM[2], N. AUMANN[2], L. LITTLE[2], C. CARLSON[2], S. DELAURA[2], E. JONES[2];
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Abstract: Parkinson's disease (PD) affects ~1% of people over the age of 65 and is the second most common neurodegenerative brain disorder after Alzheimer's. The physiological decline

associated with PD is generally thought to be caused by a marked pathological deterioration of dopaminergic neurons located in the *substantia nigra*. Mutations in several different genes have been clearly linked to PD, including *SNCA* that encodes the alpha-synuclein (⟨-syn) protein, which is predominantly expressed in the brain at presynaptic terminals. The mutation in alpha-syn at A53T renders the protein more susceptible to aggregation and accumulation, which are hallmark indicators of PD pathology. Despite its low occurrence, A53T is also one of the highly penetrant and widely studied alpha-syn mutations.
Combining cutting-edge genome-editing and induced pluripotent stem cell (iPSC) technologies offers the opportunity to study patient-specific risk factors or disease-specific mutations (such as the A53T mutation in alpha-syn) in a physiologically-relevant cell type (dopaminergic neurons) and compare the function and phenotype in a series of assays to cells derived from healthy control iPS cell lines. This is revolutionary for disease modeling and drug discovery.
In this poster, we show data comparing healthy (WT) and A53T dopaminergic neurons that demonstrate alterations at the synapse, both functionally (electrophysiological MEA readout) and anatomically (neurite outgrowth and branching). The observed differences between healthy and A53T suggest early physiological changes tilted towards producing a more connected and highly-active neuronal network. In correlation with the known disease pathology, these “aging” cultures show synaptic deterioration and dendritic atrophy.
Current studies are underway to further determine if additional hallmarks of PD patho-physiology, including alpha-syn aggregation or mitochondrial dysfunction, can be measured in these human cell models.

Disclosures: **B.W. Jarecki:** None. **K. Mangan:** None. **K. Kim:** None. **N. Aumann:** None. **L. Little:** None. **C. Carlson:** None. **S. DeLaura:** None. **E. Jones:** None.

Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

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Topic: C.03. Parkinson’s Disease

Support: DZNE Intersite project

BMBF, MITO PD, 031A430A

Eva-Theers-Stiftung

Title: iPSC-derived human neuronal knockout model of PINK1

Authors: *C. J. BUS[1,2,3], B. SCHMID[4], S. HOFFMANN[1,2], S. GEISLER[1,2], P. FALLIER-BECKER[5], K. KAPOLOU[6], J. WAGNER[6], D. O. HALIM[6], R. KRÜGER[1,2,7], T. GASSER[1,2], J. C. FITZGERALD[1,2];
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Abstract: Loss of function mutations in the PTEN induced putative kinase 1 (PINK1) gene cause autosomal recessive early-onset Parkinson's disease (PD). PINK1 is a mitochondrial kinase and acts as a key player in the initiation of Parkin-mediated mitophagy. Recent findings draw a more complex picture and indicate important Parkin-independent functions of PINK1. The revolution of gene-editing technologies enabled the generation of unique human neuronal models to investigate the pathological mechanism underlying PD. We have generated a PINK1 knockout in healthy human induced pluripotent stem cells (iPSCs) using TALEN-technology and are currently generating PINK1-mutated lines and patient iPSCs. The PINK1 knockout iPSCs and their isogenic control were differentiated into neurons and used for several established readouts to assess mitochondrial function under different stress conditions. We were able to identify distinct mitochondrial phenotypes, which support a role for PINK1 in mitochondrial quality control. Ionophores induce loss of mitochondria in isogenic control cells but this effect is significantly hampered in PINK1 knockout lines. In PINK1 knockout neurons no ubiquitinylation or phosphorylation of several outer mitochondrial membrane (OMM) proteins was observed. Thus this neurons exhibit a severe defect in the initiation of mitophagy. As a compensatory mechanism, mitochondrial biogenesis is upregulated. As a result neurons lacking PINK1 have a significantly reduced mitochondrial membrane potential and were less sensitive to mitochondrial toxins. These findings hint towards deficits in the respiratory chain. Therefore, we performed a mitochondrial stress test of the respiratory chain, which revealed changes in OXPHOS under uncoupled conditions. To investigate whether PINK1 knockout neurons are more susceptible to apoptosis, we analysed several apoptotic markers and measured LDH release. Apoptotic markers are dysregulated and the PINK1 knockout neurons display an increased vulnerability to Rotenone as compared to isogenic control. This shows that PINK1 is not only involved in the regulation of Parkin-dependent mitophagy, but also regulates the metabolic state of the mitochondria and the cellular fate after mitochondrial damage has occurred. Using this model on a high content screening platform, we will next investigate genetic and pharmacological modifiers to apply novel neuroprotective strategies.

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Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

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Topic: C.03. Parkinson's Disease

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Mito-PD BMBF 031A430A

Title: Mitochondrial chaperone Trap1 loss of function in Parkinson's disease

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Abstract: We initially identified the mitochondrial chaperone Trap1 (mitochondrial Hsp90) as an interactor of Parkinson's disease (PD)-associated mitochondrial protease HtrA2 via an unbiased mass spectrometry approach. The PD kinase PINK1 is required for the phosphorylation of HtrA2 (Plun-Favreau et al., 2007) and Trap1 (Pridgeon et al., 2007) following cellular stress but the downstream signaling involving Trap1 is not fully understood. In our model, Trap1 is protective against cell death and can rescue PD loss of function phenotypes. Loss of function of Omi and PINK1 cause Parkinsonism in humans and animal and cell models. Here we also report for the first time a mutation in TRAP1 leading to a premature stop codon in the transit sequence of TRAP1 in a patient suffering from late onset idiopathic PD and report TRAP1 variants in a PD cohort. Functional analysis in patient-derived cells suggests that loss of TRAP1 results in significant loss of mitochondrial membrane potential and sensitivity to late stage quality control such as organellar removal and apoptosis. Our data also suggests that the protective mechanism of Trap1 involves regulation of energy metabolism and we propose TRAP1 as a novel susceptibility gene for Parkinson's disease acting in a signaling pathway downstream of PINK1 and HtrA2 but likely independent of Parkin.

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Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

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Michael J Fox Foundation 11709

Title: Characterization of alpha synuclein oligomers from Parkinson's patient tissue

Authors: *C. SILKY, C. REHAK, K. MOZZONI, R. YURKO, N. IZZO, G. RISHTON, G. LOOK, H. SAFFERSTEIN, S. M. CATALANO;
Cognition Therapeut. Inc, Pittsburgh, PA

Abstract: Parkinson’s disease (PD) is characterized by motor dysfunction as well as non-motor symptoms including cognitive deficits. It is pathologically differentiated from other neurodegenerative diseases by the presence of Lewy bodies (LBs), predominantly composed of alpha-synuclein (Asyn). Native Asyn is an intrinsically-disordered pre-synaptic protein involved in vesicular trafficking and neurotransmitter release. Although fibrillar aggregates of Asyn accumulate in LBs, evidence suggests that soluble, oligomeric assemblies of Asyn are the primary neurotoxic species. Oligomeric Asyn inhibits SNARE mediated vesicle fusion (Choi ’15), disrupts synaptic plasticity and impairs long-term potentiation (Diogenes ’12; Martin ‘12) which could underlie cognitive deficits in PD.
We characterized human Asyn oligomers by immunoprecipitating PBS-soluble proteins from post-mortem Parkinson’s patient brain samples with a211 antibody directed against Asyn residues 121-125 on the C-terminus. Elution with 6M Guanidine HCL released the maximum amount of Asyn oligomer from IP columns of all the conditions examined, while preserving the oligomers’ neuroactive properties in an assay measuring membrane trafficking in cultured neurons. To characterize these samples, patient-derived oligomers were run side-by-side on

native and SDS page gels. Analysis of non-denaturing Western blots revealed low molecular weight bands at 20, 35 and 50 kDa, corresponding to monomer, dimer and trimer respectively, as well as higher molecular weight species >100 kDa. Analysis of this material on denaturing Western blots revealed high molecular weight species >80 kDa with no lower species below 50 kDa. Changes in molecular weights of oligomer species under denaturing conditions has been previously documented for other oligomeric proteins like Abeta1-42.
In order to identify a method to produce oligomers from recombinant Asyn protein that most closely match those isolated from human patient brain, we compared several methods for oligomerizing full length Asyn recombinant protein (Brenner ‘08; Danzer ‘07; Choi ‘15; Tsigelny ‘08). Only Asyn protein seeded with nanomolar concentrations of synthetic Abeta 1-42 yielded neuroactive oligomers with similar molecular weights to human patient derived oligomers on non-denaturing Western blots. Lower amounts of >100kDa species and higher amount of dimer are seen in the recombinant oligomers vs. patient-derived. Additionally, both human PD patient-derived and recombinant seeded Asyn oligomers cause deficits in membrane trafficking rate in primary neuronal cultures.

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Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

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Title: Functional characterization of dual-specificity tyrosine-phosphorylation-regulated kinase 1A to modulate parkin ubiquitin E3 ligase activity and the formation of toxic protein aggregates

Authors: S. JUNG[1], E. IM[1], *H. RHIM[2], K. CHUNG[1];
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Abstract: Mutations of parkin are linked to autosomal recessive forms of familial Parkinson's disease (PD). According to its functional relevance in abnormal protein aggregation and neuronal cell death, a number of post-translational modifications regulate the ubiquitin E3 ligase activity of parkin. Here we propose a novel inhibitory mechanism of parkin E3 ubiquitin ligase through dual-specificity tyrosine-phosphorylation-regulated kinase 1A (Dyrk1A)-mediated phosphorylation as well as its neuroprotective action against 6-hydroxydopamine (6-OHDA)-induced cell death. The present work suggests that parkin phosphorylation by Dyrk1A may affect the pathogenesis of PD under PD-inducing pathological conditions.

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Poster

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Title: Leucine-rich repeat kinase 2 (LRRK2) stimulates IL-1β-mediated inflammatory signaling through phosphorylation of a novel target p25

Authors: M. HYUN[1], K. HAN[1], W. SEOL[2], *K. C. CHUNG[1];
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Abstract: Leucine-rich repeat kinase 2 (LRRK2) is a Ser/Thr kinase having mixed lineage kinase-like and GTPase domains, controlling neurite outgrowth and neuronal cell death. Mutations of LRRK2 gene cause autosomal dominant late-onset Parkinsonism. Parkinson and Alzheimer disease-associated p21 protein is reported to modulate calcium-dependent protein dephosphorylation, consequently affecting many cellular responses, including lymphocyte activation and inflammatory signaling. Based on evidence suggesting a putative role of LRRK2 during inflammatory signaling, we investigated biochemical and functional interactions between LRRK2 and p25, and its potentially regulatory role for in IL-1β inflammatory signaling. At the meeting, recent data will be presented showing that p25 is a novel substrate of LRRK2 and acts as a positive regulator of inflammatory signaling. In conclusion, LRRK2 positively regulates inflammatory responses through p25 phosphorylation in Parkinson disease.

Disclosures: **M. Hyun:** None. **K. Han:** None. **W. Seol:** None. **K.C. Chung:** None.

Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

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Topic: C.03. Parkinson’s Disease

Support: Michael J Fox Foundation for Parkinson's Research to L.P

Title: LRRK2-mediated cellular and synaptic alterations in the striatum

Authors: *L. PARISIADOU[1], C. MAKARIOU-PIKIS[1], F. KOURI[1], H. CAI[2];
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Abstract: Mutations in LRRK2 represent a strong genetic risk for both hereditary and sporadic forms of Parkinson’s disease (PD). However, there are still several fundamental aspects of LRRK2 function that remain unresolved at this time. LRRK2 is significantly enriched in spiny projection neurons (SPNs) in the dorsal striatum. This cellular expression pattern suggests that LRRK2 mutations may contribute to striatal pathophysiology in PD. The function of LRRK2 in the striatum has remained relatively under-investigated; however, we recently showed that LRRK2 directs PKA signaling in SPNs. The pathogenic $LRRK2^{R1441C}$ mutation impairs the binding of PKA with LRRK2, leading to increased levels of PKA in the dendritic spines that, in turn, result in aberrant synaptic PKA signaling. The components of PKA enzyme in neurons are confined to sub-cellular compartments, ensuring for spatial and temporal regulations of PKA signaling which lead to specificity in fundamental striatal functions. Our data suggest that

LRRK2 is strategically located in the dendritic shaft to organize signaling events in a spatiotemporal way. Therefore, we propose that increased synaptic PKA activity of *LRRK2*^{R1441C} SPNs stems from altered subcellular compartmentalization of PKA. Furthermore, since PKA is a critical effector of dopamine receptors, our central hypothesis is that LRRK2-mediated deregulation of PKA activity results in aberrant dopaminergic signaling in SPNs, which in turn contributes to PD symptomatology. In support of this idea, we provide evidence that *LRRK2*^{R1441C} mutations impact dopaminergic signaling in SPNs. Specifically, SPNs harboring *LRRK2*^{R1441C} mutations show an abnormal elevation in PKA activity in response to dopamine receptor, Drd1, activation. In addition, *LRRK2*^{R1441C} mice show altered behavioral responses under several dopamine manipulations, suggesting a novel role of LRRK2 in dopaminergic signaling which, in turn, may direct the striatal related motor functions. Taken together, our findings emphasize that LRRK2 contributes to glutamatergic synaptic functions by directing postsynaptic signaling events in the SPNs and demonstrate a novel LRRK2-based pathogenic mechanism of striatal dysfunction in PD.

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Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

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Topic: C.03. Parkinson’s Disease

Title: In silico simulation of Lrrk2 interactions in Parkinson’s Disease using SEED

Authors: *J. W. RYAN, A. WIDENER, I. C. IKEDA, T. J. SWEENEY, A. D. LEE, B. BEHROUZ;
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Abstract: Mutations in Leucine-rich repeat kinase 2 (LRRK2) cause autosomal dominant Parkinson’s Disease (PD). LRRK2 encodes for a large protein of the same name which interacts with over 60 other proteins and can alter several molecular pathways. Lrrk2’s role in multiple different pathways makes it difficult to successfully identify therapeutics with low off-target effects. It is therefore important to understand the molecular changes involving Lrrk2 as well as the relationship of these changes with PD pathology and normal cellular function. To aid in the research and identification of PD therapeutics, we have developed a computer simulation program, Simulation Environment for Experimental Design (SEED). We created an *in silico* pathway model around Lrrk2 interactions based on information derived from peer-reviewed manuscripts and public databases. We simulated these interactions using SEED running on

Nvidia graphics processing units (GPU) with the CUDA parallel programming platform. We visualized and quantified the simulation data from Lrrk2 overexpression and knockout models and compared it with a baseline model. Furthermore, we manipulated downstream targets of Lrrk2 in order to reverse the abnormalities observed by Lrrk2 overexpression. Elucidation of these druggable targets downstream of Lrrk2 and upstream of PD pathology may help to identify therapeutic targets for both Lrrk2-mediated and idiopathic PD.

Disclosures: J.W. Ryan: None. **A. Widener:** None. **I.C. Ikeda:** None. **T.J. Sweeney:** None. **A.D. Lee:** None. **B. Behrouz:** None.

Poster

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Title: Mitophagy is impaired in carriers of Parkinson's disease-linked LRRK2 mutations

Authors: F. WAUTERS[1], T. CORNELISSEN[1], S. MARTIN[2], B. KOENTJORO[3], C. SUE[3], P. VANGHELUWE[2], *W. P. VANDENBERGHE[4,1];
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Abstract: Parkinson's disease (PD)-linked mutations in *PARK2* and *PINK1* disrupt selective autophagic elimination of damaged mitochondria (mitophagy). Mutations in *LRRK2* are a much more prevalent cause of PD than *PARK2* or *PINK1* mutations. *LRRK2* encodes an enzyme with a kinase domain, a GTPase domain and multiple protein-protein interaction domains. Here, we investigated whether PD-linked *LRRK2* mutations affect mitophagy. Using a variety of mitophagy assays we demonstrate that mitophagy is consistently impaired in primary fibroblasts obtained from PD patients with the two most common *LRRK2* mutations (G2019S and R1441C). We observed a similar mitophagy defect in fibroblasts from non-manifesting carriers of the G2019S mutation. *LRRK2* mutant fibroblasts had a decreased mitochondrial membrane potential and enhanced mitochondrial production of reactive oxygen species. Endogenous parkin-mediated ubiquitination of mitofusin-2 on depolarized mitochondria was intact in *LRRK2* mutant fibroblasts, indicating that the mitophagy defect was downstream of parkin activation and mitochondrial ubiquitination. The mitophagy defect was rescued by pharmacological inhibition

of LRRK2 kinase activity and by overexpression of Rab10, a recently identified LRRK2 kinase substrate involved in membrane trafficking and fusion. In conclusion, we identified a mitophagy defect in human cells expressing endogenous levels of mutant LRRK2, suggesting that the pathogenic effects of *LRRK2*, *PARK2* and *PINK1* mutations may converge on a common pathway.

Disclosures: **F. Wauters:** None. **T. Cornelissen:** None. **S. Martin:** None. **B. Koentjoro:** None. **C. Sue:** None. **P. Vangheluwe:** None. **W.P. Vandenberghe:** None.

Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 42.18/K11

Topic: C.03. Parkinson's Disease

Title: The relationship between LRRK2 and Rab GTPase family

Authors: ***T. FUJIMOTO**, T. KUWAHARA, T. KOMORI, T. IWATSUBO;
The Univ. of Tokyo, Tokyo, Japan

Abstract: *Leucine-rich repeat kinase 2 (LRRK2)* has been identified as the most common causative gene for autosomal dominant Parkinson's disease (PD). LRRK2 regulates neurite outgrowth, lysosome integrity and intracellular trafficking in coordination with *RAB7-like variant 1 (RAB7L1)*, a putative responsible gene for another PD risk locus *PARK16*. However, the physiological and functional relationship between LRRK2 and RAB7L1 still remains unclear. We first investigated the possibility of RAB7L1 as a substrate of LRRK2 kinase activity. In vitro, we confirmed that recombinant LRRK2 efficiently phosphorylated recombinant RAB7L1. We also revealed that LRRK2 phosphorylates RAB7L1 in HEK293 cells, but our preliminary results for phosphorylation site analyses suggested that the phosphorylation sites in vitro and in vivo were different. Recently, it has been reported that a subset of other Rab GTPases are in-cell substrates of LRRK2. In our study, some Rab GTPases, but not all, were phosphorylated by LRRK2 in cell as well as in vitro, consistent with the results of prior studies. We further examined the physical interaction between LRRK2 and a set of Rab GTPases by immunoprecipitation assays. As a result, another set of Rab GTPases including RAB7L1 interacted with LRRK2 to varying degrees, regardless of their ability as a substrate. These results suggest that LRRK2 regulates intracellular trafficking by simultaneously regulating various Rab GTPases in kinase activity-dependent or interaction-dependent manners.

Disclosures: **T. Fujimoto:** None. **T. Kuwahara:** None. **T. Komori:** None. **T. Iwatsubo:** None.

Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

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Program#/Poster#: 42.19/K12

Topic: C.03. Parkinson's Disease

Title: The dynamic changes in the localization of LRRK2 in macrophage cells

Authors: *T. EGUCHI, T. KUWAHARA, G. ITO, T. IWATSUBO;
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Abstract: Background
Leucine-rich repeat kinase 2 (LRRK2) is one of the causative genes for autosomal dominant familial Parkinson's disease. LRRK2 is composed of 2527 amino acids and has multiple domains, including GTP-binding and kinase domains. The physiological and pathological functions of LRRK2 remain to be clarified.
Recent studies have revealed that LRRK2 is highly expressed in immune cells including macrophages and that its expression is induced by IFN-γ stimulation. Furthermore, genome-wide association studies have identified LRRK2 as a risk gene for inflammatory and infectious disorders, e.g., Crohn's disease and leprosy. These results suggest that LRRK2 has a role in the immune system. To reveal the role of LRRK2 in the immune system, we analyzed the intracellular localization of LRRK2 upon phagocytosis in RAW264.7 cells, a mouse macrophage cell line, as well as in bone marrow derived macrophages (BMDM).
Method
RAW264.7 cells and BMDM were activated with IFN-γ and then fed with zymosan, a yeast cell wall. The localization of LRRK2 was analyzed by immunocytochemistry. Diacylglycerol-PKC/PKD pathway, one of the major signaling pathways on phagosomes, was pharmacologically inhibited, and LRRK2 localization was analyzed.
Result
LRRK2 was localized in the cytosol in macrophages without zymosan treatment. Upon exposure with zymosan, it was up taken by cells and LRRK2 was relocalized to zymosan-containing phagosomes, in a manner to line their outer surface. PKC/PKD inhibitors suppressed LRRK2 relocalizaiton to phagosomes. PKC/PKD agonist, phorbol 12-myristate 13-acetate, relocalized LRRK2 from the cytosol to the lysosomal compartments.
Discussion
This study suggests that LRRK2 has a role in phagocytosis, and that the PKC/PKD pathway is involved in this phenomenon. PKC/PKD pathway is implicated in the regulation of anti-bacterial responses, e.g., reactive oxygen species production, bacteria killing, and induction of inflammation. The mechanism whereby LRRK2 plays a role in immune responses and

neuroinflammation, including the effects of Parkinson-linked LRRK2 mutations, should further be examined.

Disclosures: **T. Eguchi:** None. **T. Kuwahara:** None. **G. Ito:** None. **T. Iwatsubo:** None.

Poster

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Location: Halls B-H

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Topic: C.03. Parkinson's Disease

Title: LRRK2 phosphorylates the actin nucleating complex protein WASF2 to regulate phagocytosis

Authors: *K. KIM[1], P. C. MARCOGLIESE[1], J. YANG[1], C. WEI[1], E. ABDEL-MESSIH[1], R. S. SLACK[1], M. G. SCHLOSSMACHER[1,2], D. S. PARK[1];
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Abstract: Mutations in leucine-rich repeat kinase 2 (LRRK2) are implicated in both familial and sporadic Parkinson's disease (PD) as the most common causative factor. However, LRRK2's pathophysiological role remains unknown. It was recently discovered that LRRK2 is highly expressed in immune cells relating to the innate immune system and has a genetic association the immune-related disorders: Crohn's disease and leprosy. Thus, determining LRRK2's function in the central immune system and subsequent effect in dopaminergic neuronal cell death could provide insight into the pathogenesis of PD. Here we show both in vitro and in vivo that myeloid-lineage cells such as primary microglia and bone marrow-derived macrophages (BMDMs) from LRRK2 G2019S knock-in mice display increased phagocytic activity that correlates with an increase in the protein level of the ARP2/3 complex activator, WASF2. Conversely, myeloid cells from LRRK2 null mice show impaired engulfment that correlates with a decrease in WASF2 protein. We show that LRRK2-mediated phagocytic activity is kinase dependent and that LRRK2 influences the stability of WASF2 via direct phosphorylation. Lastly, survival of TH-positive neurons is reduced in mixed, wildtype-midbrain, G2019S-BMDM culture. Taken together, these results suggest that the LRRK2-G2019S mutation may contribute to the pathogenesis of PD by increasing the microglial phagocytic response via enhanced WASF2 stability and actin polymerization.

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Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

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Topic: C.03. Parkinson's Disease

Support: Medical Research Council

Wellcome Trust

Michael J. Fox Foundation

Title: Regulation of Wnt and Ca^{2+} signalling by the Parkinson's disease protein LRRK2

Authors: *A. WETZEL[1], M. HUGHES[1], T. MCKAY[2], S. WADDINGTON[3], A. RAHIM[1], K. HARVEY[1];
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Abstract: The evolutionary highly conserved Wingless/Int (Wnt) signalling pathways have shown to be important regulators for neuronal development and maintenance. Moreover, Wnt signalling has been linked to several cell biological functions in midbrain dopaminergic neurons that are known to be affected in Parkinson's diseases (PD). Deregulation of Wnt signalling cause amongst others impaired synaptic stability in the striatum, a brain region where high levels of *leucin-rich repeat kinase 2* (*LRRK2*) expression have been found. Mutations in *LRRK2* are a common cause of inherited and sporadic PD. Interestingly, we showed previously that LRRK2 is a key component of three Wnt signalling protein complexes. It interacts with the LRP6 Wnt co-receptor at membranes (i), binds to all three cytoplasmatic dishevelled proteins, which play a central role in all branches of Wnt signalling (ii), and it has an important role as scaffolding protein in canonical Wnt signalling via an interaction with the β-catenin destruction complex (iii). The importance of Wnt signalling for midbrain dopaminergic neurons during neuroinflammatory responses in PD animal models has also recently been reported. In addition, LRRK2 was shown to inhibit immune responses via Ca^{2+} signalling and was further linked to other Ca^{2+}-dependent functions in autophagy and mitochondrial function. Here, we present the analysis of Wnt and Ca^{2+} signalling activity using the exciting new technology of tissue-targeted delivery of lentiviruses containing a transcription factor activated luciferase reporter to new born mice. This allows quantification of transcription factor activity over the lifetime of an animal. We compare wildtype mice with *LRRK2* knockout mice and with mice harbouring the most prevalent familial *LRRK2 G2019S* mutation. Our data collectively support the idea of LRRK2 as a modulator of Wnt and Ca^{2+} signalling. Increasing our understanding about the role of LRRK2

in these pathways during neuronal development and aging might contribute to the generation of novel PD treatments targeting early events in the pathogenesis of PD.

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Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

Location: Halls B-H

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Program#/Poster#: 42.22/K15

Topic: C.03. Parkinson's Disease

Title: Leucine-rich repeat kinase 2 (LRRK2) inhibitors exhibit greater pharmacological potency to reduce the Ser1292 autophosphorylation compared to Ser935 *In vitro* and *In vivo*

Authors: *H. SAMAROO[1], Y. CHEN[1], E. NEEDLE[1,2], K. WELCH[1], P. GALATSIS[3], S. STEYN[4], Z. BERGER[1], W. D. HIRST[1,5];
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Abstract: Mutations in the GTPase and kinase domains of Leucine-rich Repeat Kinase 2 (LRRK2) increase the risk for PD. Previous studies suggest these mutations result in a more active state of LRRK2 which presents an opportunity for therapeutic intervention using small-molecule kinase inhibitors. Here, we validate LRRK2 auto-phosphorylation at Ser1292 (pSer1292) *in vivo* as a robust and sensitive measure of LRRK2 kinase activity, and conferring greater pharmacological potency for diverse LRRK2 inhibitors relative to pSer935 (a conventional measure of LRRK2 inhibition). We transiently transfected HEK-293T cells with LRRK2 mutant constructs containing several familial PD mutations and measured phosphorylation at Ser1292 and Ser935. We observed pSer1292 to be elevated with familial PD activating mutations (G2019S, R1441C and Y1699C) and reduced upon pharmacological inhibition. We used non-transgenic C57BL6 (NTg) and BAC-Tg mouse brain and primary mouse astrocytes overexpressing LRRK2-WT and LRRK2-G2019S to determine dose response curves for selective LRRK2 inhibitors (PF-06447475, GNE-7915, MLi-2). Specifically, all three LRRK2 inhibitors consistently showed a 2-3 fold leftward shift in IC_{50} against pSer1292 vs pSer935. Using the same G2019S-Tg model systems, we further validated the difference in pharmacological potency by determining time-course profiles of pSer1292 and pSer935 after treatment with PF-06447475. The results illustrate higher rates of de-phosphorylation at Ser1292

relative to Ser935 in both G2019S-Tg mouse brain and primary astrocytes. These data suggest that pSer1292 is a sensitive pharmacodynamic marker which consistently predicts potency in multiple PD-relevant model systems. Furthermore, we propose that pSer1292 can be utilized to determine whether disease relevant factors such as pathologic α-synuclein and other cell stressors can activate LRRK2.

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Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

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Topic: C.03. Parkinson’s Disease

Support: Z01-AG000948

Title: Regulation of the Parkinson's kinase Leucine-rich Repeat Kinase 2 (LRRK2) by metabolic signalling

Authors: *A. MAMAIS[1], D. ROOSEN[1], R. KUMARAN[1], R. LANGSTON[1], A. BEILINA[1], H. RIDEOUT[2], K. MELACHROINOU[2], D. HAUSER[1], I. N. RUDENKO[1], Y. LI[3], M. R. COOKSON[1];
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Abstract: Genetic variability in and around the gene encoding for LRRK2 is associated with inherited and sporadic Parkinson’s disease (PD). LRRK2 has been linked to signalling events of autophagy and trans-golgi dynamics, but the regulation of this protein and role in neuropathology remain elusive. LRRK2 is a phosphoprotein and its phosphorylation is altered by PD-linked mutations in ways that are not well understood. For example, mutations in the GTPase domain of LRRK2 have been shown to diminish phosphorylation at sites that are not the result of autophosphorylation, although the mechanism(s) by which phosphorylation of LRRK2 is controlled by mutations are not resolved. Mitochondrial abnormalities are a common feature in PD pathogenesis, so we investigated whether mitochondrial impairment can alter LRRK2 function. We show, in striatal slices and a variety of cell types, that depletion of ATP via mitochondrial stress causes changes in phosphorylation and 14-3-3 binding of LRRK2. We set out to identify downstream effects of LRRK2 dephophorylation under mitochondrial

impairment, specifically on phosphorylation of small Rab family GTPases that may have implications in endosomal trafficking and autophagy. Our data place LRRK2 in pathways related to mitochondrial dysfunction, suggesting links to multiple forms of inherited PD and may have implications for development of LRRK2 kinase inhibitors as therapies for this disease.

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Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

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Topic: C.03. Parkinson's Disease

Support: NIH MD007599 to Hunter College infrastructure from NIMHD

The Graduate Center, City University of New York

Title: Calcium-dependent calpain cleavage of Parkin: relevance to Parkinson's disease

Authors: A. STOLL[1], H. WANG[1], P. ROCKWELL[1], *M. E. FIGUEIREDO-PEREIRA[2]; [1]Biol. Sci., [2]Hunter Col., New York, NY

Abstract: Parkin dysfunction during mitochondrial stress contributes to Parkinson's disease (PD) via mechanisms which are poorly delineated. In addition, mitochondrial impairment is linked to PD although the underlying mechanisms are not clearly understood. It is postulated that in neurons even a modest restriction of ATP production by mitochondria far outweighs the negligible effects of ROS. We investigated the effects of a mitochondrial inhibitor (oligomycin, Oligo) on Parkin integrity in rat midbrain and cerebral cortical cultures. Treatment with Oligo led to calpain-cleavage of Parkin. As far as we know, Parkin cleavage by calpain has not been reported. Upon calpain cleavage, Parkin migrates as a doublet. Based on computational analysis we predict two calpain cleavage sites (P1 position) in human/rat full-length Parkin: (1) Gln71 with a predicted molecular weight of 43.6, and located within the UbL domain, and (2) Ala134 with a predicted molecular weight of 36.9, and located within the linker region between the UbL and RING0 domains. Both cleavages free the partial or complete UbL domain, which could bind to proteasomes and inhibit their activity. Calpain-activation is linked to ATP-depletion and necrosis, a cell death pathway characterized by a bioenergetic catastrophe. In fact, calpain-activation was shown to be induced by electron transport chain inhibitors, such as oligomycin,

the one used in our studies. Calpain activation is calcium (Ca^{++})-dependent. While extracellular Ca^{++} is in the millimolar range, cytosolic Ca^{++} is usually less than one micromolar, except during a Ca^{++} signaling event. Ca^{++}-ATPase pumps in the plasma and ER membranes maintain this low concentration by transporting Ca^{++} away from the cytoplasm, either out of the cell or into the ER. When intracellular ATP levels are lowered, such as in the case of oligomycin treatment, Ca^{++}-ATPase pumps are impaired causing cytoplasmic Ca^{++} to be elevated leading to calpain activation. To determine whether calpain-cleavage of Parkin is Ca^{++} dependent, we treated the cells with the Ca^{++} ionophore A23187, which facilitates the transport of Ca^{++} across the plasma membrane. Treatment with A23187 mimicked the effect of Oligo by inducing calpain-cleavage of Parkin to fragments of the same size. We are addressing whether calpain cleavage of Parkin diminishes its E3 ubiquitin ligase activity. In conclusion, based on our findings we propose that stabilizing FL-Parkin could provide a novel therapeutic strategy for treating PD.

Disclosures: **A. Stoll:** None. **H. Wang:** None. **P. Rockwell:** None. **M.E. Figueiredo-Pereira:** None.

Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

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Topic: C.03. Parkinson’s Disease

Support: JSPS KAKENHI 23591265, 26461287

Title: The involvement of the calcium binding protein in aberrant calcium release from IP_3 receptor by alpha-synuclein oligomers

Authors: *K. YAMAMOTO, H. SAWADA;
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Abstract: There is emerging evidence implicating α-synuclein (α-SN) oligomers as potential culprits in the pathogenesis of Parkinson's disease and dementia with Lewy bodies (PD/DLB). Soluble oligomeric α-SN accumulated in the cytoplasm, can modify neuronal activities and intraneural Ca^{2+} dynamics, which may augment the metabolic burden in central neurons vulnerable for PD/DLB, but this hypothesis still remained to be fully tested. We evaluated how intracellular α-SN oligomers act on neuronal excitabilities and cytoplasmic Ca^{2+} dynamics by using whole-cell recording from pyramidal neurons in mouse neocortical slice. Intracellular application of α-SN incubated with dopamine, which makes stable oligomers (α-SNo), significantly reduced the spike frequency during current injection, elongated the duration of

spike afterhyperpolarization (AHP), and enlarged AHP current charge, when compared to application with α-SN incubated without dopamine. Pharmacological experiments indicated that this alteration mediated by α-SNo was dependent on the functional coupling of L-type Ca^{2+} channel, SK-type K^+ channel, and IP_3 receptor (IP_3R). α-SNo did not change Ca^{2+} current or IP_3 production, but intracellularly applied IP_3 mimicked and occluded the effect of α-SNo. These results suggested that the mechanism regulating IP_3R was targeted directly by α-SNo. Further observations using intracellularly applied Ca^{2+}-binding proteins regulating IP_3R opening or their antibodies, revealed that calcium binding protein 1 (CaBP1), but not calmodulin, was the key molecule involved with Ca^{2+}-induced Ca^{2+} release from IP_3 during spike trains mediated by α-SNo. Taken together, these findings revealed that α-SN oligomers hamper CaBP1-mediated inactivation of IP_3R and lead to spike-induced Ca^{2+} release from IP_3R without upregulating IP_3. This aberrant machinery may result in mitochondrial Ca^{2+} burden and be the molecular basis for neuronal vulnerability in PD/DLB.

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Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

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NMRC-TCR Grant (K.L. Lim)

CG Pilot Grant-NNI (J.L.Thundyil)

Title: The parkin-lipoprotein lipase link in the pathogenesis of parkinson’s disease

Authors: *J. L. THUNDYIL[1], S. Y. Q. ZHANG[2], A. NAIR[2], G. G. Y. LIM[1], T. P. YAO*[3], K. L. LIM*[1,2,4];
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Abstract: Lipids are abundantly present in the brain and play a critical role in its functioning. However, their involvement in neurodegenerative diseases is unclear. Recently parkin, a ubiquitin ligase whose mutations are causative of recessive Parkinson’s disease (PD), was shown to regulate brain lipid metabolism by modulating expression of CD36 (a fatty acid (FA) receptor) in the brain. Functionally, lipoprotein lipase (LPL-a rate limiting enzyme involved in FA

metabolism), is closely linked to CD36. Given this close functional link between CD36 and LPL in FA metabolism, we hypothesized that parkin may play a role in regulating brain LPL level. Accordingly, we explored the potential parkin-LPL link, with the view to understand its role in PD pathogenesis.
Using both parkin-deficient and parkin over-expression systems, we demonstrate that parkin indeed regulates LPL expression and this effect is consistent even at the transcript levels. Interestingly, this parkin mediated LPL regulation was independent of parkin's catalytic activity. Furthermore, our efforts to delineate the functional implications of parkin-LPL nexus demonstrated that lipid droplets (LDs- lipid rich intracellular organelles that regulate the storage and hydrolysis of neutral lipids) are significantly lower in parkin and LPL overexpressing cells in a toxin (rotenone) induced PD model. Given the extracellular role of LPL in mediating triglyceride hydrolysis and FA accumalation, this finding is indeed intriguing, and is suggestive of a novel intracellular role for LPL. Additionally, a significant increase in mitochondrial perimeter is observed in these cells, suggestive of a mitochondrial fusion phenotype in response to toxin-induced stress. Taken together, our results suggest a novel relationship between parkin and LPL. At the same time, our results also suggest a mechanism for the parkin-LPL nexus in the reduction of LDs via its effect on α-synuclein levels and subsequent mitochondrial fusion as a response to LD reduction.
References: Lim, K.L., et al., Mitochondrial dynamics and Parkinson's disease: focus on parkin. Antioxid Redox Signal, 2012. 16(9): p. 935-49.

Disclosures: J.L. Thundyil: None. **S.Y.Q. Zhang:** None. **A. Nair:** None. **G.G.Y. Lim:** None. **T.P. Yao*:** None. **K.L. Lim*:** None.

Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

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Topic: C.03. Parkinson's Disease

Support: FWO fellowship 11Y7516N

Title: Lipid induced relief of N terminal mediated autoinhibition of ATP13A2/PARK9.

Authors: *S. VAN VEEN[1], S. MARTIN[1], T. HOLEMANS[1], C. VAN DEN HAUTE[2], V. BAEKELANDT[2], P. VANGHELUWE[1];
[1]Cell. and Mol. Med., [2]Dept. of Neurosciences, KU Leuven, Leuven, Belgium

Abstract: ATP13A2/PARK9 is a lysosomal P-type transport ATPase of unknown function which has been implicated in Parkinson's disease (PD) and Kufor-Rakeb syndrome, an early-onset atypical parkinsonism. Interestingly, ATP13A2 confers protection against α-synuclein, Mn^{2+} and Zn^{2+} toxicity in various model organisms. Recently, we demonstrated that ATP13A2 undergoes auto-phosphorylation like other P-type ATPases, which can be stimulated by the lipids phosphatidic acid (PA) and phosphatidyl inositol (3,5) bisphosphate (PI(3,5)P2) by interacting at the N-terminus. Our biochemical analysis further showed that ATP13A2 resides in an inactive conformation and awaits activation by PA and PI(3,5)P2. In SHSY5Y neuroblastoma cells ATP13A2 activity is required to provide protection in conditions of mitochondrial stress (rotenone/MPP+) and heavy metal (Mn^{2+}, Zn^{2+}, Fe^{3+}) toxicity. However, when the formation of PA and PI(3,5)P2 is inhibited, the protective effect of ATP13A2 is abolished. This indicates that in cells ATP13A2 also resides in an inactive state, pending lipid-dependent activation during specific stress conditions.
To further test this model, we generated stable SHSY5Y cell lines expressing WT ATP13A2 or mutants of the three putative N-terminal lipid binding sites (LBS1-3). At the biochemical level, the LBS mutants lost the ability to auto-phosphorylate and are considered to be catalytically dead. Consequently, the LBS mutants failed to protect against both mitochondrial and metal toxicities. In contrast to WT the LBS2 and LBS3 mutants responded poorly to pharmacological inhibition of respectively PI(3,5)P2 and PA formation. Thus, our data support the view that the ATP13A2 N-terminus acts as an auto-inhibitory domain that can be relieved by binding of PI(3,5)P2 to LBS2 and of PA to LBS3. Most interestingly, mutations of LBS2 and LBS3, but not LBS1, exert a dominant negative effect on the endogenous ATP13A2 in SHSY5Y cells. In agreement, purified N-terminal protein fragments of the LBS2/3 mutants stably interact with full length ATP13A2 protein and inhibit ATP13A2 activity. These findings indicate that the dominant negative phenotype of LBS2/3 may result from a direct and stable interaction on an intramolecular inhibitory binding site of ATP13A2.
We conclude that the N-terminus emerges as a key to unlock ATP13A2's lysosomal activity and suggest that the N-terminus may serve as a novel therapeutic target to activate ATP13A2 for reducing metal and mitochondrial toxicity in PD.

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Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

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Program#/Poster#: 42.28/L4

Topic: C.03. Parkinson's Disease

Title: ATP13A2/PARK9 protects against mitochondrial stress and metal toxicity by maintaining mitochondrial network functionality.

Authors: *S. MARTIN[1], S. VAN VEEN[1], T. HOLEMANS[1], C. VAN DEN HAUTE[2], V. BAEKELANDT[2], P. AGOSTINIS[1], P. VANGHELUWE[1];
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Abstract: ATP13A2 (PARK9) is a P-type transport ATPase residing in the late endosomes and lysosomes that is implicated in Parkinson's disease (PD)[1]. The function of ATP13A2 remains unknown, yet it plays important roles in cellular homeostasis, underlying a pro-survival phenotype in both *in vitro* and *in vivo* models of PD exposed to heavy metals[1], mitochondrial (mito) toxins as well as alpha synuclein. Previous research demonstrated that ATP13A2 regulates autophagy, exosome biogenesis and mito/lysosomal functionality[1,2]. Recently, we showed that ATP13A2, like other P-type ATPases, undergoes auto-phosphorylation[2]. Moreover, the stress inducible lipids phosphatidic acid (PA) and phosphatidyl inositol (3,5) bisphosphate (PI(3,5)P2) modulate this activity by interacting at the level of the N-terminus, activating ATP13A2 responses[2]. Our biochemical analysis further showed that ATP13A2 resides in an inactive conformation and waits for further activation by PA and PI(3,5)P2[2], potentiating a pro-survival phenotype against various cellular insults and mito dysfunction[2]. Using stable SHSY5Y cell models of ATP13A2 overexpression *vs.* knockdown, we demonstrated a significant role of ATP13A2 in the maintenance of functional mitochondria, compared to control (fluc). Upon exposure to classical PD inducers (*e.g.* rotenone, MPP+, 6-OHDA) or heavy metals (Zinc, Manganese, Iron), we identified a significant protective effect of ATP13A2 on mito membrane potential and ATP output, which correlated inversely with the generation of reactive oxygen species (ROS). This ATP13A2-mediated protective effect was directly related to ROS, as its removal by the addition of anti-oxidants quenched the phenotype. Upon blockage of the functionality of the lysosomes by the addition of bafilomycin, we observed a re-sensitization of ATP13A2 overexpression towards mito toxicity and subsequent cell death. Moreover, in ATP13A2 knockdown cells we observed an increase of the mito unfolded protein response (HSP60, CHOP, CEBPβ), under both basal and rotenone-induced stress conditions, that was diminished by ATP13A2. Finally, using ATP13A2 loss-of-function patient-derived fibroblasts, we recapitulated our findings generated in the SHSY5Y cell models. In conclusion, our results provide further validity for the role of ATP13A2 in mito homeostasis and emphasize the importance of ATP13A2 activating compounds for PD prevention and protection. **References** 1. S. van Veen, *Front Mol Neurosci*, 2014, **7**, e-journal. 2. T. Holemans, *PNAS*, 2015, **29**, 9040-5.

Disclosures: **S. Martin:** None. **S. van Veen:** None. **T. Holemans:** None. **C. van den Haute:** None. **V. Baekelandt:** None. **P. Agostinis:** None. **P. Vangheluwe:** None.

Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 42.29/L5

Topic: C.03. Parkinson’s Disease

Title: Cloning the n27 dopamine cell line to improve a cell culture model of parkinson’s disease

Authors: *L. GAO;
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Abstract: Parkinson’s disease is the second most common neurodegenerative disease characterized by the death of dopamine neurons in the substantia nigra. To understand the molecular mechanisms of the disease, an *in vitro* model is important. In the 1990’s, we developed a dopaminergic cell line named as N27 cells from fetal rat mesencephalic dopamine neurons immortalized with the SV40 large T antigen. Because the original N27 cell line has been passaged many times, the line has become a mixture of cell types expressing highly variable levels of tyrosine hydroxylase (TH), a primary phenotypic marker for dopamine neurons. In this study, we performed multiple rounds of cell clonal cultures and finally identified a clone expressing high level of TH and dopamine transporter (DAT). Nearly 100% of cells in this purified N27 cells express TH, DAT and Tuj1. Western blot also confirmed that the purified N27 cells had three to four times level of TH and DAT than unpurified N27 cells. In addition, the purified N27 cells also express transcription factors of authentic dopamine neurons, such as Nurr1, En1, FoxA2 and Ptx3. As a dopaminergic cell line, the purified N27 cells were more sensitive to 6-hydroxydopamine induced toxicity as compared to unpurified N27 cells, while both cells had similar response to hydrogen peroxide induced toxicity. We conclude this purified N27 cells express high levels of dopamine neuron markers, which should provide an improved *in vitro* model for Parkinson’s research.

Disclosures: L. Gao: None.

Poster

042. Parkinson's Disease: Molecular Mechanisms and Models

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 42.30/L6

Topic: C.03. Parkinson's Disease

Support: University of Arizona Intramural funds

Title: Modeling Parkinson's disease using patient-derived primary dermal fibroblasts

Authors: *J. Y. TEVES[1], A. J. FLORES[2], V. BHARGAVA[3], M. J. CORENBLUM[4], R. JUSTINIANO[5], G. T. WONDRAK[5], J. E. SLIGH[6], C. CURIEL-LEWANDROWSKI[6], S. J. SHERMAN[4], D. A. SCHIPPER[7], Z. KHALPEY[7], L. MADHAVAN[4];
[2]Grad. Interdisciplinary Program in Physiological Sci., [3]Undergraduate Biol. Res. Program, [4]Dept. of Neurol., [5]Pharmacol. and Toxicology, [6]Dept. of Med., [7]Dept. of Surgery, [1]Univ. of Arizona, Tucson, AZ

Abstract: Patient-derived primary dermal fibroblasts are accessible peripheral cells that recapitulate the Parkinson's disease (PD) chronological and epigenetic aging history, and provide a useful system to study the disease. Here, we utilized dermal fibroblasts from late onset sporadic PD and age-matched control patients, and systematically examined the morphology, growth dynamics, response to stress, as well as mitochondrial and autophagy-related function of these cells. It was found that fibroblasts from PD patients were significantly ($p<0.05$) smaller and more circular than control cells. In terms of growth dynamics, PD fibroblasts grew faster and showed higher cell density at time of passage relative to the control cells. In addition, PD cells showed specific patterns of spatial organization in culture, which were different from control fibroblast lines. When the response of the fibroblasts to ultraviolet radiation (specifically UVA)-induced stress was examined, higher reactive oxygen species (ROS) production, particularly mitochondrial ROS (MitoSOX™ Red assay), was observed in PD cells compared to controls. To further analyze this effect, we subjected the PD and control lines to a mitochondrial stress test using the Seahorse Mito Stress Kit and Extracellular Flux analyzer. Preliminary data indicate that in comparison to control fibroblasts, respiratory control rate (RCR), proton leak, and coupling efficiency were negatively altered in the cells from individuals diagnosed with PD. Furthermore, given that increased expression of alpha-synuclein (α-syn), a substrate of autophagy, was observed in PD fibroblasts via immunocytochemistry, we analyzed specific autophagy marker proteins (LAMP1, p62, LC3-I, LC3-II) via immunoblot analysis. Preliminary data from these experiments suggest that autophagy may be impaired in the PD cells relative to controls. Currently, mitochondrial dynamics and autophagy function are further being analyzed in the patient cells. In summary, these studies indicate that patient-derived primary skin fibroblasts can act as a powerful model to study sporadic PD, and glean insights into mechanisms underlying the PD pathogenesis.

Disclosures: J.Y. Teves: None. **A.J. Flores:** None. **V. Bhargava:** None. **M.J. Corenblum:** None. **R. Justiniano:** None. **G.T. Wondrak:** None. **J.E. Sligh:** None. **C. Curiel-Lewandrowski:** None. **S.J. Sherman:** None. **D.A. Schipper:** None. **Z. Khalpey:** None. **L. Madhavan:** None.

Poster

043. Ataxia

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 43.01/L7

Topic: C.04. Movement Disorders

Support: NIH grant 1R21NS093695-01

Title: Anterior cerebellar degeneration impairs feedforward control in spinocerebellar ataxia type 6

Authors: *N. KANG, D. E. VAILLANCOURT, A. CASAMENTO-MORAN, S. H. SUBRAMONY, E. A. CHRISTOU;
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Abstract: The cerebellum has been implicated with both feedforward and feedback motor control. Spinocerebellar ataxia type 6 (SCA6), is a genetic disorder that results in the loss of Purkinje cells in the cerebellum, and loss of pyramidal track cells in the motor cortex. The purpose of this study was to investigate whether cerebellar degeneration in SCA6 influences the control of tasks that are primarily controlled by feedforward or feedback process. Fifteen individuals diagnosed with SCA6 performed fast goal-directed (feedforward) or constant force (feedback) isometric contractions with ankle dorsiflexion. Surface electromyography (EMG) and single and multi-motor unit recordings were recorded from the tibialis anterior muscle during the tasks. Moreover, we collected diffusion magnetic resonance imaging data to quantify free-water accumulation and free-water corrected fractional anisotropy in the cerebellar cortex and cerebellar peduncles, in order to examine the relation of the microstructural diffusion with force control, EMG burst activity, motor unit firing properties, and ataxia clinical assessments. Three novel findings were identified: (a) during the feedforward force control task, the SCA6 patients exhibited higher force error as well as greater EMG burst in the tibialis anterior than age-matched healthy controls (n = 9), (b) during the feedback force control task, there was no significant difference in force control and motor unit discharge rate properties between the SCA6 and control groups, and (c) elevated free-water values in the dentate, cerebellar peduncles, cerebellar lobules I-VI, vermis were found in the SCA6 group. Greater free-water in the lobule I-IV was associated with greater force error during feedforward motor control and more severe ataxia. These findings indicate that the anterior cerebellum degeneration that occurs with SCA6 interferes with feedforward motor control but not with feedback motor control.

Disclosures: N. Kang: None. **D.E. Vaillancourt:** 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, Bachmann-Strauss Foundation, Tyler's Hope Foundation. F.

Consulting Fees (e.g., advisory boards); UT Southwestern Medical Center, University of Illinois at Chicago, Scott & White, Great Lakes NeuroTechnologies. Other; Neuroimaging Solutions, LLC. **A. Casamento-Moran:** None. **S.H. Subramony:** None. **E.A. Christou:** None.

Poster

043. Ataxia

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 43.02/L8

Topic: C.04. Movement Disorders

Support: ISF

Title: Combining Atm and Wrn deficiencies in mice leads to cerebellar deterioration

Authors: *O. BIHARI[1], N. KAMINSKY[2], R. GALRON[2], B. F. JOHNSON[4], E. PIKARSKY[5], Y. SHILOH[3], A. BARZILAI[2];
[1]Sagol Sch. of Neurosci., Tel Aviv Univ., Tel Aviv-Yafo, Israel; [2]Neurobiology, Life Sci., [3]Human Mol. Genet. and Biochemistry, Sackler Sch. of Med., Tel Aviv Univ., Tel Aviv, Israel; [4]Pathology and Lab. Med., Univ. of Pennsylvania, Philadelphia, PA; [5]Immunol. and Cancer Res., Hebrew University-Hadassah Med. Sch., Jerusalem, Israel

Abstract: The primary cause of Ataxia Telangiectasia (A-T) is *ATM* mutations. The ATM protein is a multifunctional, homeostatic protein kinase with a major role in maintaining genome stability, primarily in the response to DNA double-strand breaks (DSBs). It is still unclear which ATM functions are those whose loss leads to the major symptom of A-T - cerebellar atrophy. We suggest that a critical function of ATM in this regard is its involvement in many DNA repair pathways and other aspects of genome stability. The loss of this ATM function might be particularly deleterious to the ability of Purkinje neurons to cope with excessive wear and tear on their DNA due to the unique combination of their high metabolic activity, special chromatin organization, and finite non-regenerative state. In this scenario, the critical DNA lesions are those formed during ongoing DNA transactions or induced by endogenous oxygen radicals, with DSBs having a minor share in the broad spectrum of this DNA damage.
We are using mouse models to substantiate this contention. The cerebellar abnormalities observed in *Atm*-knockout (*Atm*-KO) mice are extremely mild over the lifetime of this mouse model. However, it does enable examining additional pressure on genomic stability that might enhance the deterioration of the Atm-deficient cerebellum and make it visible within the animal's life span. Presuming that this could be abrogation of the response to the ongoing damage and aberrations of the DNA, we combined a *Wrn* null allele with Atm loss. *Wrn* encodes the murine ortholog of the human gene, *WRN*, which is mutated in the premature aging disease,

Werner syndrome (WRN) . Wrn is a bifunctional helicase/nuclease that is involved in numerous DNA transactions and DNA repair mechanisms and in the resolution of non-canonical DNA structures. *Wrn*-knockout (*Wrn*-KO) mice were reported to show no discernible phenotype. Notably, the double-knockout genotype (*Atm*-/-//*Wrn*-/-) turned out to be embryonic lethal. A viable genotype of particular interest is *Atm*-/-//*Wrn*+/-, in which a mere heterozygosity at the *Wrn* locus is added to Atm loss. Other than premature aging signs, such as early graying, cracked skin and kyphosis, these animals exhibit neuromotor dysfunction from one year of age. Histopathological examination revealed various degrees of cerebellar degeneration, with atrophy in both the neuronal cell layer and supporting astroglia. Importantly, Purkinje neurons were deformed, with heteropycnotic nuclei. This mouse model is being further developed using conditional *Atm* ablation in the CNS combined with *Wrn*-KO. This mouse is likely to provide a much needed animal model of the degenerating ATM-deficient cerebellum.

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Poster

043. Ataxia

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 43.03/L9

Topic: C.04. Movement Disorders

Support: NIH Grant R01NS082788

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National Ataxia Foundation Post-Doc Fellowship Award

JSPS KAKENHI Grant Number 26293213 (Japan)

Grants from Japan Agency for Medical Research and Development

Title: An miRNA-mediated therapy for SCA6 blocks IRES-driven translation of the CACNA1A second cistron

Authors: *Y. MIYAZAKI[1], X. DU[1], S.-I. MURAMATSU[2], C. GOMEZ[1];
[1]Dept. of Neurol., The Univ. of Chicago, Chicago, IL; [2]Div. of Neurol., Jichi Med. Univ., Shimotsuke, Japan

Abstract: Spinocerebellar ataxia type 6 (SCA6) is a dominantly-inherited neurodegenerative disease characterized by slowly progressive ataxia and Purkinje cell degeneration. SCA6 is attributable to a polyglutamine repeat expansion within a second *CACNA1A* gene product, α1ACT. α1ACT expression is under the control of an internal ribosomal entry site (IRES) present within the *CACNA1A* coding region. While SCA6 allele knock-in mice show indistinguishable phenotypes from wild-type littermates even at late phase, expression of SCA6-associated α1ACT ($\alpha1ACT_{SCA6}$) driven by Purkinje cell-specific promoter in mice produces slowly progressive ataxia and cerebellar atrophy. Here, we used *CACNA1A* IRES-driven $\alpha1ACT_{SCA6}$ expression by an adeno-associated virus (AAV)-based gene delivery system to develop an early onset ataxia model with a more pronounced phenotype, more suitable for testing *CACNA1A* IRES-targeted potential therapies. AAV9-mediated somatic gene transfer of *CACNA1A* IRES-driven $\alpha1ACT_{SCA6}$ caused expression throughout mouse brain and cerebellum. Mice expressing AAV9-delivered *CACNA1A* IRES-driven $\alpha1ACT_{SCA6}$ exhibit an early onset ataxia, motor deficits, and Purkinje cell degeneration in cerebellum from early age. We also found miR-3191-5p targeting *CACNA1A* IRES preferentially blocks the *CACNA1A* IRES-driven translation of α1ACT in an Argonaute (Ago) protein-4-dependent manner. miR-3191-5p bound to Ago4 selectively inhibits the translational initiation of α1ACT by eukaryotic initiation factors, eIF4AII and eIF4GII, which directly act on *CACNA1A* IRES to enhance α1ACT translation. Furthermore, AAV9-mediated delivery of miR-3191-5p protects from the ataxia, motor deficits, and Purkinje cell degeneration caused by *CACNA1A* IRES-driven $\alpha1ACT_{SCA6}$ in mice. Our results establish the proof of principle that therapeutic intervention with viral delivery of a disease-specific miRNA rescues SCA6 phenotypes in a mouse model that expresses *CACNA1A* IRES-driven $\alpha1ACT_{SCA6}$.

Disclosures: **Y. Miyazaki:** None. **X. Du:** None. **S. Muramatsu:** None. **C. Gomez:** None.

Poster

043. Ataxia

Location: Halls B-H

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Topic: C.04. Movement Disorders

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NIH Grant R01DC02390 (KC)

CIHR Grant MOP-42440 (KC)

McGill IPN Returning Student Award (SJ)

Title: 4-Aminopyridine alleviates ataxia and reverses cerebellar cortical output deficiency in a mouse model of spinocerebellar ataxia type 6

Authors: *S. JAYABAL[1,2], H. CHANG[3], K. E. CULLEN[3], A. J. WATT[1];
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Abstract: Spinocerebellar ataxia type 6 (SCA6) is a mid-life onset progressive neurodegenerative disease that affects motor coordination and gait and has no known treatment. This autosomal dominant disease is caused by a CAG repeat expansion in the *CACNA1A* gene; most affected people have only one copy of the mutated gene. Here to evaluate the role of cerebellar Purkinje cell firing in SCA6 pathophysiology, we studied a hyperexpanded (84Q) mouse model of SCA6.

Purkinje cell firing was first measured non-invasively with extracellular recordings in acute slices made from heterozygous $SCA6^{84Q/+}$ mice at an age when motor coordination deficits were observed (19 months). We found reduced spike timing precision compared to litter-matched wildtype (WT) cells, reflected in an enhanced coefficient of variation (CV) of interspike intervals ($P = 0.0047$) without a significant change in firing rate ($P = 0.071$). Then to determine whether homozygous $SCA6^{84Q/84Q}$ mice, which develop motor deficits earlier (7 months) are a good model for SCA6, we measured Purkinje cell firing properties from acute slices of 7-month old cerebellum. Homozygous $SCA6^{84Q/84Q}$ Purkinje cells also displayed reduced spike timing precision compared to age-matched WT cells ($P < 0.0001$), suggesting that homozygous mice are a good and practical model for SCA6.

Since the FDA-approved potassium channel blocker 4-aminopyridine (4-AP) has been shown to improve ataxia and Purkinje cell firing abnormalities in mouse models of other forms of ataxia, we acutely applied 4-AP to $SCA6^{84Q/84Q}$ Purkinje cells in acute slices and found that it improved spike timing precision ($P = 0.003$) without affecting firing rate ($P = 0.15$). To determine if 4-AP might alleviate ataxia in SCA6 mice, we provided 4-AP in drinking water to 7-month-old $SCA6^{84Q/84Q}$ and WT mice. 4-AP produced a significant partial rescue of motor coordination in $SCA6^{84Q/84Q}$ mice after a week ($P < 0.0001$), without affecting WT motor performance. This improvement lasted as long as we tested (up to 3 months). Finally, to add further support to our hypothesis that changes in Purkinje cell firing contribute to disease onset in SCA6 mice, we completed *in vivo* single unit recordings, and found that spike timing precision was indeed reduced in untreated $SCA6^{84Q/84Q}$ mice ($P = 0.04$); it could then be rescued to WT levels following 2 weeks of chronic 4-AP (not significantly different from WT, $P = 0.21$), without significant changes in firing rates ($P > 0.05$). Taken together, our results provide a novel therapeutic approach for the treatment of SCA6 with 4-AP.

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Poster

043. Ataxia

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 43.05/L11

Topic: C.04. Movement Disorders

Support: European Commission's Seventh Framework Programme FP7/2010 under grant agreement no. 264508 (TreatPolyQ)

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National Ataxia Foundation

Title: Mechanistic insights into the nucleocytoplasmic shuttling of ataxin-3

Authors: *T. SCHMIDT[1,2], A. SOWA[1,2,3], M. MARTINS[1,2], J. SCHMIDT[1,2], D. WEISHAEUPL[1,2], M. ABEDI[1,2,3], Z. WANG[1,2,3], L. LEHMANN[1,2], A. TEIXEIRA-CASTRO[4], P. MACIEL[4], H. TRICOIRE[5], O. RIESS[1,2];
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Abstract: Spinocerebellar ataxia type 3 (SCA3) or Machado-Joseph disease (MJD) is an autosomal-dominantly inherited neurodegenerative disorder caused by a CAG expansion in the *ATXN3* gene leading to a polyglutamine expansion in the encoded ataxin-3 protein. Characteristic for SCA3 and other polyglutamine diseases are the so called neuronal intranuclear inclusion bodies (NII). As ataxin-3 is predominantly located in the cytoplasm, the formation of protein aggregates in the nucleus require a nucleocytoplasmic shuttling of ataxin-3. We demonstrated *in vivo* that the toxicity of expanded ataxin-3 depends on its intracellular localization: While nuclear ataxin-3 gave rise to a strong phenotype in transgenic mouse models with a high number of protein aggregates, purely cytoplasmic ataxin-3, however, even with a highly expanded polyglutamine repeat, was not able to induce a phenotype and even did not aggregate. We further identified and characterized intracellular transport signals (two nuclear export signals, NES, and one nuclear localization signal, NLS) within the coding sequence of ataxin-3. Therefore, it is evident that proteins involved in the nucleocytoplasmic transport machinery recognize these localization signals, control the intracellular localization of ataxin-3, thereby influence the toxicity and aggregation of ataxin-3 and, thus, the pathogenesis of SCA3. We now identified a transport protein which is critically involved in the nucleocytoplasmic

shuttling of ataxin-3 and impacts typical pathogenic mechanisms of SCA3 (like the formation of aggregates) as well as the phenotype induced by expanded ataxin-3. While the overexpression of this protein moved ataxin-3 into the nucleus, its downregulation kept it out of the nucleus. We replicated this correlation *in vivo* in *Drosophila* and observed a clear link between the intracellular localization of ataxin-3 and its toxicity i.e. its ability of induce neurodegeneration and a behavioral phenotype. Likewise we even confirmed in a mouse model of SCA3 the importance of the identified transport protein as its knockout largely prevented ataxin-3 from aggregating and alleviated behavioral and movement deficits. Our results demonstrate that transport proteins are involved in the intracellular localization of ataxin-3 and the neurodegenerative processes in SCA3. The insights into the mechanisms behind the intracellular transport of ataxin-3 give us clues into the pathogenic functions of expanded ataxin-3 and ways to mediate the progression of neuronal degeneration in SCA3 patients.

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Poster

043. Ataxia

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Topic: C.04. Movement Disorders

Support: R21NS081182

R01NS033123

R37NS033123

Title: ASO-mediated reduction of ATXN2 expression after motor phenotype onset in SCA2 mice restores cerebellar Purkinje cell electrophysiological phenotypes

Authors: *D. R. SCOLES[1], P. MEERA[2], M. SCHNEIDER[1], K. FIGUEROA[1], F. RIGO[3], F. BENNETT[3], T. OTIS[2], S. PULST[1];
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Abstract: Spinocerebellar ataxia type 2 (SCA2) is caused by CAG repeat expansion in the ATXN2 gene resulting in polyglutamine expanded ATXN2 protein and pathogenic gain of toxic function. By screening 152 antisense oligonucleotides (ASOs) targeting ATXN2 we identified

ASO7 that lowered ATXN2 expression with long lasting effects without toxicity. We have characterized two SCA2 animal models, one expressing ATXN2-Q127 in Purkinje cells (PCs), the other a BAC-ATXN2-Q72 (Hansen et al., 2013, Dansithong et al., 2015). **Objective:** To demonstrate that lowering ATXN2 expression modifies the motor phenotypes of two SCA2 mouse models and to compare the ASO effects between the two mouse models for modifying neurophysiological phenotypes. **Methods:** SCA2 mice were treated by intracerebroventricular (ICV) injection of ASOs. The effects of ASO treatments on motor phenotypes were verified by rotarod testing, cerebellar molecular phenotypes were determined by qPCR, and PC firing frequencies (FFs) were determined by extracellular recordings in acute cerebellar slices. **Results:** Both ATXN2-Q127 mice and BAC-ATXN2-Q72 mice exhibit progressive age-dependent rotarod phenotypes. ATXN2-Q127 mice treated ICV with ASO7 at 8 wks of age had a significantly improved rotarod performance at 18 weeks of age vs. saline treated control mice (n=15 mice per group, $P<0.01$). At the endpoint, PC FFs were 16±1 Hz for saline treated mice, and 42±2 Hz for ASO7 treated mice (mean±SEM, per group n=2 mice, 102-107 neurons. $P<0.001$), which is similar to age-matched wildtype mice. For BAC-ATXN2-Q72 mice injected with ASO7 at 8 wks of age, we also observed a significantly improved rotarod performance at 18 weeks of age vs. saline treated mice (n=11-14 mice per group, $P<0.01$). BAC-ATXN2-Q72 mice have no PC FF phenotype until age 24 wks. Therefore, to evaluate the effect of ASO7 on BAC-ATXN2-Q72 mice we injected mice at age 30 wks, well after motor phenotype onset, and evaluated PC FFs at age 40 wks. We observed PC FFs of 49±1 Hz for ASO7 treated mice and 36±1 Hz for saline treated mice (mean±SEM, per group n=3-4 mice, 134-148 neurons. $P<0.001$). In both models, ASO7 engaged the target well resulting in reduction of hATXN2 by up to 80%. **Conclusions:** A single treatment of ASO7 lowered ATXN2 expression resulting in restoration of PC FF even well after onset of SCA2 mouse motor phenotype and significantly improved the rotarod phenotype in both mouse models.

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Poster

043. Ataxia

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Topic: C.04. Movement Disorders

Support: NIH Grant1R01NS082788

Title: The role of alpha1ACT in the early stage development of the cerebellum

Authors: *X. DU, C. GOMEZ;
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Abstract: The spinocerebellar ataxia 6 (SCA6) gene, *CACNA1A*, encodes two proteins: the calcium channel, α1A, and α1ACT, a novel transcription factor. α1ACT is critical for neurite outgrowth and cerebellar cortical development by coordinating expression of Purkinje cell genes in concert with α1A and bears an expanded polyQ in SCA6. Using RNA-seq, the global gene expression profile of α1ACT revealed that there were 166 and 717 differentially expressed transcripts in PC12 cells over-expressing α1ACT at 6 hrs and 10 days, respectively. Additionally, 39 common differentially-expressed genes were identified over 4 time points. Furthermore, ChIP-seq and RNA-seq analysis revealed an enrichment of α1ACT binding around transcription start sites and a positive correlation between α1ACT genomic occupancy and the expression of many associated genes. Gene ontology and network analysis further demonstrated that differing functions of the identified specific genes were associated with neurogenesis and neuronal differentiation in early time points, and cell cycle in late time points. Using animal models we found that a humanized exon-47 mouse *CACNA1A* allele was a hypomorph, and mice bearing a second null *CACNA1A* allele (KIKO) had delayed cerebellar development, decreased survival, and impaired motor behaviors before 12 months old of age. Transgenic expression of α1ACT in KIKO background (KIKO/PC-α1ACT) restored cerebellar development, increased survival, and improved the impaired motor behavior. The α1ACT target gene expression was decreased in KIKO mice but restored in KIKO:PC-α1ACT mice, suggesting that α1ACT directly regulates genes important for orchestrating early postnatal stages of neural development. Absence or delay of α1ACT expression leads to dysregulated expression of common target genes and cerebellar development in the mouse model. These results reveal time-dependent expression profiles for α1ACT in global transcriptional regulation and have implications for understanding mechanisms underlying function of α1ACT *in vitro* and *in vivo* relevant to developing therapies for SCA6.

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Poster

043. Ataxia

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Program#/Poster#: 43.08/L14

Topic: C.04. Movement Disorders

Support: CHOP Foerderer Grant for Excellence (HL)

FARA Center of Excellence Grant (DL)

FARA New Investigator Grant (JM)

Title: Early cerebellar mitochondrial deficits in a frataxin-deficient mouse model of Friedreich's ataxia

Authors: *H. LIN[1], C. LAO-PEREGRÍN[2], E. M. CLARK[3], Y. DONG[1], A. J. RATTELLE[1], J. MAGRANÉ[2], D. R. LYNCH[1,3];
[1]Neurol. and Pediatrics, The Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Weill Cornell Med. Col., New York, NY; [3]Perelman Sch. of Med. Univ. of Pennsylvania, Philadelphia, PA

Abstract: Frataxin is a highly conserved small mitochondrial protein crucial for iron-sulfur cluster formation and ATP production. Frataxin deficiency in Friedreich's ataxia (FRDA) causes a complex neurological and pathological phenotype of peripheral and central nervous systems. Postmortem studies show degeneration of the cerebellum within dentate nucleus in FRDA patient brain samples. However, the contribution of mitochondrial pathophysiology in cerebellar degeneration of FRDA at early stage of disease remains largely uncharacterized. Here we report early cerebellar mitochondrial deficits at presymptomatic ages in a frataxin-deficient FRDA mouse model, frataxin knockin/knockout (*KIKO*) mice. In *KIKO* double transgenic mice expressing the fluorescent protein Dendra targeted to mitochondria in neurons (*mitoDendra-KIKO)*, while the cerebellar levels of frataxin were significantly reduced, the number and levels of mitochondria labeled with Dendra in the cerebellum were significantly decreased compared with control mice at presymptomatic ages. The levels of mitochondrial respiration chain complex II protein succinate dehydrogenase subunit A (SDHA) and mitochondrial heat shock protein mortalin/GRP75 were also significantly reduced in the cerebellum of *KIKO* and *mitoDendra-KIKO* mice compared with control mice at presympotmatic ages. More interestingly, the levels of mitochondrial biogenesis master regulator PGC-1α and mitochondrial fusion protein mitofusin were markedly reduced in the cerebellum of *KIKO* and *mitoDendra-KIKO* mice at presymptomatic ages, suggesting that frataxin deficiency may lead to disruption of cerebellar mitochondrial biogenesis and network at early stage of disease in FRDA. Our findings thus demonstrate early cerebellar mitochondrial deficits in a FRDA mouse model, which may contribute to progressive ataxia that could be reversible by restoring frataxin or mitochondria integrity in FRDA patients.

Disclosures: H. Lin: None. **C. Lao-Peregrín:** None. **E.M. Clark:** None. **Y. Dong:** None. **A.J. Rattelle:** None. **J. Magrané:** None. **D.R. Lynch:** None.

Poster

043. Ataxia

Location: Halls B-H

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Topic: C.04. Movement Disorders

Support: A-T Children's Fund

Research Grants Council, HKSAR

Title: ATM kinase activity is required to maintain mitochondrial integrity

Authors: *R. P. HART[1], H. CHOW[2], K. HERRUP[2];
[1]Rutgers Univ., Piscataway, NJ; [2]Hong Kong Univ. of Sci. and Technol., Clearwater Bay, Hong Kong

Abstract: Ataxia-telangiectasia (A-T) is one of the most prominent inherited neuropathological disorders caused by defects in the DNA damage response (DDR) pathway. While neurodegeneration in A-T has been attributed to defective DDR or to epigenetic regulation, recent reports on the role of ATM in redox signaling also suggest enhanced oxidative stress and reduced anti-oxidative defense may contribute to the neurodegenerative phenotype. We have previously reported a missense mutation 7181C>T which translates to an ATM S2394L protein variant, which exhibited little or no ATM kinase activity. This variant was identified in induced pluripotent stem cells (iPSC) derived from an A-T subject having later-onset, milder symptoms. Together with an unaffected single nucleotide polymorphism (SNP), which translates to an ATM L2037F protein and is hypothesized to have a protective effect in neurodegeneration, we tested effects of each variant on mitochondrial integrity. Our preliminary data show that when overexpressed in $ATM^{-/-}$ mouse astrocytes, ectopic expression of the S2394L mutant protein resulted in enhanced mitochondrial oxidation, fragmentation, and loss of mitochondrial potential; whereas expression of the L2037F mutant showed the opposite effect. Similar experiments in A-T iPSC-derived human neurons will extend these results to a neuronal background. These results suggest that functional ATM kinase activity is indispensable for the maintenance of mitochondrial structure and function.

Disclosures: **R.P. Hart:** None. **H. Chow:** None. **K. Herrup:** None.

Poster

043. Ataxia

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Topic: C.04. Movement Disorders

Support: NIH Grant 2R37NS033123

Title: Dysfunction of mGluR1 signaling as a potential cause for ATX2-mediated purkinje neuron death in a spinocerebellar ataxia 2 mouse model

Authors: *P. MEERA[1], S. M. PULST[2], T. S. OTIS[1];
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Abstract: Spinocerebellar ataxia 2 (SCA2) is an autosomal dominant neurodegenerative disorder, characterized by progressive ataxia and cerebellar atrophy, affecting primarily cerebellar Purkinje neurons (PNs) and is caused by an expansion of triplet CAG repeats (>31 triplet repeat) in the Ataxin 2 (ATXN2) gene coding region. In our earlier work, we performed a detailed characterization of the SCA2 mouse model generated by the Pulst laboratory, where full length ATXN2-Q127 was expressed under the control of a PN specific promoter (SCA2-Q127, Hansen, Meera et al., 2013). Here, we explore molecular mechanisms that underlie SCA2 pathophysiology in this SCA2-Q127 mouse model using simultaneous electrical recording and Ca^{2+} imaging. Using two photon microscopy and the Ca^{2+} dye Oregon Green BAPTA 1(OGB1), loaded into the PNs with the recording pipette, we find that the basal Ca^{2+} levels in PNs of SCA2 mice are increased when compared to WT PNs. To further elucidate potential mechanisms of SCA2 pathology, we focused on mGluR1-mediated signaling. The mGluR1 agonist DHPG leads to an age-dependent progressive increase in SCA2 PN firing frequency with only minimal effects on WT PNs. We also show that Parallel Fiber (PF)-mediated mGuR1 Ca^{2+} signals are dramatically and progressively enhanced in SCA2-Q127 mice. We further show that synaptic mGluR1 EPSCs are determined by internal free [Ca^{2+}]. Clamping [Ca^{2+}] close to 100 nM leads to indistinguishable EPSCs both in WT and SCA2 and raising internal Ca^{2+} close to 500 nM increases peak mGlur1 EPSCs in WT, similar to those found in SCA2. These findings support our overarching hypothesis that a key aspect of SCA2 pathology involves positive feedback between elevated basal Ca^{2+} and mGluR activity. Ca^{2+} elevations are known to potentiate mGluR function in PNs, suggesting that chronically elevated Ca^{2+} levels caused by hyperactive IP3Rs could lead to enhanced mGluR function. Such changes would be expected to exacerbate the elevated Ca^{2+}, and possibly lead to decreased firing frequency through the activation of SK channels, and eventually contribute to PN death. This PN cell loss likely leads to cerebellar circuit dysfunction responsible for the behavioral signs of ataxia characteristic of the disease.

Disclosures: **P. Meera:** None. **S.M. Pulst:** None. **T.S. Otis:** None.

Poster

043. Ataxia

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Topic: C.04. Movement Disorders

Support: NIH R01 NS038712

MiBrain Initiative Graduate Fellowship in Neuroscience

Ionis Pharmaceuticals

Title: Widespread *In vivo* suppression of mutant ATXN3 by antisense oligonucleotides in transgenic mouse models of SCA3

Authors: ***L. MOORE**[1], H. S. MCLOUGHLIN[2], I. DILLINGHAM[2], R. KOMLO[2], M. QUTOB[2], G. RAJPAL[2], H. KORDASIEWICZ[3], H. L. PAULSON[2];
[1]Neurosci. Grad. Program Univ. of Michigan, Ann Arbor, MI; [2]Neurol., Univ. of Michigan, Ann Arbor, MI; [3]Ionis Pharmaceuticals, Carslbad, CA

Abstract: Spinocerebellar ataxia type 3 (SCA3) is a progressive, fatal neurodegenerative disorder caused by a repeat expansion of the trinucleotide sequence CAG encoding the amino acid glutamine. In SCA3, the mutant polyglutamine expansion occurs in the gene ATXN3 encoding the deubiquitinating enzyme ATXN3. Currently there is no effective treatment or cure for SCA3. Given the still limited understanding of specific pathogenic processes leading to neuronal loss, disease protein-lowering strategies have emerged as a powerful potential therapeutic strategy to prevent or slow genetic neurodegenerative diseases through targeted suppression of mutant protein expression. Here we tested target engagement and potential toxicity of antisense oligonucleotides (ASOs) targeting human ATXN3 in two transgenic mouse models of SCA3, the YACQ84.2 model expressing the full human disease gene with 84 CAG repeats and the CMV-MJDQ135 model expressing the human ATXN3 cDNA with 135 CAG repeats. Anti-ATXN3 ASOs were administered via a unilateral intracerebroventricular bolus injection. Histological analysis confirmed widespread delivery of ASOs throughout the brain, including regions highly affected in SCA3 such as pontine nuclei and deep cerebellar nuclei, with no evidence of ASO toxicity at effective doses. ASO candidates achieved knockdown of mutant ATXN3 in cervical spinal cord, cerebellum, brainstem and forebrain by western blot and histochemical analysis four weeks following delivery, and one ASO exhibited even greater

ATXN3 knockdown at 8 weeks post operation. We are currently further investigating ASOs in both mouse models and in a novel SCA3 disease-specific human embryonic stem cell line and derived neuronal populations. Our results demonstrate that an ASO strategy to reduce mutant ATXN3 levels effectively lowers ATXN3 in relevant tissues.

Disclosures: **L. Moore:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Ionis Pharmaceuticals. **H.S. McLoughlin:** None. **I. Dillingham:** None. **R. Komlo:** None. **M. Qutob:** None. **G. Rajpal:** None. **H. Kordasiewicz:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Ionis Pharmaceuticals. **H.L. Paulson:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Ionis Pharmaceuticals.

Poster

043. Ataxia

Location: Halls B-H

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Topic: C.04. Movement Disorders

Support: Brain Health Research Centre

HT Orr, University of Minnesota

University of Otago PhD scholarship

OSMS Dean's bequest fund

Department of Physiology PhD scholarship

Title: Impact of ataxin-1 expression during postnatal cerebellar Purkinje neuron development and the role of metabotropic glutamate receptor type 1 for progression of SpinoCerebellar Ataxia Type 1

Authors: ***E. M. POWER**, M. F. IBRAHIM, R. M. EMPSON;
Dept. of Physiol., Univ. of Otago, Dunedin, New Zealand

Abstract: Spino-cerebellar ataxia type 1 (SCA1) is an incurable, autosomal dominant neurodegenerative motor disorder resulting from a CAG trinucleotide expansion of ataxin-1. In this study, we use a conditional transgenic mouse model of SCA1, where the CAG repeat expansion is restricted to cerebellar Purkinje neurons (PNs) and can be repressed via administration of doxycycline (doxy) in the food.

SCA1 mice exhibited a disruption of motor performance on the accelerating rotarod compared with controls at six weeks of age (two-way-ANOVA, $F_{1,25} = 5.78$, $P < 0.024$), and as the disease progresses motor performance becomes further impaired. However, when mutant ataxin-1 is repressed via doxy for the first 6 weeks of life there is a marked improvement in motor performance, compared to WT, up to 24 weeks of age (2-way-ANOVA, $P=0.071$, $F_{1,29} = 3.50$). This highlights the impact of mutant ataxin-1 expression during early postnatal cerebellar PN development.
PNs from pre-symptomatic doxy treated SCA1 mice at 12 weeks of age (Doxy administration in utero and for the first 6 weeks of life) exhibited intact dendritic morphology (Sholl analysis) (two-way-ANOVA $F_{96, 1536} = 2.36$, $P < 0.0001$) and normal phasic firing (one-way-ANOVA, $F_{2, 36} = 17.73$, $P < 0.0001$) when compared with PNs from 12 week SCA1 and WT doxy treated mice. The PNs did however demonstrate a significantly lengthened Parallel fibre evoked mGluR1-dependent current (one-way-ANOVA $F_{2,15} = 6.75$, $P = 0.008$) that may arise from early loss of the PN glutamate transporter EAAT4 occurring at 3 weeks of age in these SCA1 mice. Our results emphasize the importance of early intervention to prevent the progression of SCA1 and highlight mGluR1 receptors as a potential target for future therapies.

Disclosures: **E.M. Power:** None. **M.F. Ibrahim:** None. **R.M. Empson:** None.

Poster

043. Ataxia

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 43.13/M5

Topic: C.04. Movement Disorders

Support: Muscular Dystrophy Association, Grant #MDA186994

Title: Peripheral blood gene expression biomarkers in Friedreich's Ataxia

Authors: ***D. NACHUN**[1], F. GAO[1], C. ISAACS[3], Z. YANG[1], D. DOKURU[1], V. VAN BERLO[1], R. SEARS[4], J. FARMER[5], S. PERLMAN[2], D. LYNCH[3], G. COPPOLA[1,2];
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Abstract: Friedreich's ataxia (FRDA) is a rare recessive genetic disorder caused by a trinucleotide repeat expansion in the frataxin gene (FXN), which results in FXN deficiency. We studied gene expression profiles in peripheral blood in 392 patients, 217 carriers, and 80 controls, the largest FRDA cohort ever collected. Rich phenotypic data is available for most of

these subjects, and longitudinal data is available for a subset. Data analysis included correlation with expansion length, differential expression, network analysis, and machine learning approaches. We identified a robust peripheral signature associated with disease which partially overlaps with published datasets from both human FRDA patients and animal models of frataxin deficiency. A subset of transcripts showed longitudinal changes over time. Enriched ontologies and pathways in our signature included protein translation, mitochondrial metabolism, inflammation and cell stress. These findings will contribute to the identification of biomarkers for use in diagnostics and therapeutics.

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Poster

043. Ataxia

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National Key Basic Research Program of China (2013CB530900)

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Hong Kong PhD Fellowship

Title: ATM is located on synaptic vesicles and its deficit leads to failsures in synaptic plasticity

Authors: *A. CHENG[1], G. VAIL[2], T. ZHAO[1], Y. HAN[2], S. DU[1], M. LOY[1], K. HERRUP[1], M. PLUMMER[2];
[1]HKUST, Kowloon, Hong Kong; [2]Rutgers Univ., New Brunswick, NJ

Abstract: Ataxia-telangiectasia is a multi-systemic disorder that includes a devastating neurodegeneration phenotype. The ATM (ataxia-telangiectasia mutated) protein is well-known for its role in the DNA damage response. Yet ATM is also found in association with cytoplasmic vesicular structures - endosomes and lysosomes as well as neuronal synaptic vesicles. In keeping with this latter association, electrical stimulation of the Schaffer collateral pathway in

hippocampal slices from ATM-deficient mice does not elicit normal long term potentiation (LTP). The current study was undertaken to assess the nature of this deficit. Theta burst-induced LTP was reduced in Atm-/- animals with the reduction most pronounced at burst stimuli that included six or greater trains. To assess whether the deficit was associated with a pre- or post-synaptic failure, we analyzed paired-pulse facilitation and found that it too was significantly reduced in Atm-/- mice. This indicates a deficit in presynaptic function. As further evidence that these synaptic effects of ATM deficiency were presynaptic, we used stochastic optical reconstruction microscopy (STORM). Three-dimensional reconstruction revealed that ATM is significantly more closely associated with Piccolo (a pre-synaptic marker) than with Homer1 (a post-synaptic marker). These results underline how, in addition to its nuclear functions, ATM plays an important functional role in the neuronal synapse where it participates in the regulation of presynaptic vesicle physiology.

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Poster

043. Ataxia

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 43.15/M7

Topic: C.04. Movement Disorders

Title: A new simple automatic and non invasive method for measurement of chemically induced tremors in rats

Authors: *D. AMRUTKAR, T. A. JACOBSEN, K. S. NIELSEN;
Saniona A/S, Ballerup, Denmark

Abstract: Essential tremor (ET) is regarded as one of the most common neurological disorders with a prevalence similar to or greater than that of stroke, Alzheimer disease, migraine and lumbosacral pain syndromes and is as much as 20 times more prevalent than Parkinson's disease. The incidence of ET increases with advancing age, but it is fairly common in all age groups and almost equal in men and women. Due to lack of understanding of the basic mechanism, origin of tremors and predictive animal models, it is difficult to develop and screen novel pharmacological agents with selective and specific antitremor activity. A new simple method of quantifying harmaline induced tremors in male Sprague-Dawley (SD) rats is described. Tremor activity (shaking movement of fore paws) is detected by movement of small metal band placed on one of the fore limb of the rats. A signal is generated as the band breaks the electromagnetic field of a loop antenna located under the rat and processed through an algorithm that determines tremor

activity using 1) amplitude, 2) zero-voltage crossing, and 3) signal duration. Tremors are summed and stored over a selected collection interval throughout the assay for later analysis as described by Yaksh TL. et al (2000). Harmaline induced significant tremors at 10mg/kg upon intraperitoneal administration. The method is validated by propranolol, diazepam and primidone, drugs used in the clinic to treat ET. The reliability and robustness of the automatic method is confirmed by manual visual assessment. In addition, riluzole, a neuroprotective, sodium channel stabilizer potently and dose-dependently inhibited the harmaline induced tremors as previously shown in the literature. The advantages of the method are: 1) It is less labour intensive as compared to manual evaluation of tremor activity 2) The data can be collected for longer periods of time and 3) The animals are not restrained and can move freely in the glass chamber unlike for instance tremor boxes. A limitation of the method is, that only fore limb tremors are counted and not the whole body tremors. In conclusion, a new simple automatic and non-invasive method for measurement of chemically induced tremors in rats can be useful for developing and screening novel treatment for human movement disorder. Keywords: Tremors, GABA, harmaline

Disclosures: **D. Amrutkar:** A. Employment/Salary (full or part-time): Saniona A/s. **T.A. Jacobsen:** None. **K.S. Nielsen:** None.

Poster

043. Ataxia

Location: Halls B-H

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Program#/Poster#: 43.16/M8

Topic: C.04. Movement Disorders

Support: 9550301675

Title: Use of the scale for the assessment and rating of ataxia (sara) in healthy volunteer and subjects with schizophrenia.

Authors: S. SYED[1], N. SHAAFI KABIRI[2], T. BALI[3], D. KARLIN[4], B. BINNEMAN[4], Y. TAN[5], *K. THOMAS[2];
[1]Tufts Med. Center, Pfizer Neurosci. and Pain Res. Unit, Boston, MA; [2]Anat. and Neurobio., Boston Univ. Sch. of Med., Boston, MA; [3]Dept. of Neurol., Massachusetts Gen. Hosp., Boston, MA; [4]Pfizer Neurosci. and Pain Res. Unit, Cambridge, MA; [5]Pfizer Inc. Res. Statistics, Worldwide Res. & Develop., Cambridge, MA

Abstract: Objective: Schizophrenia is associated with soft neurological signs and symptoms, including motor incoordination. The Scale for the Assessment and Rating of Ataxia (SARA) is a semi-quantitative assessment that is used to evaluate impairment level of cerebellar ataxia. The

goal of these studies was to assess the inter-subject variability and inter-rater reliability of SARA in a Healthy Volunteer (HV) group and subjects with Schizophrenia (SCZ).
Methods: Two studies were completed to collect SARA scores, in a HV group and in a stable SCZ group receiving background maintenance atypical antipsychotic monotherapy. The HV study was approved by Schulman Associates Institutional Review Board, Inc., and the SCZ study by Boston University School of Medicine Institutional Review board; both studies conformed to the Declaration of Helsinki. 177 HVs (18-65 years) and 16 SCZs (18-58 years) provided written consent and were assessed using the SARA. Of 177 HV subjects, 88 had test-retest (within 3 days of initial visit) while all 16 SCZ had three test-retest performances (within a 14 days of initial visit).
Results: For the HV group, the mean score for SARA on visit-1 was 0.38±0.70, and 0.34±0.61 for visit-2. The Pearson Correlation Coefficient between visit-1 and visit-2 was 0.686. For the SCZ group, the mean score for SARA on visit-1 was 0.6±0.65, and 0.8±1.19 for visit-2, and 0.8±0.94 for visit-3. The Pearson Correlation Coefficient between visit-1 and visit-2 was 0.655, between visit-1 and visit-3 was 0.663 and between visit-2 and visit-3 was 0.761.
Conclusions: When comparing across these 2 studies, the HV group demonstrated minimal symptoms of ataxia, similar to the SCZ group. The HV group also demonstrated similar inter-subject variability to the SCZ group. Acceptable inter-rater reliability was observed within the HV and SCZ groups respectively.
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Poster

043. Ataxia

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Topic: C.04. Movement Disorders

Support: NIH Grant F31 NS090725-02

Title: A CK2 dependent phosphorylation mechanism is required to trigger attacks in an episodic ataxia type 2 mouse model.

Authors: *A. VITENZON, K. KHODAKHAH;
Albert Einstein Col. of Medicince, Bronx, NY

Abstract: Episodic ataxia type 2 (EA2) is a neurological disorder caused by mutations in the *CACNA1A* gene in which patients exhibit transient attacks of severe ataxia and dyskinesia triggered by different stressors such as, physical or emotional stress and caffeine or alcohol consumption. The *tottering* mouse, which carries a spontaneous loss-of-function mutation in the *CACNA1A* gene, is a well-established rodent model of EA2 that, like patients, exhibits motor attacks induced by stress, caffeine and alcohol. Work from our lab and others have shown that the cerebellum plays a critical role in the pathology of EA2. Expressing the human *CACNA1A* mutation only in Purkinje cells (PC) of wild-type mice is sufficient to recapitulate all the symptoms of the disorder, while killing the PCs of *tottering* mice alleviates much of the motor symptoms. Previous work in our lab has shown that the motor attacks in *tottering* mice are correlated with aberrant output from the cerebellum driven by high frequency burst firing of PCs, which normally fire regularly at 50 spikes per second *in vivo*. *CACNA1A* encodes the pore forming subunit of the P/Q type calcium channel ($Ca_v2.1$). The tonic firing pattern of PCs is partially governed by the tight coupling of $Ca_v2.1$ channels to SK2 channels that contribute to the after-hyperpolarization of PCs. It has been shown that block of SK2 channels promotes burst firing of wild type PCs. Thus, we hypothesized that attacks in the *tottering* mice might be a consequence of a further reduction in SK2 activity in PCs. One mechanism by which the activity of SK2 channels is modulated is their phosphorylation. Casein kinase 2 (CK2) is part of a protein complex that resides on the intracellular domains of SK2 channels and phosphorylates the calmodulin associated with SK2 thereby reducing its affinity for calcium, thereby decreasing SK2 channel open probability. We thus explored whether a decrease in the SK channel conductance by its CK2-dependent phosphorylation triggers Purkinje cell burst firing and attacks of dyskinesia in the tottering mice. To test this hypothesis, we used short hairpin RNA (shRNA) to knock down CK2. We find that knocking down CK2 in the cerebellum of *tottering* mice with two different shRNAs is sufficient to prevent attacks induced by stress, caffeine or ethanol. These data suggest that motor attacks in *tottering* mice are triggered by a reduction in SK2 activity by a CK2 dependent phosphorylation mechanism. This finding further delineates the events which mediate the episodic motor attacks in EA2 and opens new potential targets for drug intervention.

Disclosures: **A. Vitenzon:** None. **K. Khodakhah:** None.

Poster

043. Ataxia

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 43.18/M10

Topic: C.04. Movement Disorders

Support: Great Ormond Street Hospital Children's Charity

Title: Impact of SNX14 mutations on endocytic trafficking and autophagy

Authors: *D. T. BRYANT[1], C. DEMETRIOU[1], E. PESKETT[1], M. ISHIDA[1], M. SEDA[1], D. JENKINS[1], R. SCOTT[2], S. SOUSA[3], M. BITNER-GLINDZICZ[1,2], G. MOORE[1], P. STANIER[1]; [1]Inst. of Child Hlth., Univ. Col. London, London, United Kingdom; [2]Great Ormond Street Hosp., London, United Kingdom; [3]Hosp. Pediátrico de Coimbra, Coimbra, Portugal

Abstract: A large number of conditions have been described where severe intellectual disability and ataxia are found in patients with cerebellar hypotrophy. Individually, most types are rare and without known molecular pathology. Mutations in SNX14 have recently been reported to cause a distinct autosomal-recessive cerebellar ataxia with moderate to severe intellectual disability, early-onset cerebellar atrophy, sensorineural hearing loss and coarsened facial features. Patient-derived fibroblasts were acquired from individuals of three unrelated consanguineous families in Turkey and Portugal. These cell lines have mutations that result in either truncation or loss of the SNX14 protein. The SNX14 protein contains a Phox (PX) domain and a regulator of G protein signalling (RGS) domain. In one patient, loss of a single exon (containing the PX-domain) alone was sufficient to result in the associated pathology. This suggests that the PX domain is critical for normal SNX14 function. SNX14 mutations lead to the accumulation of vesicular inclusions in patient cells, indicating that there is a disturbance in protein metabolism and-/-or vesicle mediated transport. Analysis of cellular processes associated with autophagy provides additional evidence of this. However, the precise function of SNX14 remains unknown. We are currently investigating the precise role of SNX14 in subcellular trafficking by monitoring these processes in cell based models of the disease. Loss of SNX14 appears to impact cholesterol distribution and we are investigating the events leading to this. Additionally, we are examining zebrafish with homozygous null mutations in the snx14 gene to establish it as a research platform with which to explore therapeutic intervention into the neurological deficits associated with the disease.

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Poster

043. Ataxia

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Simultaneus ablation of polyamine catabolizing enzymes Spermine/Spermidine-N^1-Acetyltransferase and Spermine Oxidase causes cerebellar injury and ataxia

Authors: *M. SOLEIMANI[1], M. BROOKS[1], S. L. BARONE[1], T. MURRAY-STEWART[2], C. DESTEFANO-SHIELDS[2], J. L. CLEVELAND[3], R. A. CASERO[2], K. ZAHEDI[1];
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Abstract: Polyamines, spermidine and spermine, are aliphatic cations with multiple indispensable roles in the regulation of DNA structure, protein-nucleic acid interactions, and cell growth and viability. The cellular content of polyamines is tightly regulated through the balance of their synthesis, degradation, import and export. Catabolism of polyamines is accomplished via their back conversion through the activity of spermine/spermidine-N^1-acetyltransferase (SSAT)/acetylpolyamine oxidase enzyme cascade, and the oxidation of spermine, by spermine oxidase (SMO). The expression of SSAT and SMOX increases in injured tissues and contributes to the induction of organ damage and dysfunction in response to a variety of insults (e.g. ischemia/reperfusion, traumatic and toxic). The importance of polyamine catabolic enzymes and their role in maintenance of tissue polyamine levels under normal conditions is not completely defined. In order to address this, we developed SSAT/SMO double knockout (SSAT/SMOX-dKO) mice. Examination of the SSAT/SMOX dKO mice did not reveal any overt growth or developmental deficits. These mice also have a build up of polyamines in the organs that were examined. The SSAT/SMOX dKO mice begin to develop neural deficits at approximately 3 months of age (average ataxia score of 5.07+/-0.26), while WT, SSAT KO and SMOX KO mice do not show any neural deficits (average ataxia scores of 0.51+/-0.07, 0.86+/-0.17 and 0.66+/-0.12). The comparison of brain and spinal cord histology of WT and SSAT/SMOX dKO mice revealed an increase in the presence of intensely stained contracted nerve cell clusters, and abnormalities (vacuolization) in the granular layer and white matter of the cerebellum. Furthermore, our results show that compared to WT mice, calbindin-1 staining of SSAT/SMOX dKO Purkinje cells is significantly increased. The increase in calbindin-1, in addition to amplified polyamine levels and their established role in the regulation of $[Ca^{2+}]_i$ suggests that there is an increase in $[Ca^{2+}]_i$ flux in SSAT/SMOX dKO mice that is being buffered by increased levels of calbindin-1. Further studies to examine the $[Ca^{2+}]_I$ levels, Lewy Body formation and the excitotoxic effect of polyamine accumulation in SSAT/SMOX dKO animals are underway. The results presented here indicate that polyamine catabolism is important in the maintenance of normal cellular polyamine levels, and that accumulation of polyamines due to the disruption of their catabolism is cytotoxic, especially in nerve cells. These disturbances to the polyamine catabolic pathway manifest with histological alterations in the cerebellum, as well as overt ataxia, in SSAT/SMOX dKO mice.

Disclosures: M. Soleimani: None. **M. Brooks:** None. **S.L. Barone:** None. **T. Murray-Stewart:** None. **C. DeStefano-Shields:** None. **J.L. Cleveland:** None. **R.A. Casero:** None. **K. Zahedi:** None.

Poster

043. Ataxia

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 43.20/M12

Topic: C.04. Movement Disorders

Support: the Funding Program for Next Generation World-Leading Researchers LS021

JSPS KAKENHI 25430003

Title: Progressive impairment of metabotropic glutamate receptor (mGluR)-mediated signaling and cerebellar synaptic plasticity in spinocerebellar ataxia type 1 (SCA1) model mice

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Abstract: Spinocerebellar ataxia type 1 (SCA1) exhibits cerebellar ataxia and progressive atrophy of Purkinje cells (PCs). SCA1 is an inherited progressive neurodegenerative disease caused by abnormal CAG repeat expansion (polyglutamine tract) in the gene of Ataxin-1. Ataxin-1 interacts indirectly with a transcriptional factor, retinoid-related orphan receptor α (RORα), and abnormal disease-causing mutant Ataxin-1 is suggested to disrupt RORα-mediated gene regulation. Our previous study has shown that RORα-deficient ataxic mice lack functional metabotropic glutamate receptor type 1 (mGluR1)-mediated signaling in the cerebellum. Moreover, previous molecular studies indicated that SCA1 involves downregulated expression of the metabotropic glutamate receptor type 1 (mGluR1), which is indispensable for synaptic plasticity and motor coordination. Thus, considering a shared signaling pathway between Ataxin-1 and RORα, we hypothesize that abnormal mGluR signaling underlie SCA1 pathology. However, it remains unknown whether mGluR-mediated signaling is functionally abnormal in SCA1 and which synaptic defect contributes to SCA1 pathology in the cerebellum. Using transgenic SCA1 model mice carrying the disease-causing mutant ataxin-1 with expanded polyglutamine tract, we show that those mice develop progressive deficits in mGluR-mediated signaling at cerebellar parallel fiber (PF)-PC synapses. SCA1 mice older but not younger than 5 weeks, exhibited impaired mGluR-mediated slow synaptic responses, loss of endocannabinoid-mediated retrograde short-term synaptic plasticity and lack of long-term synaptic depression (LTD). These types of synaptic plasticity are known to require mGluR1-mediated Ca^{2+} signals in PC dendrites. Our fast confocal Ca^{2+} imaging revealed that mGluR-mediated dendritic Ca^{2+} signals are also progressively impaired in PCs of SCA1 model mice older than 5 weeks (still prior to PC death). Interestingly, reduction in the dendritic Ca^{2+} signals in SCA1 mice were linearly correlated with small but substantial progressive PC atrophy, which was detected by measurement of the PC membrane capacitance (i.e. the cell surface area). These results suggest

that disruption of mGluR signaling including slow synaptic responses, dendritic Ca^{2+} signals and synaptic plasticity such as LTD at PF-PC synapses may underlie cerebellar ataxia in SCA1 pathology, and that progressive impairment of dendritic mGluR1-mediated Ca^{2+} signaling might accompany slight progressive PC atrophy in SCA1.

Disclosures: **N. Hosoi:** None. **A. Shuvaev:** None. **H. Hirai:** None.

Poster

043. Ataxia

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MIND Institute Intellectual and Developmental Disabilities Research Center (U54 HD079125) to RFB

Title: Neuron-specific contributions to pathology in a dox-inducible mouse model of FXTAS

Authors: *M. M. FOOTE[1], C. VIERIA[1], E. NEVEROVA[1], K. VALENTINE[1], L. BROCHARD[1], M. CAREAGA[2,3], E. DOISY[1], R. WILLEMSEN[4], R. HUKEMA[4], J. KEITER[2,3], S. C. NOCTOR[2,3], R. F. BERMAN[1,3];
[1]Neurolog. Surgery, [2]Psychiatry, Univ. of California Davis, Davis, CA; [3]M.I.N.D. Inst., Univ. of California Davis, Sacramento, CA; [4]Clin. Genet., Erasmus Med. Ctr., Rotterdam, Netherlands

Abstract: The *Fragile X Mental Retardation (FMR1)* gene codes for the Fragile X Mental Retardation protein (FMRP) which is essential for proper neuronal development and function. A CGG trinucleotide sequence is located in the 5'UTR of *FMR1* and is normally repeated 5-54 times. Carriers of the Fragile X premutation (PM) have the CGG repeat sequence expanded to between 55-200 and are at risk for developing the late onset Fragile X-associated tremor/ataxia syndrome (FXTAS). Carriers of the full mutation have CGG repeat sequences over 200 and develop Fragile X syndrome (FS). In the brain, intranuclear inclusions that stain for ubiquitin are considered to be a pathological hallmark of FXTAS and are observed in both astrocytes and neurons. In order to examine the contribution of neuronal pathology to overall FXTAS pathology we developed a doxycycline (dox)-inducible mouse that expresses a CGG90 repeat sequence limited to neurons using the CaMKII-rtTA promoter in mice expressing a TetO-CGG(90)-eGFP transgene. By exposing inducible mice to dox at different ages, and then removing the dox, we are able to assess FXTAS disease progression (comparing 8 vs 20 wks on) and the potential for

disease reversibility (8 wks on, followed by a 12 wk off). Mice were tested using a battery of behavioral assays and subsequently characterized using immunohistological, stereological and western blot techniques. Inducible CGG(90) mice show behavioral deficits after exposure to dox (8 or 20 weeks), including increased anxiety related behaviors, hyperactivity and impaired cognitive abilities. After only 8 wks of dox treatment, neuron-specific expression of the ectopic CGG(90) was widespread and sufficient to produce the FXTAS-associated intranuclear inclusions. Brain atrophy is associated with FXTAS, and consistent with this finding the dox-inducible CGG(90) mice show significant cell loss in key brain regions, including the ventral and dorsal lateral geniculate nucleus. Western blot analyses showed that 8 wks of dox treatment was sufficient to lead to alterations in NR2A and GAD2 expression levels in key brain regions of the CGG(90) mice, which suggested an excitation/inhibitory imbalance in brain activity. Collectively, these results indicate that ectopic CGG(90) expression in neurons is sufficient to produce key elements of FXTAS-associated pathology in mice.

Disclosures: **M.M. Foote:** None. **C. Vieria:** None. **E. Neverova:** None. **K. Valentine:** None. **L. Brochard:** None. **M. Careaga:** None. **E. Doisy:** None. **R. Willemsen:** None. **R. Hukema:** None. **J. Keiter:** None. **S.C. Noctor:** None. **R.F. Berman:** None.

Poster

043. Ataxia

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Support: NIH R01 NS058500 to DMP

National Ataxia Foundation Postdoctoral Fellowship to FAI

Jennifer S. Buchwald Graduate Fellowship in Physiology to BU and JYH

Title: Rapid atrophy and hyperexcitability in cerebellar Purkinje cells expressing infant-onset spinocerebellar ataxia type 13 Kv3.3. mutation

Authors: *D. M. PAPAZIAN[1], B. N. ULRICH[2], J.-Y. HSIEH[2], F. A. ISSA[2], B. BROWN[2], M.-C. A. LIN[2];
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Abstract: Spinocerebellar ataxia 13 (SCA13) is caused by mutations in Kv3.3, which promotes rapid firing in neurons and contributes to spontaneous tonic firing and complex spiking in cerebellar Purkinje cells (PCs). Different Kv3.3 mutations cause distinct forms of SCA13 that

differ in the age of onset. Infant-onset SCA13 is typified by severe cerebellar atrophy early in life, persistent motor problems, and cognitive impairment, whereas adult-onset SCA13 is characterized by progressive cerebellar degeneration and ataxia beginning in adulthood. Infant-onset mutations alter channel gating whereas the adult-onset mutation suppresses current amplitude without altering functional properties. To test the hypothesis that infant- and adult-onset mutations have distinct effects on PC excitability and viability, we expressed two S4 voltage sensor mutations, R3H and R4H, which cause adult- and infant-onset SCA13, respectively, in cerebellar PCs in zebrafish; membrane-bound EGFP (mEGFP) was co-expressed. Zebrafish PCs are born at 3 days post-fertilization (dpf) and begin firing spontaneously within hours. By 5 dpf, PCs receive parallel and climbing fiber input, and exhibit regular tonic firing and complex spiking. Expression of the R4H infant-onset mutation causes dramatic hyperexcitability by 4 dpf. In contrast, the R3H adult-onset mutation does not alter basal firing properties, but reduces excitability when PCs are driven by mossy fiber input. The effects of R3H and R4H on PC morphology were characterized by live confocal imaging starting at 3.5 dpf. PCs expressing the infant-onset R4H mutation lacked branched processes and dendritic spines, rapidly atrophied, and disappeared by ~4-5 dpf. In contrast, PCs expressing the adult-onset R3H mutation, exogenous wild-type Kv3.3, or mEGFP alone developed extensive, branched arbors and numerous spines, with no indication of reduced viability. To determine the role of hyperexcitability in the degeneration of R4H-expressing PCs, we treated zebrafish with NS13001, an SK channel agonist, or co-expressed $K_{IR}2.1$. Preliminary results suggest that reducing the excitability of R4H-expressing PCs stabilizes processes and increases cell survival. Our data suggest that in infant-onset SCA13, hyperexcitability triggers PC atrophy and death.

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Poster

044. Motor Neuron Disease

Location: Halls B-H

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Program#/Poster#: 44.01/M15

Topic: C.05. Neuromuscular Diseases

Title: PTBP1 and PTBP2 regulate conserved and nonconserved cryptic exons.

Authors: *R. CHHABRA[1], J. P. LING[1], J. D. MERRAN[2], P. M. SCHAUGHENCY[3], S. J. WHEELAN[3], J. L. CORDEN[4], P. C. WONG[1,4];
[1]Dept. of Pathology, [2]Mol. Biol. and Genet., [3]Oncology Biostatistics and Bioinformatics, [4]Neurosci., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: RNA splicing is essential for gene regulation and tightly controlled with nucleotide precision. It is thus not surprising that interruption of this delicate system underlies a variety of human diseases. In addition to their role in mRNA regulation and gene expression, various studies have revealed that the RNA binding proteins, polypyrimidine tract-binding protein 1 (PTBP1) and its neuronal homolog polypyrimidine tract-binding protein 2 (PTBP2), act as splicing factors. PTBP1 and PTBP2 both contain four RNA-recognition motifs (RRMs) with high affinity for CU-rich sequences. Intriguingly, the expression pattern of PTBP1 and PTBP2 are mutually exclusive, whereby PTBP1 downregulates PTBP2 by repressing exon 10 in PTBP2 leading to nonsense mediated decay of its transcript. PTBP1 is highly expressed in most of the tissues except certain organs such as brain, where PTBP2 is highly expressed. We previously reported that TDP-43, an RNA binding protein involved in a variety of neurodegenerative diseases, binds to UG dinucleotide repeats to repress nonconserved cryptic exons. As PTBP1 and PTBP2 are also splicing repressors, which binds to CU dinucleotide repeats, we reasoned that these two splicing factors might also repress cryptic exons. We performed RNA-seq analysis in PTBP1/2 knockdown HeLa and HEK 293 cells and searched for previously unidentified cryptic exons. Furthermore, we examined RNA-seq data set from brain of mice lacking *ptbp2* in neurons. Our results revealed that PTBP1 and PTBP2 bind CU dinucleotide repeats to regulate both conserved and nonconserved cryptic exons. Moreover, PTBP1 and PTBP2 seem to promote neuronal differentiation by governing the extent of CU repeat-associated repression. Together, our results establish that PTBP1 and PTBP2, along with TDP-43, belong to a novel family of cryptic exon repressors that serve to maintain the integrity of introns.

Disclosures: **R. Chhabra:** A. Employment/Salary (full or part-time): International Graduate School in Molecular Medicine, Ulm University, Ulm, Germany. **J.P. Ling:** None. **J.D. Merran:** None. **P.M. Schaughency:** None. **S.J. Wheelan:** None. **J.L. Corden:** None. **P.C. Wong:** None.

Poster

044. Motor Neuron Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

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Topic: C.05. Neuromuscular Diseases

Support: ALS Association

Title: Exploring G4C2 repeat expansions using long-read next generation sequencing

Authors: ***J. GREGORY**[1], K. KANG[1,2], G. DEIKUS[2,3], M. HARMS[3], N. SHNEIDER[3], R. SEBRA[2], H. PHATNANI[1];

[1]New York Genome Ctr., New York, NY; [2]Icahn Sch. of Med. at Mt. Sinai, New York, NY; [3]Columbia Univ. Med. Ctr., New York, NY

Abstract: We are developing methods to sequence and assemble the G_4C_2 hexanucleotide repeat expansion (RE) in chromosome 9 open reading frame 72 (C9orf72), which is the most common hereditary cause of familial ALS-FTD. In control populations, the RE is relatively short and can be identified with routine molecular biology and sequencing. However, ALS-FTLD patients have 100's to 1000's of repeats and, unfortunately, high throughput methods to determine the exact length, sequence, and heterogeneity of the C9orf72 RE do not currently exist; the current state-of-the-art uses laborious protocols that yield only gross approximations. The 100% GC content, length, and repetitive nature of the expansion have been particularly difficult to overcome for sequencing applications. There is considerable clinical heterogeneity in age of onset, severity, and clinical symptoms among C9orf72 RE carriers, some of which may be explained by differences in the C9orf72 repeat expansion. Preliminary data from PacBio and Oxford Nanopore sequencing using novel enrichment strategies for targeted sequencing of G_4C_2 repeat expansions will be discussed.

Disclosures: J. Gregory: None. **K. Kang:** None. **G. Deikus:** None. **M. Harms:** None. **N. Shneider:** None. **R. Sebra:** None. **H. Phatnani:** None.

Poster

044. Motor Neuron Disease

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Topic: C.05. Neuromuscular Diseases

Support: MDA Grant MDA255293

NIH Grant NS091299

Arnold and Mabel Beckman Foundation

Title: Investigating effects of TDP-43 on metabolic gene expression in a *Drosophila* model of amyotrophic lateral sclerosis

Authors: *J. BARROWS[1], E. MANZO[1], A. JOARDAR[1], A. COYNE[1,2], D. C. ZARNESCU[1,2,3];
[1]Mol. and Cell. Biol., [2]Neurosci., [3]Neurol., Univ. of Arizona, Tucson, AZ

Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that primarily affects motor neurons, disrupting muscle function, which eventually leads to death by respiratory failure. TDP-43, a DNA/RNA binding protein that plays numerous roles in RNA processing, has been associated with RNA stress granule pathology in over 95% of ALS cases. TDP-43 has also been linked to dysregulation of specific mRNA targets at both the transcriptional and translational levels. Metabolomic analyses demonstrated that several metabolites in glycolysis and the TCA cycle are altered in *Drosophila* larvae expressing human TDP-43 compared to controls, suggesting that enzymes within these metabolic pathways are being affected in ALS. To confirm this hypothesis, we are using the GAL4-UAS system in *Drosophila* to express TDP-43 specifically in the motor neurons or glia. Indeed, transcriptional profiling shows that phosphofructokinase (PFK; Pfk in *Drosophila*) and glucose-6-phosphate dehydrogenase (G6PD; Zw in *Drosophila*) expression levels are altered in the context of TDP^{WT} or disease associated TDP^{G298S}. These findings are consistent with increased glycolysis and high levels of pyruvate identified using metabolomics in TDP-43 expressing flies. Current experiments are aimed at identifying additional transcriptional and translational alterations in metabolic pathways that control cellular energetics. Next, we will use genetic approaches to reduce (in the case of upregulation) or increase (in the case of downregulation) the expression levels of metabolic genes in the context of TDP-43. These experiments will determine whether restoring specific targets can rescue TDP-43 dependent phenotypes. This combination of molecular and genetic analyses will establish physiologically significant targets of TDP-43 that can be used for developing novel therapeutic strategies in the future.

Disclosures: **J. Barrows:** None. **E. Manzo:** None. **A. Joardar:** None. **A. Coyne:** None. **D.C. Zarnescu:** None.

Poster

044. Motor Neuron Disease

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Topic: C.05. Neuromuscular Diseases

Support: CCHMC Trustee Award

ALS Association Starter Grant T7EMDY

Title: V2a neurons drive accessory respiratory muscle activity and degenerate in mouse models of ALS

Authors: *V. N. JENSEN[1], S. H. ROMER[2], K. SEEDLE[2], S. M. TURNER[2], S. A. CRONE[2]; [1]Grad. Neurosci. Program, Univ. of Cincinnati, Cincinnati, OH; [2]Neurosurg., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstract: Respiratory failure is the leading cause of death in amyotrophic lateral sclerosis (ALS) and the only current treatment to improve breathing is mechanical ventilation. Despite progressive degeneration of phrenic motor neurons innervating the diaphragm, ALS patients and rodent models are able to maintain ventilation at early stages of disease, but experience a sharp decline at late disease stages. The mechanisms that maintain ventilation at early stages or lead to ventilation failure at late disease stages are unknown. We propose that compensatory mechanisms including recruitment of accessory respiratory muscles (ARMs) help to maintain ventilation at early stages of disease. Using a customized system for simultaneous non-invasive measurement of ARM electromyograph (EMG) activity and breathing (whole body plethysmography), we demonstrate that ARMs are recruited in early stage ALS model mice and increase ventilation. Surprisingly, ARMs are not used for breathing at late disease stages, even though they are used to perform other motor functions. In addition, we have identified a glutamatergic neuron class in the spinal cord and brainstem, the V2a class, that is able to drive accessory respiratory muscle activity and that degenerates in mouse models of ALS. These results support the hypothesis that a central deficit in respiratory circuitry underlies the failure to activate ARMs at late stages of disease. Our studies suggest that therapies to protect, replace, or improve the function of V2a neurons could help to restore or maintain ventilation in patients with ALS, other neuromuscular diseases, or spinal cord injury.

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Poster

044. Motor Neuron Disease

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Topic: C.05. Neuromuscular Diseases

Support: Les Turner ALS Foundation

Target ALS

Muscular Dystrophy Association

Title: Mechanisms of RAN translation in a C9orf72 heterologous expression system

Authors: *E. L. DALEY, J. A. ORTEGA, D. SANTOS, E. KISKINIS;
Neurosci., Northwestern Univ. - Chicago, Chicago, IL

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder that is characterized by degeneration of motor neurons and for which there is no effective therapy. Recently, the hexanucleotide repeat expansion GGGGCC within the first intron of C9*orf*72 was discovered to be the largest genetic contributor to ALS. Toxic mechanisms underlying pathogenesis driven by this mutation include C9*orf*72 haploinsufficiency, the accumulation of C9*orf*72 mRNA repeat transcripts and subsequent sequestration of RNA binding proteins, and production of dipeptide repeat proteins (DPRs) by the mechanism of repeat-associated non-ATG (RAN) translation. There is much that is still unknown about RAN translation. Past studies have demonstrated that RAN translation machinery likely varies based on location within the mRNA transcript, frame position, constituent bases comprising the repeat, and secondary structures formed by repeat nucleotide interactions. The current study sought further mechanistic understanding underlying RAN translation in the context of the GGGGCC repeat. Here, we report the development of a heterologous expression system in which 58 GGGGCC repeats, in the absence of an ATG and a Kozak sequence, stably expressed in HEK293 cells, demonstrated the accumulation of RNA foci and DPRs associated with this mutation. We report on the preliminary data of an unbiased genetic screen that identifies proteins necessary for RAN translation to occur within this system.

Disclosures: **E.L. Daley:** None. **J.A. Ortega:** None. **D. Santos:** None. **E. Kiskinis:** None.

Poster

044. Motor Neuron Disease

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NINDS Grant NS089544

NIA Grant AG050471

Alzheimer Association

Brightfocus Foundation

CureAlzheimer Foundation

Title: Environmental toxicants upregulate TDP-43 expression through the Aryl Hydrocarbon Receptor

Authors: *P. E. ASH[1], E. A. STANFORD[2], G. J. MURPHY[3], D. H. SHERR[2], B. WOLOZIN[1]; [1]Dept of Pharmacol., Boston Univ. Med. Ctr., Boston, MA; [2]Dept. of Envrn. Hlth., Boston Univ. Sch. of Publ. Hlth., Boston, MA; [3]CReM, Boston Univ., Boston, MA

Abstract: Amyotrophic lateral sclerosis (ALS) is a debilitating neurodegenerative condition that is pathologically characterized by progressive loss of motor neurons and the accumulation of aggregated TAR DNA Binding Protein-43 (TDP-43). The causes of ALS are poorly understood, but environmental factors are established contributors to the risk of ALS. Recent epidemiological studies identified environmental toxicants, such as dioxins and polychlorinated biphenyls (PCBs), as a risk factor for ALS. Dioxins activate the aryl hydrocarbon receptor (AHR), which is a ligand-activated transcription factor. The TAR DNA Binding Protein (*TARDBP*) promoter contains clusters of AHR response elements. We report that ligands of the AHR, including known dioxin family toxicants, acutely increase *TARDBP* transcription, while AHR knockdown prevents such increases. Chronic treatment with AHR agonists for 1 week leads to over a 2-fold accumulation of soluble TDP-43 in cultured neuroblastoma cells. Similar sustained treatment of human iPSC-derived neurons (from an ALS patient carrying the *TARDBP* G298S mutation) increases the accumulation of insoluble monomeric, high molecular weight and C-terminal fragments of TDP-43. TDP-43 levels increase in the murine brain following intraperitoneal injection of an agonist of the AHR, demonstrating CNS penetration of these toxicants. These results provide the first lines of evidence that dioxins can increase levels of the principle pathological protein associated with ALS, which suggest a novel mechanism through which dioxins might contribute to the risk of ALS. The widespread distribution of dioxins and other AHR ligands across industrialized societies raises the possibility that these environmental toxicants might represent a significant public health concern for the risk of ALS.

Disclosures: P.E. Ash: None. **E.A. Stanford:** None. **G.J. Murphy:** None. **D.H. Sherr:** None. **B. Wolozin:** None.

Poster

044. Motor Neuron Disease

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Topic: C.05. Neuromuscular Diseases

Support: R01GM118530

USU SQ75QF

Title: Phosphorylation of the intrinsically disordered domain of fused-in-sarcoma inhibits aggregation

Authors: *Z. MONAHAN[1], V. RYAN[2], K. BURKE[2], F. SHEWMAKER[1], N. FAWZI[2]; [1]Pharmacol., Uniformed Services Univ. of the Hlth. Scienc, Bethesda, MD; [2]Dept. of Mol. Pharmacology, Physiology, and Biotech., Brown Univ., Providence, RI

Abstract: Mutations of the RNA-binding protein fused-in-sarcoma (FUS) correlate with familial amyotrophic lateral sclerosis (ALS), a fatal motor neuron disease for which there is no effective treatment. Diseased motor neurons show a classic pattern of FUS cytosolic aggregation. Intriguingly, FUS contains a long N-terminal intrinsically disordered region of low sequence complexity, resembling the amino acid composition of canonical yeast prion proteins as well as other aggregation-prone human proteins implicated in numerous neurodegenerative diseases. These "prion-like" domains (PLDs) are characterized by an abundance of uncharged, hydrophilic amino acids, and propensity toward forming self-propagating aggregates. Here, we show that FUS is phosphorylated by the nuclear kinase DNA-PK *in vitro* and that the N-terminal PLD of FUS is specifically phosphorylated in mammalian cell culture following cell stress, thus reducing the prion-like character of the domain and its propensity to aggregate. Moreover, we show that specifically targeted phosphomimetic substitutions on serine or threonine residues within the PLD of FUS is correlated with a reduction in aggregation and cytotoxicity in a *Saccharomyces cerevisiae* model system. Although nuclear magnetic resonance spectroscopy demonstrates that the intrinsically disordered structure of the domain is preserved after phosphorylation, self-interaction of the prion-like domain is inhibited by phosphorylation. Conversion of *in vitro* liquid phase-separated FUS to solid aggregates is completely abrogated by phosphomimetic substitution in the prion-like domain. These results suggest that phosphorylation of prion-like low-complexity domains reduces protein aggregation propensity and cytotoxicity. Hence, post translational modification may be a mechanism by which cells control physiological assembly and prevent pathological protein aggregation, offering potential treatment pathway amenable to pharmacologic modulation.

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Poster

044. Motor Neuron Disease

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Topic: C.05. Neuromuscular Diseases

Support: Australian National Health and Medical Research Council (Grant 1044407)

Title: In the superoxide dismutase 1 ($hSOD1^{G93A}$) mouse model, cortical glucose metabolism is impaired while astrocytic TCA cycling is unaffected at onset of disease

Authors: *T. W. TEFERA, K. BORGES;
Sch. of Biomed. Sci., The Univ. of Queensland, Brisbane, Australia

Abstract: Altered glucose metabolism has been shown in various brain regions and spinal cord of patients with amyotrophic lateral sclerosis (ALS). However, the specific biochemical changes in neuronal and astrocytic glycolytic and TCA cycle pathways were unknown. We therefore investigated [1-^{13}C] glucose and [1,2-^{13}C] acetate metabolism in neurons and astrocytes in brain extracts from cortex of $hSOD1^{G93A}$ mice at onset of disease (80 days). Wild-type (n=15) and $hSOD1^{G93A}$ mice (n=10) at onset were simultaneously injected with 543 mg/kg [1-^{13}C]glucose and 504 mg/kg [1,2-^{13}C]acetate (i.p.) followed by microwave fixation of the head 15 minutes later. Cerebral cortices were removed and extracted with methanol-chloroform. The total concentrations of metabolites, amino acids and ^{13}C labelling in cortex extract were quantified using high-pressure liquid chromatography (HPLC), ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Levels of various glycolytic derived metabolites were reduced in $hSOD1^{G93A}$ mice, namely [3-^{13}C]lactate by 53%, total lactate by 43%, [3-^{13}C]alanine by 45% and total alanine by 20% ($p<0.03$). This signifies impairment in the glycolytic pathway in $hSOD1^{G93A}$ mice. We also found reductions in the amounts of glutamate by 27% and succinate by 23% in $hSOD1^{G93A}$ mice cortex compared to control mice ($p=0.02$), while, other total metabolite levels including those of glutamine, aspartate and GABA were unchanged. In addition, the labelling in glutamate ([4-^{13}C]glutamate), glutamine ([4-^{13}C]glutamine) and GABA ([2-^{13}C]GABA) from [1-^{13}C] glucose was diminished by 30% ($p<0.04$). This could be explained by the impairments in glycolysis, however additional abnormalities in TCA cycle reactions or enzymes metabolizing 2-oxoglutarate cannot be ruled out. Astrocytic metabolism of ^{13}C-acetate was unchanged as evidenced by unaltered incorporation of ^{13}C in [4,5-^{13}C]glutamate and [4,5-^{13}C]glutamine from [1,2-^{13}C]acetate. However, we found reduced labelling in [1,2-^{13}C]GABA, indicating that metabolism in GABAergic neurons is impaired, which can contribute to excitotoxicity in these mice. In conclusion, glucose metabolism is compromised in glutamatergic and GABAergic neurons, while astrocytic TCA cycling and glutamine-glutamate shuttling appears to be normal in the $hSOD1^{G93A}$ mouse model of amyotrophic lateral sclerosis at onset of

disease. These results justify additional research into alternative fuels to glucose to delay progression of ALS.

Disclosures: T.W. Tefera: None. **K. Borges:** A. Employment/Salary (full or part-time): The University of Queensland.

Poster

044. Motor Neuron Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 44.09/N5

Topic: C.05. Neuromuscular Diseases

Support: ALSA

NIH

Target ALS

Robert Packard Center for ALS Research

Title: Pharmacologic and pathophysiologic readouts of c9orf72 therapy in iPS neurons

Authors: *L. R. HAYES[1], T. GENDRON[2], L. PETRUCELLI[2], M. DISNEY[3], J. ROTHSTEIN[1]; [1]Sch. of Med., Johns Hopkins Univ., Baltimore, MD; [2]Dept. of Neurosci., Mayo Clin., Jacksonville, FL; [3]Departments of Chem. and Neurosci., The Scripps Res. Inst., Jupiter, FL

Abstract: A hexanucleotide repeat expansion in *C9ORF72* is now regarded as the most common genetic cause of ALS and FTD. Antisense oligonucleotide (ASO) therapy and small molecule agents are in development for C9*,* and have shown promising results in vitro and in vivo. Our early data show that ASO treatment of C9 iPSN ameliorates glutamate toxicity and deficits in nucleocytoplasmic transport. We have also demonstrated that glycine-proline (GP), one of six *C9ORF72* repeat-associated, non-ATG initiated (RAN) proteins, provides a dose-dependent readout of ASO efficacy in culture media from induced pluripotent stem cell-derived neurons (iPSN). We are in the process of working out the correlates between these pathophysiologic and pharmacodynamic readouts, which will be critical for understanding how to titrate therapy. Efforts are underway to carry out a systematic characterization of time- and dose-dependent effects of ASO and small molecule therapy on RAN protein levels in the culture media and measures of C9 toxicity, including sensitivity to glutamate, ER stress, nucleocytoplasmic transport defects, and nuclear pore pathology. These results will provide important preclinical data to help move these agents forward to clinical trial.

Disclosures: **L.R. Hayes:** None. **T. Gendron:** None. **L. Petrucelli:** None. **M. Disney:** None. **J. Rothstein:** None.

Poster

044. Motor Neuron Disease

Location: Halls B-H

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Program#/Poster#: 44.10/N6

Topic: C.05. Neuromuscular Diseases

Title: Assessing the role of Nurr1 in a murine model of amyotrophic lateral sclerosis

Authors: ***M. M. BOIDO**, V. VALSECCHI, M. GUGLIELMOTTO, N. GIONCHIGLIA, E. TAMAGNO, A. VERCELLI;
Univ. of Turin, Orbassano (TO), Italy

Abstract: The nuclear receptor related 1 protein (Nurr1), also known as NR4A2, belongs to the steroid nuclear hormone receptor class, but it is considered an orphan receptor since its activity is not regulated by ligands. The role of Nurr1 is still debated, but it seems implicated both in neuroprotection and immunomodulation in different neurodegenerative diseases, as Parkinson's disease and multiple sclerosis. By cooperating with the CoREST complex, Nurr1 can repress the activity of the pro-inflammatory transcription factor NF-kB, therefore playing an anti-inflammatory role. Neuroinflammation is a pathological hallmark of many neurodegenerative diseases including amyotrophic lateral sclerosis (ALS): here we aimed to unravel the role of Nurr1 in ALS, in order to identify a new therapeutic target.
By following the progression of disease in SOD1 G93A mice (the most widely used animal model of ALS), we have correlated the Nurr1 expression with the severity of the disease, compared to age-matched WT mice that were considered as the baseline. Behavioral tests (rotarod and paw grip endurance test) have been employed to identify three different phases of the disease (pre-symtomatic, early and late symptomatic). RT-PCR showed that Nurr1 mRNA expression is strongly up-regulated at the onset of the pathology and decreases in the last phase. Moreover in nuclear extracts from the lumbar spinal cord of SOD mice, we measured a decreased level of NF-kB subunits (in particular the P50 one) in the early symptomatic phase. We also evaluated the Nurr1 expression by immunofluorescence reactions, in order to clarify which cells were specifically involved in this pathway: our results revealed a significant contribution of astroglial cells. Finally we quantified motoneuron number and astro/microgliosis, correlating this parameters to the progression of the disease.
Our overarching hypothesis is that Nurr1 activation aims to modulate neuroinflammation and to protect motor neurons, at least at the onset of disease. Our observations, in association with the

pharmacological modulation of Nurr1, could clarify the role of Nurr1 in such devastating disease.

Disclosures: **M.M. Boido:** None. **V. Valsecchi:** None. **M. Guglielmotto:** None. **N. Gionchiglia:** None. **E. Tamagno:** None. **A. Vercelli:** None.

Poster

044. Motor Neuron Disease

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Topic: C.05. Neuromuscular Diseases

Support: NIH-R21- NS085750-01

The ALS association

Les Turner ALS Foundation

Title: Cellular and Molecular Analysis of Corticospinal Motor Neurons that become vulnerable due to hTDP-43^{A315T} mutation

Authors: ***M. GAUTAM**[1], L. A. LABOISSONNIERE[3], M. KANDPAL[2], J. H. JARA[1], Y. BI[2], K. D. KIM[1], J. M. TRIMARCHI[3], R. V. DAVULURI[2], P. H. OZDINLER[1];
[1]Neurol., [2]Preventive Medicine-Health and Biomed. Informatics, Northwestern Univ., Chicago, IL; [3]Dept. of Genetics, Develop. and Cell Biol., Iowa State Univ., Ames, IA

Abstract: Corticospinal motor neurons (CSMN) are unique in their ability to collect and integrate signals from different regions of the cerebral cortex, and to transmit this information to distinct segments of the spinal cord. Their proper function is thus pivotal for motor functions, and CSMN degeneration is an important aspect of motor neuron diseases, in which voluntary movement is impaired. Transactive Response (TAR) DNA binding protein 43 (TDP-43) is an evolutionarily conserved DNA/RNA binding nucleoprotein involved in various functions, most importantly in RNA metabolism. Numerous mutations in the conserved region of TDP-43 have been detected in sporadic and familial cases of ALS patients. Of all the mouse models generated to date, the one overexpressing the hTDP-43^{A315T}mutation under the control of the prion promoter recapitulates many aspects of ALS pathology. Here, we investigate the health and stability of CSMN that express mutant TDP-43 and display TDP-43 pathology. We previously generated and characterized UCHL1-eGFP mice, in which CSMN are genetically labelled with eGFP expression that is stable and long lasting. Now, in an attempt to distinguish CSMN from other neurons and cells in the cerebral cortex, and to study their cell biology with high precision

and clarity, we generated a CSMN reporter line of *TDP-43^{A315T}* by crossing *UCHL1-eGFP* and hTDP-43^{A315T} mice. Our ongoing studies suggest that CSMN undergo progressive degeneration in the presence of TDP-43 pathology, and CSMN loss is accompanied by compromised motor behaviour, such as poor performance on a rotating rod and weak hind limb strength. Increased astrogliosis and microgliosis display an evoked immune response in the motor cortex, and immunohistochemistry coupled with electron microscopy reveals mitochondrial aggregation as well as cyto-architectural defects. RNA-Seq analysis of FACS purified healthy and diseased CSMN provides unique insights into the differentially regulated gene pathways in diseased CSMN, and enables identification of canonical pathways that are selectively modulated with respect to TDP-43 pathology, as well as potential alternative splice variations of key genes. Upon completion, our studies will reveal the unique importance of the *TDP-43^{A315T}* mutation on CSMN vulnerability and progressive degeneration.

Disclosures: **M. Gautam:** None. **L.A. Laboissonniere:** None. **M. Kandpal:** None. **J.H. Jara:** None. **Y. Bi:** None. **K.D. Kim:** None. **J.M. Trimarchi:** None. **R.V. Davuluri:** None. **P.H. Ozdinler:** None.

Poster

044. Motor Neuron Disease

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Program#/Poster#: 44.12/N8

Topic: C.05. Neuromuscular Diseases

Support: Above & Beyond, LLC.

Title: Investigating the role of a novel variant in the copper transporter ATP7A detected in an ALS patient-parent trio

Authors: *A. P. STARR[1], Z. T. MCEACHIN[2], N. BAKKAR[1], I. LORENZINI[1], D. C. ZARNESCU[3], G. J. BASSELL[2], C. M. HALES[2], W. ROSSOLL[2], R. SATTLER[1], N. M. BOULIS[2], R. BOWSER[1];
[1]Div. of Neurobio., Barrow Neurolog. Inst., Phoenix, AZ; [2]Emory Univ., Atlanta, GA; [3]Univ. of Arizona, Tucson, AZ

Abstract: Copper regulation is vital to the operation of a number of cellular processes, including mitochondrial electron transport, iron transfer, and antioxidant activity. The copper transporting P-type ATPase (ATP7A) is known to function in most human cell types in two primary ways; it chaperones copper to proteins in the trans-Golgi apparatus and exports excess copper at the plasma membrane. Mutations in the ATP7A gene are responsible for a number of neurological

disorders including Menkes' disease, occipital horn syndrome, and distal motor neuropathies. Here, we report a case of a male patient presenting with brachial amyotrophic diplegia, an atypical form of amyotrophic lateral sclerosis (ALS). Whole genome sequencing of the proband and his parents revealed a maternally inherited variant of unknown significance in the X-linked ATP7A gene. The hemizygous c.3931A>G variant results in a nonsynonymous p.M1311V substitution. This amino acid alteration occurs next to the ATP binding site of the protein. Given the frequency of neurological symptoms in ATP7A-related diseases and ATP7A's function as a copper chaperone to the ALS-associated enzyme superoxide dismutase (SOD1), we investigated the effect of this mutation on ATP7A expression, location, and activity. In patient-derived fibroblasts, ATP7A transcript and protein expression levels showed no difference from control fibroblasts. Similarly, no change was observed in cell viability, SOD activity or cytochrome c oxidase function. Immuno-colocalization analysis, however, did show that ATP7A relocation in response to elevated copper levels was reduced in patient fibroblasts. Patient-derived induced pluripotent stem cells (iPSCs) differentiated into neurons and *Drosophila* models overexpressing ATP7A-M1311V are being generated to further investigate the role of ATP7A-M1311V in more disease-relevant models.

Disclosures: **A.P. Starr:** None. **Z.T. McEachin:** 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; Above & Beyond, LLC.. **N. Bakkar:** None. **I. Lorenzini:** None. **D.C. Zarnescu:** None. **G.J. Bassell:** None. **C.M. Hales:** None. **W. Rossoll:** None. **R. Sattler:** None. **N.M. Boulis:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Above & Beyond, Inc. **R. Bowser:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Iron Horse Daignostics, Inc.. F. Consulting Fees (e.g., advisory boards); Above & Beyond, LLC..

Poster

044. Motor Neuron Disease

Location: Halls B-H

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Program#/Poster#: 44.13/N9

Topic: C.05. Neuromuscular Diseases

Support: Department of Defense W81XWH-11-1-0689

NIH-NINDS: R01NS078375

NIH-NINDS: R21 NS084185

Title: Complement and microglia mediate sensory-motor synaptic loss in Spinal Muscular Atrophy

Authors: *A. VUKOJICIC[1,2], N. DELESTREE[1,2], E. V. FLETCHER[1,2], S. SANKARANARAYANAN[3], T. YEDNOCK[3], B. A. BARRES[4,3], G. Z. MENTIS[1];
[1]Ctr. for Motor Neuron Biol. and Dis., [2]Depts. of Pathology & Cell Biol. and Neurol., Columbia Univ., New York, NY; [3]Annexon Biosci., South San Francisco, CA; [4]Dept. of Neurobio., Stanford Univ. Sch. of Med., Stanford, CA

Abstract: Spinal muscular atrophy (SMA) is a neurodegenerative disease caused by reduced levels of the ubiquitously expressed SMN protein. The hallmarks of SMA are loss of motor neurons and abnormal postural reflexes. We have previously shown that decreased number of select synapses and sensory-motor circuit dysfunction precedes motor neuron loss. The mechanisms leading to this selective synapse loss on SMN deficient motor neurons remain unknown. Here we investigated whether complement-dependent pathways are activated and cause synapse elimination in SMA. Immunohistochemical assays in a mouse model of SMA, revealed that C1q, the initiating protein of the classical complement cascade, associates abnormally with excitatory - but not inhibitory - synapses on motor neurons. We show further that both C1q and C3, a downstream complement protein, are tagging proprioceptive (VGluT1+) synapses on vulnerable motor neurons. Furthermore we show that synaptic elimination is mediated by phagocytic activity of reactive microglia. Additionally, we find that microglia is the major source of C1q in the SMN deficient spinal cord. Finally, we asked whether immunotherapy by *in vivo* blockade of C1q with a monoclonal anti-C1q antibody, rescues synapses destined to be eliminated and whether prevention of early synaptic loss alleviates the severe SMA mouse phenotype. Strikingly, behavioral and morphological analysis revealed significant rescue of proprioceptive synapses, improved righting times, posture and lifespan. Importantly, functional assays employing the spinal cord *ex vivo* preparation demonstrated that synapses rescued from elimination are functional, providing further evidence that SMA is a disease of motor circuits. Collectively, our findings suggest that aberrant activation of classical complement pathway and microglial phagocytic activity mediate synaptic loss in a mouse model of SMA and identify blockade of C1q as a novel therapeutic target.

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Poster

044. Motor Neuron Disease

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Topic: C.05. Neuromuscular Diseases

Support: NIH NS061867

Title: Blood-CSF barrier disruptions in ALS

Authors: *N. BAKKAR, R. KOTHUR, R. BOWSER;
Gregory W. Fulton ALS and Neuromuscular Res. Ctr., St Joseph Hosp. and Med. Center/Barrow Neurolog. Inst., Phoenix, AZ

Abstract: The blood-CSF (BCSF) barrier is structurally comprised of an endothelial cell layer and a polarized epithelial cell layer named the choroid plexus (CP). The BCSF barrier lines the inside of the brain ventricles, separating the blood in the vascular system from the cerebrospinal fluid (CSF) that is in direct contact with neurons. The CP mainly functions in CSF production and turnover as well as selective transport of nutrients from the systemic compartment, removal of metabolic products out of the brain, regulation of cell trafficking into the CSF. Under inflammatory conditions, the CP epithelium expresses factors involved in mediating an immune response thus serving as a primary point of entry of immune cells into the nervous system. Aging and neurodegeneration have been reported to affect CP morphology and function, decreasing CSF production and turnover by as much as 50%, altering levels of proteins involved in energy production and free radical scavenging, and increasing protein leakage from blood to the CSF. ALS is similarly associated with increased oxidative stress markers, neuronal loss, and metabolic disturbances that may also impede CP function. Our group and others have reported increased levels of many proteins in CSF from ALS patients, including inflammatory targets, cytoskeletal and extracellular matrix proteins, as well as aggregated proteins. Having these proteins and factors abnormally localized to the CSF compartment suggests impaired BCSF barrier permeability, increased protein leakage from blood to the CSF and decreased clearance of metabolic products pointing to a dysfunctional BCSF barrier. To date, there have been few studies investigating BCSF barrier or CP alterations in ALS patients. We hereby have investigated BCSF integrity in ALS. We hypothesized that this physical barrier is disrupted in ALS possibly via excess metalloproteinase (MMP) activation, thus allowing an influx of immune cells into the CSF and conversely into the nervous system**.** We have investigated the morphology and distribution of various cell junction and cell adhesion proteins such as cadherins, occludins, and claudins in ALS CP compared to control CP by immunohistochemistry. We show that levels and distribution of many of these markers is altered in ALS. Results were confirmed by real-time PCR analysis. In addition, we have examined levels of various MMPs in ALS CP compared to controls, as a potential cause for BCSF barrier breakdown. By investigating BCSF barrier disruption in ALS patients, our study provides novel mechanisms of how toxic metabolites and immune modulators spread to the CSF potentially providing new targets for therapy development.

Disclosures: **N. Bakkar:** None. **R. Kothur:** None. **R. Bowser:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Iron Horse Diagnostics.

Poster

044. Motor Neuron Disease

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Topic: C.05. Neuromuscular Diseases

Support: TargetALS

Packard Center for ALS

NIH R01-NS095969

ALS Association

Title: TDP-43 overexpression leads to the repression of conserved exons

Authors: *J. P. LING, W. W. TSAO, P. C. WONG;
Johns Hopkins Univ., Baltimore, MD

Abstract: Cytoplasmic aggregation of transactivation response element DNA-binding protein 43 (TDP-43) is a key pathological hallmark of amyotrophic lateral sclerosis and frontotemporal dementia (ALS-FTD). We recently discovered that TDP-43 plays an important role in repressing the splicing of nonconserved cryptic exons. Furthermore, we demonstrated that restoring the repression of cryptic exons rescues cells from cell death induced by TDP-43 loss of function. However, strong overexpression of TDP-43 in animal models also leads to cellular toxicity—the mechanisms of which remain poorly understood. Here, we suggest that under conditions of TDP-43 overexpression, excess amounts of TDP-43 protein may bind to suboptimal UG repeats on conserved exons to induce exon skipping. Repression of conserved exons may explain the toxicity observed with TDP-43 overexpression.

Disclosures: J.P. Ling: None. **W.W. Tsao:** None. **P.C. Wong:** None.

Poster

044. Motor Neuron Disease

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Topic: C.05. Neuromuscular Diseases

Support: NIH NS061867

ARCS Fellowship

Title: Alterations in protein-protein interactions caused by ALS associated mutations in Matrin 3

Authors: *A. BOEHRINGER[1], K. GARCIA[2], P. PIRROTTE[2], R. BOWSER[1];
[1]Barrow Neurolog. Inst., Phoenix, AZ; [2]Translational Genomics Inst., Phoenix, AZ

Abstract: We and others have recently reported mutations in the RNA binding protein Matrin 3 in amyotrophic lateral sclerosis (ALS) patients. ALS is a fatal, progressive, neurodegenerative disease resulting in loss of motor neurons and consequently progressive weakness and paralysis. As the mechanism by which Matrin 3 mutations result in ALS is currently unknown, we sought to identify protein-protein interactions (PPIs) with Matrin-3 and to determine if these associations are altered by mutations in the MATR3 gene. Altered PPIs caused by mutations may identify pathways that contribute to disease. We performed immunoprecipitation followed by mass spectrometry in NSC-34 cells stably expressing either human wildtype Matrin 3 or one of four ALS associated mutations in Matrin 3 (S85C, F115C, P154S and T622A). All experiments were performed in triplicate and each experiment repeated twice. Approximately 300 Matrin 3 interacting proteins were identified in each experiment. Proteins identified were further scored by probability of interaction using SAINTexpress. On average across all experiments, 50 protein binding partners were identified at medium confidence, and 20 binding partners at high confidence. A subset of these putative Matrin 3 interactors were confirmed by western blot and found to co-localize with Matrin-3 using immunocytochemistry. These two methods also highlighted differences in protein interactions with wildtype Matrin 3 as compared to ALS associated mutants. Putative binding partners were subjected to pathway analysis with the most significant pathway among wildtype and mutant being eukaryotic initiation factor 2 (eIF2) signaling. Otherwise, pathways differed greatly between wildtype and mutant Matrin 3, with some pathways found only in either wildtype or mutant experiments. Gene ontology annotation highlighted binding partners predominately involved in RNA processing and RNA splicing, though the number of proteins in each category and the specific RNA functions differed between wildtype and mutant.

Disclosures: **A. Boehringer:** None. **K. Garcia:** None. **P. Pirrotte:** None. **R. Bowser:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Iron Horse Diagnostics, Inc..

Poster

044. Motor Neuron Disease

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Support: NIH Grant NS091299

MDA Grant 255293

NIH Grant NS078429

Title: Rescue of neurotoxicity in a TDP-43-based *Drosophila* model of ALS by a 4-aminoquinoline analog

Authors: *B. ZAEPFEL[1], A. COYNE[1], J. A. CASSEL[2], A. B. REITZ[2], D. C. ZARNESCU[1]; [1]Univ. of Arizona, Tucson, AZ; [2]ALS BioPharma, LLC, Doylestown, PA

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease affecting upper and lower motor neurons. TAR DNA-binding protein (TDP-43) is an RNA and DNA binding protein that has been implicated in ALS, both as a causative factor and at the level of pathology. Although TDP-43 has been shown to play a key role in RNA metabolism, its mechanism is not fully understood. Specifically, evidence has shown that TDP-43 binds TG-rich sequences within RNA targets. In the context of disease, this binding can lead to alterations in splicing and/or proper regulation of its targets, and is partially responsible for the neurotoxicity that is associated with ALS. A 4-aminoquinoline analog (i.e. AAQ-2) has been shown to inhibit the binding of TDP-43 to TG oligonucleotides. To evaluate the effect of this small molecule in vivo, we administered AAQ probes to larvae expressing wild-type or mutant TDP-43 in motor neurons (D42 GAL4>TDP-43). Our experiments show that feeding of AAQ-2, but not a structurally similar negative control (AAQ-9), rescues the lethality caused by overexpression of TDP-43 in motor neurons. Additionally, AAQ-2 feeding also leads to improved locomotor function of larvae, as well as increased lifespan of flies overexpressing both wild-type and mutant TDP-43. In exploring the role of TDP-43-associated proteins in mediating the protective effect of AAQ-2 in motor neurons, we find that rescue of TDP-43-induced neurotoxicity by AAQ-2 is dependent in Fragile X Mental Retardation Protein (FMRP) in a TDP-43 variant-dependent manner. Currently, experiments are being performed to determine the effect of AAQ-2 on the solubility of full-length TDP-43 and its cleaved C-terminal fragment within motor neurons. These results provide insight into the role of TDP-43 in RNA metabolism, as well as suggest a possible therapeutic strategy for TDP-43-based ALS and related neurodegenerative diseases.

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Poster

044. Motor Neuron Disease

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Topic: C.05. Neuromuscular Diseases

Support: NIH Grant NS095157

Title: A computational model for investigating early alterations in motor neuron and reflex excitability in amyotrophic lateral sclerosis

Authors: ***S. VENUGOPAL**, S. CHANDLER;
Integrative Biol. and Physiol., Univ. of California Los Angeles, Los Angeles, CA

Abstract: We recently showed complex changes in excitability of disease vulnerable trigeminal motor neurons (TMNs) at early postnatal stage (P8-P12) in the $SOD1^{G93A}$ mouse model for Amyotrophic Lateral Sclerosis (ALS). The observed changes in excitability were specific to putative motor unit types and there appeared to be a homeostatic dysregulation of proportions of TMNs with distinct discharge patterns in jaw closer motor pools. Additionally we have noted hyper-excitable shifts in sensory Mesencephalic (Mes V) neurons. Similar studies on brainstem and spinal MNs showing early excitability changes together suggest multiple channelopathies and homeostatic dysregulation of membrane properties in sensorimotor circuits. Such changes representing some of the earliest abnormalities in animal models of ALS could lead to dysfunctional reflex control and neuromuscular system. Furthermore, channelopathies leading to dysregulation of intracellular calcium can lead to abnormalities in a variety of cellular functions involving metabolism, neuroprotection and excitability. We begin an integrative approach to track progressive abnormalities in disease development and their behavioral consequences with the development of a computational reflex circuit model that incorporates known ionic currents in TMNs and Mes V neurons of the brainstem trigeminal system. The conductance-based TMN models reproduce the heterogeneous discharge properties (Type I: Linear, Type II: Adapting, Type III: Sustained Linear, Type IV: Hysteretic) and Mes V model reproduces complex burst firing. The TMNs form a trigeminal jaw closer motor pool consisting of putative fast and slow motor units distinguished based on their recruitment thresholds and input resistance. The sensory Mes V neurons drive the TMN motor pool that in turn drives a simplified muscle model. Using this reflex circuit model, we examined consequences of altered ionic conductances involving persistent inward currents, calcium-activated potassium currents in TMNs as well as persistent

and resurgent sodium currents in Mes V neurons. Our model results reproduce experimentally observed changes in TMN rheobase, input resistance and proportions shifts in turn predicting correlative changes in ionic conductances and their consequences on reflex excitability and muscle force development.

Disclosures: S. Venugopal: None. **S. Chandler:** None.

Poster

044. Motor Neuron Disease

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Topic: C.05. Neuromuscular Diseases

Support: Target ALS

ALS Association

Taube/Koret Foundation

NINDS

CIRM

Title: Identification of therapeutic targets for cytoskeletal defects in amyotrophic lateral sclerosis

Authors: *A. JAVAHERIAN[1], P. GOYAL[1], K. SHAH[1], E. MOUNT[1], M. HSIAO[1], S. BROSKI[1,2], C. FALLINI[2], E. DANIELSON[2], J. LANDERS[2], S. FINKBEINER[1]; [1]Gladstone Inst., San Francisco, CA; [2]Univ. of Massachusetts Med. Sch., Worcester, MA

Abstract: The pathogenesis of ALS and the mechanisms that lead to selective motor neuron degeneration are still unknown. This lack of knowledge hinders the development of an effective therapy to prevent or stop progression of the disease. Identification of ALS causative genes has helped to identify potential pathogenic pathways involved in the development of familial and sporadic ALS that can be targeted for therapeutic intervention. We recently identified mutations in two cytoskeletal genes, the actin binding protein profilin 1 (PFN1) and the microtubule subunit α-tubulin 4A (TUBA4A) as causative for familial ALS. These observations suggest that alterations affecting the cytoskeleton architecture, dynamics, and function are important in ALS pathogenesis. Our central hypothesis is that alterations to cytoskeleton structure and dynamics disrupt essential cellular functions, such as synaptic plasticity, vesicle recycling, axonal transport, and neuronal plasticity, which are necessary for the maintenance of motor neurons. We

have developed novel primary neuron cellular models of ALS based on TUBA4A and PFN1 mutations using a custom-built automated longitudinal imaging platform. We apply these cellular models to screen a subset of the druggable genome siRNA library focused on cytoskeletal genes and genes that have direct interactions with known ALS-linked cytoskeletal genes. Screening this RNAi library allows us to identify genes that play a role in the cytoskeleton pathway and act as disease modifiers. We have found several modifiers that rescue neurodegeneration caused by mutant PFN1, TUBA4A and TDP43 and could reveal common mechanisms underlying neurodegeneration caused by these mutations. Furthermore, these genes could serve as potential therapeutic targets.

Disclosures: **A. Javaherian:** None. **P. Goyal:** None. **K. Shah:** None. **E. Mount:** None. **M. Hsiao:** None. **S. Broski:** None. **C. Fallini:** None. **E. Danielson:** None. **J. Landers:** None. **S. Finkbeiner:** None.

Poster

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Program#/Poster#: 44.20/N16

Topic: C.05. Neuromuscular Diseases

Support: Emory Medicine Catalyst Award

Title: Intracellular transport defects as emerging disease mechanisms in ALS/FTD

Authors: C.-C. CHOU[1], Y. ZHANG[4], M. E. UMOH[2], Y. CHEN[1], S. VAUGHAN[5], J. PAREE[5], M. A. POWERS[1], N. SEYFRIED[3], J. D. GLASS[2], D. C. ZARNESCU[5], *W. ROSSOLL[1];
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Abstract: Among the growing number of known amyotrophic lateral sclerosis (ALS) disease proteins, TAR DNA binding protein 43 (TDP-43) has emerged as a key player. Nearly all sporadic and familial ALS cases are characterized by cytoplasmic aggregations of hyper-phosphorylated, ubiquitinated, and cleaved TDP-43 fragments and the loss of nuclear TDP-43. Although the specific composition of these detergent-insoluble inclusions may hold important clues to the pathological process in TDP-43 proteinopathies, it was previously unknown.
To address this question, we have adapted a new technique for proximity-dependent biotin-labeling and identification of proteins (BioID). This approach is based on the expression of a fusion protein of a promiscuous biotin ligase (BirA*) to the target protein. Our results show that

addition of excess biotin led to efficient biotinyation of endogenous proteins that were co-aggregating with BirA*-TDP-CTF in live cells. This novel application of the BioID method allowed us to efficiently isolate biotinylated proteins present in detergent-insoluble pathological aggregates by affinity capture under denaturing conditions, and to identify them via mass spectrometry.
Quantitative proteomics and gene ontology analysis show that the TDP-CTF interactome can be functionally categorized in translation, RNA processing, protein degradation, and intracellular transport. Immunofluorescence experiments confirmed the co-aggregation of TDP-CTF with numerous highly disordered proteins involved in intracellular transport pathways. Our findings demonstrate that expression of TDP-CTF and full length TDP-43 carrying ALS-specific point mutations cause defects in the localization of proteins within the cell. This is further supported by genetic interaction data from a Drosophila model of TDP-43 proteinopathy and staining of human brain tissue. Taken together, our data show that TDP-43 toxicity causes the disruption of intracellular transport pathways may contribute to the neurodegeneration observed in ALS/FTD, and potentially other neurologic disorders.

Disclosures: **C. Chou:** None. **Y. Zhang:** None. **M.E. Umoh:** None. **Y. Chen:** None. **S. Vaughan:** None. **J. Paree:** None. **M.A. Powers:** None. **N. Seyfried:** None. **J.D. Glass:** None. **D.C. Zarnescu:** None. **W. Rossoll:** None.

Poster

044. Motor Neuron Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 44.21/N17

Topic: C.05. Neuromuscular Diseases

Support: Barrow Neurological Institute

ALSA

NINDS

MDA

Robert Packard Center for ALS Research at Johns Hopkins University

Title: Glua2 editing deficiency and synaptic dysfunction in C9orf72 als and ftd.

Authors: ***I. LORENZINI**[1], E. MENDEZ[2], I. VARELA[2], J. ROTHSTEIN[2], J. CHEW[3], L. PETRUCELLI[3], R. SATTLER[1];

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Abstract: The hexanucleotide repeat expansion GGGGCC (G4C2) in the noncoding region of the C9orf72 (C9) gene represents the most common cause of sporadic and familial amyotrophic lateral sclerosis (ALS) and of frontotemporal dementia (FTD). Recent studies proposed a deficit in nuclear-cytoplasmic trafficking of RNA and nuclear proteins as a disease mechanism for mutant C9orf72. In agreement with these findings, we discovered a significant mislocalization of the RNA editing enzyme ADAR2, which is known to edit the glutamate receptor subunit GluA2 at its Q-R site. Analysis of C9 patient postmortem brain and spinal cord tissue samples, C9 human induced pluripotent stem cell (hiPSC) derived motor neurons and a novel C9 mouse model confirmed this mislocalization and the subsequent misediting of the GluA2 Q/R site. Lack of editing at the GluA2 Q/R site leads to increased Ca^{2+} permeability of AMPA receptors, which has been shown to regulate surface expression of GluA2 containing receptors. Changes in AMPA receptor trafficking and localization are known to play a significant role in synaptic transmission and plasticity, and aberrant AMPA receptor function is therefore thought to lead to synaptic dysfunction and cognitive impairment in C9 patients. When analyzing C9 hiPSC neurons for signs of synaptopathy, we found a significant decrease in spine density and dendritic branching proximal to the neuronal cell body when compared to healthy control hiPSC neurons. In addition, we see a decrease in presynaptic marker protein expression synapsin 1 and VGlut1. Based on these results we propose that the C9orf72 mutation leads to GluA2 editing deficiency via mislocalization of ADAR2, which results in dendritic remodeling, cortical dysfunction and may therefore contribute to the development of cognitive impairment observed in C9ORF72 ALS and FTD patients.

Disclosures: **I. Lorenzini:** None. **E. Mendez:** None. **I. Varela:** None. **J. Rothstein:** None. **J. Chew:** None. **L. Petrucelli:** None. **R. Sattler:** None.

Poster

044. Motor Neuron Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 44.22/N18

Topic: C.05. Neuromuscular Diseases

Support: Muscular Dystrophy Association

Title: A proteomic perspective: Amyotrophic lateral sclerosis and frontotemporal dementia disease overlap

Authors: *M. E. UMOH[1,2], E. DAMMER[2], M. GEARING[2], D. DUONG[2], N. T. SEYFRIED[2], J. D. GLASS[2];
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Abstract: Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are progressive neurodegenerative diseases with substantial clinical, pathological, and genetic overlap. A hexanucleotide repeat expansion in the C9orf72 gene is the most frequent reported genetic cause of ALS and FTD. The objective of this study was to identify pathways involved in disease pathogenesis that are unique to each clinical phenotype and those that are similar across this spectrum. We performed an unbiased, quantitative proteomic screen using post-mortem brain tissue from patients clinically diagnosed with ALS (n=19), FTD (n=12), and patients who had both ALS and FTD (n=10), compared to normal (without neurological disease) controls (n=10). Patient tissue included those with and without the C9orf72 expansion mutation. Our data identified several pathways that differentiated these four clinically defined groups using weighted correlation network analysis. These included RNA binding proteins, astrocytic markers, proteins involved in nucleocytoplasmic transport, and synaptic proteins amongst other pathways. Using principal component analysis, we found that the proteomic signatures segregated out by clinical diagnosis. The presence of a C9orf72 expansion mutation was not identified as an independent variable associated with proteomic differences. Future detailed validation of these observed differences will clue us into the molecular underpinnings of these overlapping diseases. Based on our findings, we conclude that there are overlapping cellular pathways implicated in the diseases along this spectrum but also specific differences unique to clinical phenotypes separating ALS, FTD and ALS-FTD.

Disclosures: M.E. Umoh: None. **E. Dammer:** None. **M. Gearing:** None. **D. Duong:** None. **N.T. Seyfried:** None. **J.D. Glass:** None.

Poster

044. Motor Neuron Disease

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 44.23/O1

Topic: C.05. Neuromuscular Diseases

Title: Functional screening of ALS and ALS-dementia-linked Ubiquilin 2 (UBQLN2) mutations

Authors: *K. N. MCFARLAND[1], Y. ZHANG[2], D. RYU[2], C. CEBALLOS[3], D. RINCON-LIMAS[2], N. MCFARLAND[2];
[1]Dept. of Neurol., McKnight Brain Institute, Univ. of Florida, Gainesville, FL; [2]Neurol., [3]Neurosci., Univ. of Florida, Gainesville, FL

Abstract: Multiple mutations in *Ubiquilin 2* (*UBQLN2*) are found in individuals with X-linked amyotrophic lateral sclerosis (ALS), fronto-temporal dementia (FTD) or a combination of both (ALS-FTD). Since the initial study that identified a link between UBQLN2 and ALS/FTD, over 20 UBQLN2 mutations have been identified in familial as well as sporadic ALS/FTD patients. While initial mutations were identified in the proline-rich region of UBQLN2, additional mutations are scattered throughout in multiple domains outside the PXX region. UBQLN2 is a member of the ubiquitin-like family of proteins and is implicated in the functioning of the ubiquitin proteasome pathway (UPS), and possibly also in autophagy and the regulation of ER-associated protein degradation (ERAD). Both viral-mediated and transgenic overexpression of identified ALS-linked UBQLN2 mutant proteins results in widespread neuronal inclusion pathology in mouse models. In addition, a number of ALS-linked proteins are found within UBQLN2 inclusions in pathological samples, including ubiquitin, p62, TDP-43, VCP and optineurin. Furthermore, ALS-linked proteins of the hnRNP family interact with UBQLN2. These studies suggest converging pathogenic mechanisms involving UBQLN2 for abnormal proteostasis and RNA metabolism in ALS and ALS-dementia. Yet, the pathological mechanisms of UBQLN2 and its ALS-associated mutants remain unclear. The goal of our study is to determine the role of UBQLN2 in normal cellular proteostasis and RNA metabolism and to determine whether identified UBQLN2 mutations alter this using a combined approach of cellular and in vivo models. A dominant-negative effect has been proposed for UBQLN2 mutants affecting UPS function, but recent studies suggest also a toxic gain-of-function. Indeed, in our cell models expression of different UBQLN2 mutants results in varied inclusion pathology and differential effects on proteasomal function. Results from these studies lend insight into the function of UBQLN2 in cellular proteostasis and RNA metabolism and could identify proteins for targeted interventions.

Disclosures: K.N. McFarland: None. **Y. Zhang:** None. **D. Ryu:** None. **C. Ceballos:** None. **D. Rincon-Limas:** None. **N. McFarland:** None.

Poster

045. Therapeutic Potential in Motor Neuron Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 45.01/O2

Topic: C.05. Neuromuscular Diseases

Support: GAČR 14-10504P

GACR 15-06958S

GACR P304/12/G069

Title: Stem cells for treatment of Amyotrophic lateral sclerosis. Preclinical and clinical study.

Authors: M. SYKA[1,2], S. FOROSTYAK[1,3], A. HOMOLA[3], S. KONRADOVA[1,2], K. RUZICKOVA[1,2], P. JENDELOVA[1,3], M. BOJAR[3], I. VORISEK[1], *E. M. SYKOVA[1,3];
[1]Inst. Exptl. Med. ASCR, Prague, Czech Republic; [2]Bioinova, s.r.o., Prague, Czech Republic;
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Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder leading to the death of upper and lower motoneurons (MN). Cell therapy may present new possibilities to cure ALS by providing neurotrophic support to host MN, dysfunctional glial cells and by influencing the immune system. In our preclinical study human mesenchymal stem cells (hMSCs), were delivered intrathecally into symptomatic SOD1 G93A transgenic rats (n=11). Survival in the hMSC-treated group was prolonged by 13.6 days compared with the sham-treated group (n=9). We found that the cell-treated rats showed significantly better motility and grip strength. Quantitative analyses of wisteria floribunda (WFA) fluorescence intensity measured in the ventral horns of the spinal cord, revealed significantly greater numbers of perineuronal nets (PNNs) in the hMSCs-treated animals. The clinical study (sponsored by Bioinova) was designed as a prospective, non-randomized, open-label study (phase I/IIa, EudraCT No. 2011–000362–35) to assess the safety and efficacy of autologous multipotent bone marrow (BM) MSCs in the treatment of ALS. Investigational product (IP) with $15\pm4.5\times10^6$ of MSCs (manufactured by Bioinova) was applied via lumbar puncture into the cerebrospinal fluid. During the 18 month follow-up period potential adverse reactions were assessed by clinical, laboratory and MR examination. The clinical outcome was evaluated using the ALS functional rating scale (ALSFRS), Norris spinal and bulbar scale (NSS and NSB), forced vital capacity (FVC) and weakness scale (WS). To date, 26 patients were enrolled in the study. One quarter (27%) of these patients experienced mild/moderate headache resembling headache after standard lumbar puncture. No suspected serious adverse reactions (SUSAR) were observed. In the group of 19 patients suitable for efficacy analysis a significant reduction in the ALSFRS decline at 3 months ($p<0.01$) and at 6 months ($p<0.05$) after IP application was found. Moreover, stabilization of FVC decline between 3 and 6 months was observed. A subgroup of 14 patients, with remarkable pretreatment decline in functional scales (ALSFRS + NSS), had significant reduction/stabilization in their functional scales decline at 3 months after IP application ($p<0.001$ in ALSFRS, $p<0.05$ in NSS), which was less pronounced at 6 months ($p<0.05$ in ALSFRS, $p<0.07$ in NSS) and at 9 months ($p<0.05$ in ALSFRS + NSS). In this group we also observed stable WS values for a time period of 3 months after application. Our results demonstrate that the intrathecal application of BM-MSC in ALS patients is safe and can, at least temporarily, slow progression of the disease.

Disclosures: **M. Syka:** A. Employment/Salary (full or part-time): Bioinova s.r.o.. **S. Forostyak:** None. **A. Homola:** None. **S. Konradova:** A. Employment/Salary (full or part-time): Bioinova s.r.o. **K. Ruzickova:** A. Employment/Salary (full or part-time): Bioinova s.r.o.. **P. Jendelova:** None. **M. Bojar:** None. **I. Vorisek:** None. **E.M. Sykova:** None.

Poster

045. Therapeutic Potential in Motor Neuron Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 45.02/O3

Topic: C.05. Neuromuscular Diseases

Title: Selection and prioritization of candidate drug targets for amyotrophic lateral sclerosis through a meta-analysis approach

Authors: *G. MORELLO[1], A. SPAMPINATO[1], F. CONFORTI[2], V. D'AGATA[3], S. CAVALLARO[1];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive and incurable neurodegenerative disease. Although several compounds have shown promising results in preclinical studies, their translation into clinical trials has failed. This clinical failure is likely due to the inadequacy of the animal models that do not sufficiently reflect the human disease. Therefore, it is important to optimize drug target selection by identifying those that overlap in human and mouse pathology. We have recently characterized the transcriptional profiles of motor cortex samples from sporadic ALS (SALS) patients and differentiated these into two sub-groups based on differentially expressed genes, which encode 70 potential therapeutic targets. To prioritize drug target selection, we investigated their degree of conservation in superoxide dismutase 1 (SOD1) G93A transgenic mice, the most widely used ALS animal model. Interspecies comparison of our human expression data with those of eight different SOD1G93A datasets present in public repositories revealed the presence of commonly deregulated targets and related biological processes. Moreover, deregulated expression of the majority of our candidate targets occurred at the onset of the disease, offering the possibility to use them for an early and more effective diagnosis and therapy. In addition to highlighting the existence of common key drivers in human and mouse pathology, our study represents the basis for a rational preclinical drug development.

Disclosures: G. Morello: None. **A. Spampinato:** None. **F. Conforti:** None. **V. D'Agata:** None. **S. Cavallaro:** None.

Poster

045. Therapeutic Potential in Motor Neuron Disease

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

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Topic: C.05. Neuromuscular Diseases

Support: ALS Worldwide

Title: Microneurotrophins improve survival in motor neuron-astrocyte co-cultures but do not improve disease phenotypes in a mutant SOD1 mouse model of Amyotrophic Lateral Sclerosis

Authors: *K. E. GLAJCH[1], L. FERRAIUOLO[2], K. A. MUELLER[1], A. GRAVANIS[3], P. J. SHAW[2], G. SADRI-VAKILI[1];
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease 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 only extends lifespan by a few months, thus highlighting the need for new and improved therapies. Neurotrophic factors (NTFs) are important for 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 blood brain barrier (BBB) penetration. In this study we assessed the potential neuroprotective effects of a novel class of compounds known as MicroNeurotrophins (MNTs). MNTs are derivatives of Dehydroepiandrosterone (DHEA), an endogenous neurosteroid that can cross the BBB and bind to tyrosine kinase receptors mimicking the pro-survival effects of NTFs. Here we sought to determine whether MNTs were neuroprotective in two different models of ALS. Our results demonstrate that BNN27 (10 μM) attenuated loss of motor neurons co-cultured with astrocytes derived from human ALS patients with SOD1 mutations. Additionally, in the G93A SOD1 mouse, BNN27 (10 mg/kg) treatment attenuated motor behavioral impairment in the paw grip endurance and rotarod tasks at postnatal day 95 in female but not male mice. In contrast, BNN27 (10 mg/kg and 50 mg/kg) treatment did not alter any other behavioral outcome or neuropathological marker in male or female mice. Lastly, BNN27 was not detected in post-mortem brain or spinal cord tissue of treated mice and *in vitro* experiments demonstrated that BNN27 is metabolized at a faster rate by mouse hepatocytes relative to human hepatocytes. Together, these findings demonstrate that BNN27 treatment failed to yield significant neuroprotective effects in the G93A SOD1 model likely due to its rapid rate of metabolism in mice.

Disclosures: **K.E. Glajch:** None. **L. Ferraiuolo:** None. **K.A. Mueller:** None. **A. Gravanis:** None. **P.J. Shaw:** None. **G. Sadri-Vakili:** None.

Poster

045. Therapeutic Potential in Motor Neuron Disease

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Topic: C.05. Neuromuscular Diseases

Support: GACR 15-06958S

GACR 14-10504P

GACR P304/12/G069

Title: The effects of different applications of mesenchymal stem cells in the treatment of amyotrophic lateral sclerosis

Authors: ***P. JENDELOVA**[1,2], M. SENEKLOVA[1,2], S. FOROSTYAK[1,2], Y. PETRENKO[1], E. SYKOVA[1,2];
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Abstract: Amyotrophic lateral sclerosis (ALS) is characterized by the degeneration of upper and lower motor neurons, muscle atrophy and paralysis. Although the mechanisms responsible for motor neuron degeneration in ALS remain unclear, mesenchymal stem cell (MSC) transplantation is considered a promising approach for promoting neuroprotective and neuroregenerative repairs. However, the stem cells exert their effects in different manners depending on the way they are applied. In the first group, symptomatic SOD1 G93A transgenic rats received 2 injections of 10^5 cells MSCs intraspinally at the Th 10 level and $2x10^6$ intravenously (i.v.). The second group of rats received $5x10^5$ human MSCs (hMSCs) suspended in 50µl of growth media intrathecally (via the cistern magna) in a single injection, or repetitively 3 times via lumbar puncture. The third group of animals received 3 times 50µl of hMSC conditioned medium (CM-MSC). The fourth group of rats received hMSCs intramuscularly (three injections of $2x10^5$ cells into m. quadriceps femoris into both hind limbs) to prevent axonal degeneration. Sham operated animals were injected with growth media, using the same delivery route. The time course of the disease and functional scores after cell transplantation were tested behaviourally by BBB score, rotarod test, gript strength tests and motorater data analysis. We followed body weight and performed immunohistochemical analysis. Quantitative

polymerase chain reaction (qPCR) was used to evaluate the expression of growth factors in spinal tissue, apoptosis-related genes (BAX, BCL-2 and Casp-3) and necroptosis-related genes (RIP1, RIP3, MLKL). In comparison with the sham-treated group, overall survival in the i.v. MSC-treated group was prolonged by 11 days, a single intrathecal dose of hMSCs increased survival by 13.6 days and repetitive hMSCs application resulted in prolonged life spam by 19.6 days. All cell-treated rats showed significantly better motility and grip strength than controls. In the implanted rats we found a greater number of MNs at the thoracic and lumbar level and the apoptotic process assessed by the intensity of TUNEL staining decreased. CM-MSC or hMSCs intramuscular injection did not significantly prolong the life spam of SOD1 animals. Our results suggest that MSC application slows down disease progression and repeated application prolongs the effect. It is therefore reasonable to assume that repeated application can be more beneficial for potential clinical study in ALS patients.

Disclosures: P. Jendelova: None. **M. Seneklova:** None. **S. Forostyak:** None. **Y. Petrenko:** None. **E. Sykova:** None.

Poster

045. Therapeutic Potential in Motor Neuron Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 45.05/O6

Topic: C.05. Neuromuscular Diseases

Support: ALS Finding a Cure

Title: Therapeutically targeting the motor cortex in ALS using human neural progenitor cells expressing GDNF

Authors: *G. M. THOMSEN, A. MA, P. AVALOS, P. HARO, O. SHELEST, C. N. SVENDSEN;
Cedars-Sinai Med. Ctr., West Hollywood, CA

Abstract: Recently, there has been an increased focus on the role of the motor cortex in amyotrophic lateral sclerosis (ALS). We have shown that the transplantation of human neural progenitor cells expressing glial cell line-derived neurotrophic factor (hNPC-GDNF) into the motor cortex of the SOD1 rat model of ALS leads to a delayed disease onset and extension in survival. Here, we show that this targeting strategy also leads to enhanced survival of large spinal motor neurons in both the lumbar and cervical spinal cord in SOD1 rats transplanted with hNPC-GDNF relative to non-injected rats when euthanized at ~165 days of age. The difference in spinal motor neurons was not evident when rats were euthanized at endpoint, which is

unsurprising given that rats are at a similar anatomical disease state. Analysis of L5 ventral root axons was not significantly different in those rats receiving injections of hNPC-GDNF in the brain relative to non-injected controls at either time point. Variability in data suggests, however, that there may be a dose-response range whereby the extent of injected cell survival and migration throughout the cortex influences the magnitude of downstream effects, the delay in disease onset and the extension in survival. Assessment of graft survival and GDNF expression in one rat surviving 273 days (3.9 standard deviations above the mean survival of 182 +/- 23 days in non-injected SOD1 rats in this study) has revealed robust cell survival, high GDNF expression, and widespread migration throughout the rostral-caudal axis of the motor cortex. Cells in these long-term grafts primarily differentiated into GFAP-expressing astrocytes, with a number of them appearing morphologically as mature astrocytes. Additionally, host cells in the transplanted regions appeared to take-up GDNF. Two SOD1 rats that received cortical injections of hNPC-GDNF in this study are currently surviving at 315 days of age. Transplanted cells in all rats are assessed for survival, migration, differentiation and GDNF expression, and these parameters are correlated with disease onset and lifespan. Transplanting hNPC-GDNF into the motor cortex has significant beneficial functional effects in a rat model of ALS. As such, studies with a single non-human primate are underway to test the safety of delivering hNPC-GDNF to the motor cortex in a large animal model. Collectively, these preclinical studies should permit translation of targeting the brain with these cells to treat ALS. This promising treatment could involve targeting the brain alone, or in combination with spinal cord and/or muscle.

Disclosures: G.M. Thomsen: None. **A. Ma:** None. **P. Avalos:** None. **P. Haro:** None. **O. Shelest:** None. **C.N. Svendsen:** None.

Poster

045. Therapeutic Potential in Motor Neuron Disease

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Program#/Poster#: 45.06/O7

Topic: C.05. Neuromuscular Diseases

Support: Muscular Dystrophy Association “Gene Therapy Approaches to CMT2D” MDA351564

NIH U54 OD020351 The Jackson Laboratory Center For Precision Genetics

Philanthropic Support

Title: A personalized gene therapy approach for charcot-marie-tooth disease type 2d

Authors: *K. H. MORELLI[1,2], J. S. DOMIRE[3], N. PYNE[3], A. FOWLER[3], S. HARPER[3], R. BURGESS[1,2];
[1]The Jackson Lab., Bar Harbor, ME; [2]Grad. Sch. of Biomed. Sci. and Engin., The Univ. of Maine, Orono, ME; [3]Ctr. for Gene Therapy, The Res. Inst. at Nationwide Children's Hosp., Columbus, OH

Abstract: Charcot-Marie-Tooth disease is a collection of inherited polyneuropathies caused by at least 80 loci in the human genome. To date, thirteen different mutations in *GARS (*glycyl-tRNA synthetase, GlyRS*)* have been identified in patients with autosomal dominant CMT Type 2D (CMT2D). Although, the mechanisms through which mutant forms of GARS cause axon degeneration remain controversial. Preliminary data from CMT2D patients and mouse models of the disease ($Gars^{C201R/+}$ and $Gars^{P278KY/+}$) are consistent with the expression of mutant GlyRS causing toxic gain-of-function or dominant negative effects in peripheral nerves. These data suggest that the selective silencing of mutant *GARS* expression should be of therapeutic benefit for patients with this disorder. However, considering there are currently no strategies for accomplishing this pharmacologically, we have developed a gene therapy strategy that reduces mutant *Gars* transcripts through allele-specific RNAi. To test the proof-of-principle of this approach, microRNA vectors that specifically target the mutant mouse *Gars* allele, P278KY (mi-P278KY) have been developed and cloned into self-complementary adeno-associated viral vectors (scAAV9) for *in vivo* delivery. Preliminary data suggest that when injected at birth, AAV9-mi-P278KY leads to significantly improved gross motor function and nerve conduction velocity in $Gars^{P278KY/+}$ mice. Treated mice will be further evaluated for additional primary outcome measures of neuropathy, including axon atrophy, axon loss, and neuromuscular junction abnormalities to confirm the efficacy of the gene therapy approach. Future directions for this study include testing the ability of AAV9-mi-P278KY to arrest or reverse the neuropathy when delivered post onset in $Gars^{P278KY/+}$ mice, and to test efficacy of patient-specific vectors in "humanized" mouse models that have CMT2D-patient-associated mutations introduced into the mouse *Gars* gene. Success with this approach would provide a promising avenue for treatment of CMT2D and other dominantly inherited neuromuscular diseases.

Disclosures: K.H. Morelli: None. **J.S. Domire:** None. **N. Pyne:** None. **A. Fowler:** None. **S. Harper:** None. **R. Burgess:** None.

Poster

045. Therapeutic Potential in Motor Neuron Disease

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Topic: C.05. Neuromuscular Diseases

Support: SAF2013-48431-R

TERCEL

CIBERNED

Title: Il37 reduces inflammation and ameliorates pathophysiology in amyotrophic lateral sclerosis

Authors: *A. MARTINEZ-MURIANA[1], C. A. DINARELLO[2,3], R. LOPEZ-VALES[1];
[1]Cell biology, physiology and immunology, Univ. Autonoma De Barcelona, Bellaterra, Spain; [2]Div. of Infectious Dis., Univ. of Colorado Denver, Aurora, CO; [3]Dept. of Med., Radboud Univ. Med. Ctr., Nijmegen, Netherlands

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder that affects upper and lower motor neurons (MNs). MNs loss results in skeletal muscle weakness, spasticity and eventual paralysis, leading to the death of patients by respiratory failure 3 to 5 years after diagnosis. As occurs in several neurological disorders, inflammation is a hallmarks also found in ALS patients. Several reports provide evidence that glial cells accelerate the course of the disease in animal models of ALS, suggesting that therapies aimed at targeting inflammation may be a valuable approach for the treatment of ALS. IL-37 is a member of the IL-1 family that exerts broad anti-inflammatory effects over innate and acquired immunity. Transgenic mice for human IL-37 (hIL-37tg) exhibit reduced disease severity in models of endotoxemia, acute lung injury, chemical colitis, myocardial ischemia, and sleep disturbance. We have demonstrated for the first time that this cytokine mediates marked anti-inflammatory actions in the injured central nervous system, and leads to reduced tissue damage and functional deficits in a mouse model of spinal cord contusion injury. However, whether IL-37 also exerts similar beneficial effects in neurodegenerative conditions is not known yet. In the present study, we investigated whether IL-37 suppresses inflammation and slows the clinical course of the disease in a mouse model of ALS ($SOD1^{G93A}$ mouse). Since IL-37 is not expressed in the mouse, we crossed hIL-37tg mice with $SOD1^{G93A}$ mice. Our data reveals that transgenic expression of IL-37 resulted in reduced microgliosis and astrogliosis in the lumbar spinal cord of $SOD1^{G93A}$ mice. Interestingly, we also observed that IL-37 slowed ALS disease progression and increased lifespan of these mice, which correlated with an increased preservation of spinal MN. Our findings provide the first evidence of the anti-inflammatory and beneficial actions of IL-37 in a neurodegenerative disease, and supports the therapeutic potential of this cytokine for the treatment of ALS.

Disclosures: **A. Martinez-Muriana:** None. **C.A. Dinarello:** None. **R. Lopez-Vales:** None.

Poster

045. Therapeutic Potential in Motor Neuron Disease

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Program#/Poster#: 45.08/O9

Topic: C.05. Neuromuscular Diseases

Title: Gene therapy for familial amyotrophic lateral sclerosis using adeno-associated virus serotype-9 mediated silencing of mutant SOD1

Authors: *T. IANNITTI[1], J. M. SCARROTT[1], I. R. P. COLDICOTT[1], B. K. KASPAR[2], L. FERRAIUOLO[1], P. J. SHAW[1], M. AZZOUZ[1];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder resulting in death of brain and spinal cord motor neurons. Some familial cases are caused by missense mutations in the gene encoding the Cu/Zn superoxide dismutase 1 (SOD1) conferring a toxic gain of function to this protein. We hypothesised that specifically silencing expression of the mutant form of the human SOD1 gene would alleviate SOD1-linked ALS symptoms. The aim of our study was to evaluate the therapeutic efficacy of clinical AAV9-shRNA mediated SOD1 silencing in the SOD1G93A mouse model. Animals were treated either at postnatal day 1 (P1, pre-onset) or P40 (onset). scAAV9-hSOD1si or scrambled control scAAV9-hSOD1ssi were delivered using 2 routes of delivery, facial vein or cisterna magna. Mice were then tested using behavioural tests including weekly rotarod runs, neurological scoring and CatWalk gait analysis. Weekly body weight was also collected. We observed an improvement in rotarod performance in mice treated with scAAV9-hSOD1si when compared to scAAV9-hSOD1ssi and untreated controls. Survival analysis revealed that a therapeutic dose of $2.5x10^{13}$vg/kg body weight increased median survival from 129 days, to a maximum survival of over 240 days. Clinical AAV9-shRNA vector mediated SOD1 silencing through cisterna magna improved motor performance and led to remarkable life span extension in the SOD1G93A mouse model. Our gene therapy approach offers promising strategy for clinical application in SOD1-linked familial ALS.

Disclosures: T. Iannitti: None. **J.M. Scarrott:** None. **I.R.P. Coldicott:** None. **B.K. Kaspar:** None. **L. Ferraiuolo:** None. **P.J. Shaw:** None. **M. Azzouz:** None.

Poster

045. Therapeutic Potential in Motor Neuron Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 45.09/O10

Topic: C.05. Neuromuscular Diseases

Support: NIH RO1NS088689

NIH R21NS089979

Title: Artificial microRNA silences C9ORF72 variants and decreases dipeptides in BAC transgenic mouse

Authors: *G. TORO[1], O. M. PETERS[2], T. F. GENDRON[4], C. MUELLER[3], R. H. BROWN, Jr[2];
[1]Horae Gene Therapy Ctr. , UMASS Med. Sch., Worcester, MA; [2]Neurol., [3]Horae Gene Therapy center, UMASS Med. Sch., Worcester, MA; [4]Neurol., Mayo Clin., Jacksonville, FL

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease that targets upper and lower motor neurons causing progressive muscle weakening. Respiratory failure is the common cause of death approximately 2-5 years after symptom onset. An expanded hexanucleotide repeat located in chromosome 9 open reading frame 72(C9ORF72) accounts for the majority of familial ALS cases as well as frontotemporal dementia (FTD) cases. Post mortem patient brain tissues have shown that in the presence of the expansion, C9ORF72 mRNA is reduced and RNA sequences complimentary to the expansion aggregate into nuclear foci. Simultaneously, this expanded RNA can be translated into long dipeptide chains by non-ATG repeat associated (RAN) translation. These findings have led to three hypotheses on the pathogenesis of C9ORF72: 1) haploinsufficiency, postulating that decreased levels of mRNA lead to insufficient gene product; 2) adverse effects of intranuclear RNAi foci (eg. protein sequestration); 3) RAN translation from the repeat expansion generating toxic poly-dipeptide proteins. Our group has generated 2 lines of mice containing a bacterial artificial chromosome (BAC). The first line is composed of exons 1-6 of the human C9ORF72 gene and a 500 repeat hexanucleotide expansion. The second is composed of all 12 exons and a smaller, 100 repeat expansion. These mouse models recapitulate the major histopathological features seen in human ALS/FTD caused by C9orf72 mutations: lower levels of C9ORF72 mRNA, RNA nuclear foci, and the RAN translation products. We have used these mice as a platform in which to test RNAi therapeutic strategies. We have designed artificial microRNAs that target the human C9ORF72 gene with the purpose of decreasing the mRNA levels, the toxic RNA foci and/or RNA dipeptide proteins. We packaged our lead microRNA into a recombinant adeno-associated virus (rAAV) serotype 9 to use with primary neuron cultures and *in vivo* experiments. Silencing was initially validated in primary cortical neurons from the first C9ORF72 mouse line. Our results suggest

that AAV9-mediated microRNA not only reduced the mRNA levels of C9ORF72 but also the most abundant poly dipeptide (GP). Experiments are now underway to silence C9ORF72 *in vivo* via intracranial ventricular (ICV) injection of this microRNA into C9ORF72 transgenic pups; results will be presented.

Disclosures: **G. Toro:** None. **O.M. Peters:** None. **T.F. Gendron:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Licensed for distribution of an antibody to the poly(GP) dipeptide. **C. Mueller:** F. Consulting Fees (e.g., advisory boards); Consultant for Voyager. Inventor on a patent filed for the use of rAAV mediated silencing of C9ORF72. **R.H. Brown:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Consultant for Voyager. Inventor on a patent filed for the use of rAAV mediated silencing of C9ORF72.

Poster

045. Therapeutic Potential in Motor Neuron Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 45.10/O11

Topic: C.05. Neuromuscular Diseases

Support: Telethon Grant to MN

ARISLA Grant to MN

JPND to SC

Ministery of Health to GC

Title: Antisense oligonucleotides-based strategy as a therapy for the development of genetic Motor Neuron Diseases Antisense oligonucleotides-based strategy as a therapy for the development of genetic Motor Neuron Diseases

Authors: M. NIZZARDO[1], M. BUCCHIA[2], A. RAMIREZ[2], F. RIZZO[2], M. RIZZUTI[2], P. RINCHETTI[2], G. ULZI[1], A. BORDONI[2], N. BRESOLIN[1], *G. P. COMI[1], S. CORTI[1]; [1]Univ. of Milan, Ospedale Maggiore Policlinico, Milan, Italy; [2]Univ. of Milan, Milan, Italy

Abstract: Motor neuron diseases (MNDs) are fatal incurable disorders characterized by relentless motor neuron (MN) degeneration. No effective approved treatments are presently available. The genetic MNDs forms can offer a solid ground for research since, at least in these cases, the etiopathogenetic primum movens is identified. In this study, we developed novel

oligonucleotides with morpholino (MOs) chemistry designed to reduce the synthesis of human SOD1 and C9ORF72, the most frequent Amyotrophic Lateral Sclerosis (ALS) causative genes, or to up-regulated the SMN full length level, the causative molecular defect of Spinal Muscular Atrophy (SMA). To investigate the effectiveness of MO approach in a human model, we treated human familial (f)ALS (SOD1/C9ORF72) and SMA induced pluripotent stem cell (iPSC) and their derived motor neurons with MOs. The oligonucleotides can effectively modulate the expression of the target protein downregulating SOD1/C9ORF72 in fALS and up-regulating SMN protein level in SMA cells. Indeed, after treatment, SOD1 and SMA motor neurons displayed increased survival and reduced expression of apoptotic markers, while C9ORF72 cells showed a significant reduction of toxic dipeptides accumulation. At the same time, we studied the efficacy of the already validated MO sequence targeting (MO-10-34) SMN2 in vivo in the SMAΔ7 mouse model after its conjugation with four cell-penetrating peptides (Tat, R6, r6 and (RXRRBR)2XB) to increase the cellular and tissue uptake and pharmacological profile. The compounds obtained were administered in a small pilot group of pre-symptomatic SMA mice to determine the best one by its ability to rescue the SMN protein levels by western blot and to perform further studies in presymptomatic and symptomatic SMA mice to assess MO therapeutic potential. Overall, our results support the efficacy of MO-mediated therapeutic strategy in MND models, opening the path for human development of this approach.

Disclosures: **M. Nizzardo:** None. **M. Bucchia:** None. **A. Ramirez:** None. **F. Rizzo:** None. **M. Rizzuti:** None. **P. Rinchetti:** None. **G. Ulzi:** None. **A. Bordoni:** None. **N. Bresolin:** None. **G.P. Comi:** None. **S. Corti:** None.

Poster

045. Therapeutic Potential in Motor Neuron Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 45.11/O12

Topic: C.05. Neuromuscular Diseases

Support: Packard Center for ALS Research at Johns Hopkins

CIHR

Title: Mitigation of TDP-43 proteinopathy through recombinant single chain antibodies.

Authors: *S. POZZI, S. S. THAMMISSETTY, C. GRAVEL, J. KRIZ, J.-P. JULIEN; CRIUSMQ, Québec, QC, Canada

Abstract: A landmark in ALS was the discovery in 2006 of TDP-43 as a major component of ubiquitinated inclusions in ALS cases. TDP-43 is predominantly a nuclear protein that has been implicated in regulating mRNA splicing, stability and gene transcription but the pathogenic pathways of TDP-43 in ALS are not well understood. We discovered that TDP-43 acts as a co-activator of p65 subunit of nuclear factor kappa B (NF-kB). p65 interacts with the RNA recognition motif 1 (RRM1) domain of TDP-43. We propose to develop a therapy for ALS based on the delivery of single chain (scFv) antibodies that can target specifically the TDP-43 RRM1 domain with the dual action to block the TDP-43 interaction with p65 NF-κB and to attenuate formation of TDP-43 aggregates. From monoclonal antibodies, specifically binding the RRM1 domain of TDP-43, we generated vectors expressing two recombinant scFv antibodies. Vectors include a human myc epitope as a localization signal and an IgK domain which allows scFv secretion. We transfected cells with scFv expressing vectors and verify the ability of scFv to recognize TDP-43, disrupt TDP-43/p65 interaction and reduce TDP-43 aggregation. We finally generated scAAV2/9 viral vectors producing the single chain antibodies, injected them in the frontal cortex of mice expressing mutant TDP-43 and analysed the disease progression in terms of motor performances and cognitive impairments. ScFv can be produced and released in the medium of transfected cells and are able to bind recombinant TDP-43 fragment and to disrupt TDP-43/p65 interaction. Experiments in BV2 microglial cells provide evidences that scFv antibodies can attenuate the activity of p65/NF-kB and modulate cytokine expression levels after LPS induction. Moreover, transfection of scFv antibodies into Hek293 attenuates TDP-43 insolubility after treatment with Ethacrynic Acid. Mutant TDP-43 mice, injected with the virus expressing scFv antibodies, show improvements in cognitive behavioural tests like novel object recognition and open field test. These results support the feasibility to use these scFv antibodies for attenuating TDP-43 proteinopathy.

Disclosures: **S. Pozzi:** 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; Project is founded by Packard Center for ALS Research at Johns Hopkins and CIHR. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); papent with Laval University. **S.S. Thammissetty:** None. **C. Gravel:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent with Laval University. **J. Kriz:** None. **J. Julien:** 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; Project is founded by Packard Center for ALS Research at Johns Hopkins and CIHR. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent with Laval University. Other; Imstar therapeutics.

Poster

045. Therapeutic Potential in Motor Neuron Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 45.12/O13

Topic: C.05. Neuromuscular Diseases

Support: Centre for Regenerative Medicine Fellowship, Mayo Clinic

Title: Development of clinical grade neurotrophic factor secreting autologous mesenchymal stem cells for treatment of amyotrophic lateral sclerosis

Authors: *S. RAMESH, N. N. MADIGAN, K. J. CLARK, M. J. POLZIN, S. P. EKKER, A. J. WINDEBANK, N. P. STAFF;
Mayo Clin., Rochester, MN

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder affecting the motor neurons leading to progressive paralysis. In most cases, the cause is sporadic (90%) and leads to death within 3-5 years after disease onset. Recent work has shown that delivery of mesenchymal stem cells (MSCs) to the spinal cord is safe and could potentially slow motor neuron degeneration. Neurotrophic factors (NTFs) have long been considered neuroprotective in animal models of motor neuron disease. However, in human clinical trials NTFs have not been beneficial for ALS possibly due to poor central nervous system penetrance. Based on previous work, we hypothesize that controlled release of NTFs in an autologous MSC based delivery system could be a potential therapeutic solution for patients suffering from ALS. Our objective is to conduct targeted gene insertion in patient adipose derived MSCs at 'safe-harbor' sites using transcription activator like effector nuclease technology (TALEN). NTF secreting MSCs (MSC-NTFs) will then be evaluated for safety and efficacy in *in vitro* ALS models. Two pair of TALENs for each of the two safe-harbor sites (AAVS1 and CLYBL) was cloned. Various delivery methods including nucleofection and polyethylenimine based transfection systems were explored in this study. One TALEN pair for AAVS1 was chosen and cell viability, transformation efficiency, and TALEN pair cutting efficiency was measured 48 hours after nucleofection. We aim to develop clinical grade NTF-secreting adipose-derived MSCs that can be delivered back to ALS patients in a safe manner. Development of this system may be beneficial not only to treat ALS but potentially could also be used to treat other neurodegenerative disorders that are responsive to NTFs.

Disclosures: S. Ramesh: None. **N.N. Madigan:** None. **K.J. Clark:** None. **M.J. Polzin:** None. **S.P. Ekker:** None. **A.J. Windebank:** None. **N.P. Staff:** None.

Poster

045. Therapeutic Potential in Motor Neuron Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 45.13/O14

Topic: C.05. Neuromuscular Diseases

Support: Revert Onlus

Fondazione Cellule Staminali, Terni

Assicurazioni Generali

Associazione Pro Roberto

Carit

Fondazione Borgonovo

Fondazione Milan

Title: Data from pre-clinical and completed phase I clinical studies with intraspinal injection of human neural stem cells in amyotrophic lateral sclerosis

Authors: L. MAZZINI[1], M. GELATI[2,3,4], D. C. PROFICO[3], D. FERRARI[2], C. ZALFA[2], G. SGARAVIZZI[4], M. PROJETTI PENSI[4], G. MUZI[4], C. RICCIOLINI[4], S. CARLETTI[5], C. GIORGI[6], C. SPERA[5], M. COPETTI[3], M. BOIDO[7], L. ROTA NODARI[2], G. QUERIN[8], E. VACCHI[2], E. BERSANO[1], E. BINDA[3], V. GARLATTI[2], F. PINOS[2], I. BICCHI[4], D. FRONDIZI[5], I. PIRISINU[4], F. PETRUZZELLI[3], A. STECCO[1], G. SORARÙ[8], A. VERCELLI[7], N. BOULIS[9], *A. L. VESCOVI[2,3,4];
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is an incurable disease that targets motor neurons (MNs). Non cell-autonomous mechanisms, particularly micro and macro-gliosis are implicated in MNs degeneration. We describe here the results from studies testing the ability of human neural stem cells (hNSCs) – whose clinical GMP-status was granted by the Italian Medicines Agency (AIFA) – to elicit anti-inflammatory effects upon transplantation in the SOD1

rat ALS model and data emerging from a standardized phase I clinical trial on ALS patients. SOD1 experiments: hNSCs were injected bilaterally into the anterior horns of the spinal cord (4 grafts; L3-L4 tract) of early symptomatic SOD1 rats (n=15). Control groups: HBSS treated SOD1 (HBSS, n=15) and untreated ones (Ctrl, n=22). hNSCs migrated rostro-caudally up to 3.27±1.05 cm differentiating into neurons and glia. At late-symptomatic stage, hNSCs SOD1 displayed higher spinal MNs density (L3-L4) and reduced astroglial and microglial cell numbers ($p \leq 0.05$ vs HBSS and $p \leq 0.01$ vs Ctrl). hNSCs slowed body weight loss and motor performances deterioration ($p \leq 0.05$ vs HBSS and $p < 0.0001$ vs Ctrl). hNSCs SOD1 (n=5) survived more respect to HBSS (n=5, HR: 0.08 95%CI=0.01-0.72 p=0.024) and Ctrl (n=14, HR: 0.08 95%CI=0.01-0.65 p=0.018). Phase I clinical trial: In 2012 the Italian Institute of Health (ISS) and competent ethical committees approved a phase 1 clinical trial (*EudraCT* Nr 2009-014484-39) to assess the safety and feasibility of intraspinal transplantation of hNSCs in 18 ALS subjects. The trial has now been completed. Stereotactic microinjection procedures (n=6 thoracolumbar [T10/11], n=12 cervical [C3-5]) delivered hNSCs into definite ALS patients (5F and 13 M, Median age 49yrs), in three cohorts. Each injection series comprised 3 injections of 15 µl of 50,000cells/µl suspension. All patients were immunosuppressed for 6 months after surgery and are monitored monthly by both clinical and radiological standardised assessment. No patients manifested severe treatment-related adverse events (post-surgical mild pain disappearing in ~4days), no structural changes (including tumor or syrinx formation) and no evidence of acceleration of disease progression due to the treatment has been evidenced so far. Eleven patients showed a transitory improvement of MRC score respectively in the leg and in the arm. Nine patients died 8-35 months after surgery due to natural history of the disease. This study confirms the safety of this procedure and shows no evidence of immediate or delayed toxicity related to hNSCs. Based on these results we are now submitting to the ISS a request to proceed with a phase II trial examining therapeutic efficacy.

Disclosures: **L. Mazzini:** None. **M. Gelati:** None. **D.C. Profico:** None. **D. Ferrari:** None. **C. Zalfa:** None. **G. Sgaravizzi:** None. **M. Projetti Pensi:** None. **G. Muzi:** None. **C. Ricciolini:** None. **S. Carletti:** None. **C. Giorgi:** None. **C. Spera:** None. **M. Copetti:** None. **M. Boido:** None. **L. Rota Nodari:** None. **G. Querin:** None. **E. Vacchi:** None. **E. Bersano:** None. **E. Binda:** None. **V. Garlatti:** None. **F. Pinos:** None. **I. Bicchi:** None. **D. Frondizi:** None. **I. Pirisinu:** None. **F. Petruzzelli:** None. **A. Stecco:** None. **G. Sorarù:** None. **A. Vercelli:** None. **N. Boulis:** None. **A.L. Vescovi:** None.

Poster

045. Therapeutic Potential in Motor Neuron Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 45.14/O15

Topic: C.05. Neuromuscular Diseases

Title: Cryptic exon repression by tdp-43 as a novel approach to a disease modification biomarker in als, ftd, and ad

Authors: *R. KESILMAN (KORN);
Early Discovery Neurosci., Merck & Co., Inc., West Point, PA

Abstract: Dysregulation of transactivation response element DNA-binding protein 43 (TDP-43) is a common pathophysiological hallmark of Amyotrophic lateral sclerosis (ALS) and Frontotemporal dementia (FTD). Cytoplasmic mislocalization and aggregation of TDP-43 is distributed across distinct populations of neurons and is thought to reflect the pathological progression of ALS -FTD. Recently it was discovered that TDP-43 functions to repress cryptic exon expression in pre-mRNA transcripts of key target genes. Cryptic exons are small intronic regions of DNA normally removed by the spliceosome prior to mature mRNA production. A loss of TDP-43 function, resulting from nuclear mislocalization, truncation, or aggregation during the disease process, is proposed to cause abnormal inclusion of cryptic exons in target proteins. The purpose of the experiments described in this presentation was to validate cryptic exon expression following TDP-43 knock-down in a human neuroblastoma cell line, and in human post-mortem disease tissue. Data from these experiments confirmed and extended initial findings and demonstrated that TDP-43 knock-down in SH-SY5Y cells results in a robust increase in cryptic exon expression in key target mRNA. In most cases, cryptic exon incorporation results in premature stop codons and nonsense mediated decay of RNA. However, cryptic exons can also be spliced in-frame and result in translation of a cryptic peptide sequence. To explore this, cryptic peptide expression following TDP-43 knock-down in SH-SY5Y cells is being explored using mass spectrometry to characterize novel disease-specific biomarkers. Finally, we continue to evaluate and characterize cryptic exon and cryptic peptide expression in post-mortem disease brain tissue, plasma, and csf to determine the robustness and reliability of expression in patients versus control tissue. Together, these findings point to a novel biomarker of TDP-43 proteinopathy, and may be used to develop PET ligands, antibody or mass spectrometry-based biomarker assays of disease progression.

Disclosures: R. Kesilman (korn): None.

Poster

045. Therapeutic Potential in Motor Neuron Disease

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 45.15/O16

Topic: C.05. Neuromuscular Diseases

Support: NIH-SBIR/NIDCD 1R43DC015192-01

Title: Chronic laryngeal nerve stimulation to improve swallow function and survival in an ALS mouse model

Authors: M. M. HANEY[1], K. L. ROBBINS[2], J. H. ALLEN[3], A. THIESSEN[3], I. DENINGER[2], K. FLYNN[2], V. CAYWOOD[2], A. MOK[1], D. OHLHAUSEN[2], N. KHODAPARAST[4], *T. E. LEVER[3];
[1]Vet. Pathobiology, [3]Otolaryngology - Head and Neck Surgery, [2]Univ. of Missouri Columbia, Columbia, MO; [4]Nuviant Med., Dallas, TX

Abstract: Objectives: Many neurologic diseases, such as amyotrophic lateral sclerosis (ALS), result in swallowing impairment, also known as dysphagia. Dysphagia is highly correlated with malnutrition and aspiration pneumonia, leading to poor quality of life and increased morbidity and mortality. Current treatments are palliative and target the clinical symptoms of dysphagia. There are little to no treatments that address the underlying neuro-pathophysiology responsible for dysphagia. To address this, we have developed an experimental protocol to investigate the efficacy and safety of chronic superior laryngeal nerve (SLN) stimulation to improve swallow function and survival in the low copy number (LCN) *SOD1-G93A* transgenic ALS mouse model that has phenotypic variation relative to limb paralysis. This experiment is based on our previous experiments using acute SLN stimulation to immediately rescue swallow function in end-stage *SOD1-G93A* mice, enabling dysphagic mice to swallow at stimulus frequencies similar to control mice.
Methods: 80 mice (40 *SOD1-G93A* and 40 controls) underwent a freely-behaving videofluoroscopic swallow study assay to characterize dysphagia over the lifespan. 20 additional control mice were utilized for surgical protocol development for chronic SLN stimulation with a custom made nano-cuff electrode.
Results: *LCN-SOD1-G93A* mice develop dysphagia at 6 months of age that worsens with disease progression; however, dysphagia severity is worse for mice with predominant forelimb paralysis. At dysphagia onset, mice are undergoing our surgical protocol for nano-cuff placement that includes an anterior cervical approach. The SLN is isolated and the nano-cuff is positioned around the nerve to stimulate swallowing. A micro-patch electrode is placed on the anterior digastric muscle for EMG detection of swallows. Electrode leads are tunneled subcutaneously and attached to a skull-mounted head stage and connected to an electrical stimulator and bioamplifier. Using this protocol, we are establishing optimal SLN stimulation parameters that evoke swallowing in freely-behaving mice.
Conclusions: If ALS-affected mice maintain healthy swallow function and survive significantly longer as a result of chronic SLN stimulation, this study will lay the groundwork for using an implantable SLN stimulator for long-term treatment of dysphagia in patients with ALS and other chronic neurological conditions. While we hypothesize this novel treatment strategy will improve swallow function regardless of ALS phenotype, we expect mice with predominant forelimb paralysis (i.e., the most severe dysphagia) will demonstrate the best treatment outcomes.

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Poster

046. Modulating and Tracking Neuroinflammation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 46.01/O17

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: DTRA

Title: 3a-diol is neuroprotective and attenuates pro-inflammatory cytokines in the brain following soman exposure in mice.

Authors: ***K. LAITIPAYA**, J. K. CHANDLER, T. M. FERRARA-BOWENS, J. F. IRWIN, D. D. PALMER, L. J. SHUMWAY, E. A. JOHNSON;
USAMRICD, Gunpowder, MD

Abstract: Severe neuropathology and behavioral impairment result from prolonged status epilepticus (SE) caused by organophosphorus nerve agents such as soman (GD). GD, a potent acetylcholinesterase inhibitor, causes prominent cell death in the hippocampus, thalamus, piriform cortex, and amygdala, leading to the activation of the neuroinflammatory cascade. Neuroinflammation can exacerbate tissue injury or promote healing, depending on the intricate interaction of multiple cells, inflammatory factors, and receptors as the injury progresses. Few studies have explored the effects of the neuroendocrine system on brain injury caused by SE. Prior studies have shown that reductions in neuroinflammation can confer neuroprotection and that androgen therapy also has neuroprotective properties. In particular, testosterone and its metabolites dihydrotestosterone (DHT) and 3α-androstanediol (3α-diol) have been shown to attenuate seizure probability and damage caused by GD insult while also increasing seizure threshold. We have previously shown that pretreatment with 3α-diol prior to GD exposure, outperforms testosterone and testosterone with letrozole in those regards. In this study, the effects of 3α-diol on numerous pro- and anti-inflammatory markers in specific brain regions via fluorescent detection quantifiable multiplex speed assay were investigated to elucidate the possible mechanisms of 3α-diol neuroprotectivity. Brain regions protected by 3α-diol also have attenuated levels of well-known pro-inflammatory markers, such as interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNFα).

Disclosures: **K. Laitipaya:** None. **J.K. Chandler:** None. **T.M. Ferrara-Bowens:** None. **J.F. Irwin:** None. **D.D. Palmer:** None. **L.J. Shumway:** None. **E.A. Johnson:** None.

Poster

046. Modulating and Tracking Neuroinflammation

Location: Halls B-H

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Program#/Poster#: 46.02/O18

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant GM060665 (RISE Training Program at Hunter College from NIGMS to T. J.-L.)

NIMHD Grant MD007599

Graduate Center, City University of New York

Title: Increased amyloidogenic processing of APP potentially induced by the cyclooxygenase product of inflammation prostaglandin J2

Authors: *T. JEAN-LOUIS[1,2], P. ROCKWELL[1], M. FIGUEIREDO PEREIRA[1];
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Abstract: Senile plaques (SP) are pathological hallmarks of Alzheimer disease (AD). The main component of SPs is Aβ, generated via cleavage of the amyloid precursor protein (APP) by β- (BACE) and γ-secretases in the amyloidogenic pathway. Alternatively, APP processing by α- and γ-secretases in the non-amyloidogenic pathway prevents Aβ formation. The upstream events that regulate APP processing by the amyloidogenic and non-amyloidogenic pathways are poorly defined. We propose that inflammation contributes to the shift in balance towards APP amyloidogenic processing. Inflammation plays a major role in AD. Investigating how specific factors of inflammation mediate neurodegeneration in AD is crucial. Our studies focus on prostaglandin products of cyclooxygenases, which are key enzymes in inflammation and highly relevant to AD. In particular, we are investigating how the neurotoxic prostaglandin J2 (PGJ2) alters processing, trafficking, and post-translational modifications of APP. Neuronal APP occurs as mature forms exhibiting both *N*- and *O*-glycosylation, as well as an immature form displaying only *N*-glycosylation. Our previous studies with rat cerebral cortical neuronal cultures showed that PGJ2 induces a significant decrease in the *O*-glycosylated forms of APP. Our current data demonstrate that this decline in *O*-glycosylation is not due to PGJ2 stimulating APP cleavage by cellular proteases, such as proteasomes, lysosomal proteases, calpains and caspases, or by the α and γ secretases. Instead, PGJ2-treatment altered APP trafficking along the secretory pathway

and neuronal processes. Accordingly, PGJ2 induced a decline in APP levels at the ER and Golgi, as well as the accumulation of APP into large clusters at neuronal processes. Moreover, APP trafficking from the trans-Golgi network (TGN) to the cell surface is known to decline when its *O*-glycosylation is impaired. Deficits in APP *O*-glycosylation promote its trafficking to early endosomes, where APP is degraded by BACE and γ-secretase to produce Aβ and sAPP, which can then be secreted. These data together with our finding that PGJ2 alters APP *O*-glycosylation suggests that PGJ2 could promote the amyloidogenic processing of APP by re-directing its trafficking to early endosomes, where APP processing by BACE and γ-secretase would increase secreted Aβ levels. Since upregulation of cyclooxygenase-2 has emerged as an important determinant of the cytotoxicity associated with neuroinflammation, our studies address a mechanism possibly linking neuroinflammation with increased Aβ production in AD.

Disclosures: T. Jean-Louis: None. **P. Rockwell:** None. **M. Figueiredo Pereira:** None.

Poster

046. Modulating and Tracking Neuroinflammation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 46.03/P1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Tumor necrosis factor-α enhances vascular cell adhesion molecule-1 expression and promotes monocyte adhesion in human glioblastoma cells

Authors: *Y.-S. LIU[1], C. LIN[2], C.-F. TSAI[3], D.-Y. LU[4];
[1]Grad. Institue of Basic Med. Sci., China Med. Univ., Taichung, Taiwan; [2]Dept. of Physiology, Sch. of Medicine, China Med. Univ., Taichung, Taiwan; [3]Dept. of Biotechnology, Asia Univ., Taichung, Taiwan; [4]Dept. of pharmacology, Sch. of Medicine, China Med. Univ., Taichung, Taiwan

Abstract: Cytokines are abundantly in tumor microenvironment and involve in tumor progression. Vascular cell adhesion molecule-1 (VCAM-1) is a cytokine-induced adhesion molecule whose expression on the surface of cancer cell and interact with various immune cells. However, the role of VCAM-1 in monocyte/macrophage adhesion to human glioblastoma (GBM) is mostly unknown. Analysis of GSE4290 glioma patient dataset showed that VCAM-1 expression was correlated with clinicopathologic grade of gliomas. TNF-α induced VCAM-1 expression and thus promoted human monocyte adhesion to GBM. Knockdown of VCAM-1 abolished TNF-α-enhanced monocyte adhesion to GBM. In immunohistochemistry staining, the expression of VCAM-1 in glioma patients was correlated with tumor stage and higher than that in the normal brain tissue. The inductive effect of EGFR phosphorylation was observed time-

dependently after TNF-α stimulation. In addition, induction of TNF-α-enhanced monocyte adhesion to GBM was antagonized by treatment of EGFR inhibitor. TNF-α decreased miRNA-181a/b expression in GBM. Moreover, transfection with miRNA-181a/b mimics reversed the TNF-α-induced VCAM-1 expression. On the other hand, miRNA-181a/b reduced the PP2A phosphatase activity in GBM. TNF-α-induced VCAM-1 expression and monocyte adhesion were antagonized by using the PP2A pharmacological inhibitor okadaic acid (OA) or small interfering RNA (siRNA) against PP2A. Taken together, these results indicate that TNF-α-induced VCAM-1 expression through miRNA-181a/b, PP2A, and EGFR resulting in contributing to the human monocytes adhesion to GBM and promote GBM invasion. These results suggested that VCAM-1 is a potential target for improving GBM therapy.

Disclosures: **Y. Liu:** None. **C. Lin:** None. **C. Tsai:** None. **D. Lu:** None.

Poster

046. Modulating and Tracking Neuroinflammation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 46.04/P2

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: The brain penetrant phosphodiesterase-4 inhibitor, ABI-4, blocked endotoxin and age related pro-inflammatory effects in the plasma and CNS

Authors: *J. R. HEDDE;
Neurosci. & Pain Res. Unit, Pfizer Global Res., Cambridge, MA

Abstract: Inhibitors of phosphodiesterase-4 (PDE4) have been approved for the treatment of peripheral inflammatory disorders. There is a remarkably high incidence of co-morbid depression-like symptoms in patients suffering from disorders such as psoriasis which could reflect a central component of inflammation. Furthermore, symptoms of apathy and amotivation affect many healthy elderly people and are common in patients suffering from neurodegenerative disorders. In the current studies we have investigated the potential for the novel brain penetrant PDE4 inhibitor, ABI-4, to attenuate neuroinflammation and thus differentiate from the 2 approved PDE4 inhibitors which have poor CNS permeability. The anti-inflammatory effects of PDE4 were initially evaluated in vitro. Incubation of human primary blood monocytes (PBMCs) and primary murine microglial cells with lipopolysaccharide (LPS, 100 ng/ml) caused TNF-α release that was inhibited by co-incubation with PDE4 inhibitors (IC50: roflumilast 4.2 nM, rolipram 35.6 nM, ABI-4 14.7 nM). The effects of LPS (1 or 10 mg/kg i.p.) in CNS and periphery, and their modulation by PDE4 inhibition were also evaluated in vivo. LPS caused robust increases in plasma and brain TNF-α, IL-6 and IL-1β in mice. ABI-4 (0.1- 1 mg/kg s.c.)

reduced LPS-induced brain and plasma cytokines. Repeated administration of LPS (0.32 mg/kg i.p.) for 5 days resulted in microglial activation, assessed by ex vivo binding of a translocator protein (TSPO) ligand. This increase in TSPO binding in the brain was prevented by co-administration of ABI-4 (1 mg/kg s.c.). Central administration of LPS (2 ng, i.c.v.) stimulated cytokine levels in mouse brain and plasma 4 h later. Administration of ABI-4 attenuated the pro-inflammatory effects of LPS in the brain. The brain impaired PDE4 inhibitor, roflumilast, did not attenuate the CNS or systemic effects of centrally administered LPS. In naïve aged mice administration of ABI-4 (0.32 mg/kg, s.c. x 14 days) significantly decreased the plasma and brain levels of the proinflammatory cytokines TNF-α and IL-1β. These data indicate that the PDE4 inhibitor ABI-4 blocked the in vitro, and in vivo induction of proinflammatory cytokines by LPS, in brain and plasma. These data suggest that ABI-4 could represent a novel approach to achieving anti-inflammatory effects which could abrogate CNS consequences associated with inflammation.

Disclosures: **J.R. Hedde:** A. Employment/Salary (full or part-time): Pfizer Inc.

Poster

046. Modulating and Tracking Neuroinflammation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 46.05/P3

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Emergence of CNS biomarkers for lysosomal abnormalities in aging progranulin deficient mice

Authors: ***L. H. MARTENS**[1], F. ALBAYYA[1], J. H. SOPER[1], V. KING[1], D. E. SLEAT[2], P. LOBEL[2], M. TOWNSEND[1], L. LEVENTHAL[1], G. KOENIG[1], H. PATZKE[1];
[1]FORUM Pharmaceuticals, Waltham, MA; [2]Ctr. for Advanced Biotech. and Med., Piscataway, NJ

Abstract: Loss of function mutations in the progranulin (*GRN*) gene cause frontotemporal dementia (FTD). FTD is the second most common neurodegenerative disorder and is characterized by alterations in behavior and/or language. In patients, the disease is caused by haploinsufficiency of progranulin protein (PGRN) resulting in extensive neuronal loss, TDP-43 pathology, and gliosis. Patients homozygous for PGRN mutations develop a lysosomal storage disorder (LSD) at a young age that results in vision impairment and ataxia. Several mouse models of PGRN deficiency have been developed by knocking out the *Grn* gene. The haploinsufficent/heterozygous mice that mimic the decrease PGRN levels of patients appear relatively normal at the molecular and pathological level. The PGRN knockout mice consistently

exhibit a hyperactivation of the inflammatory response and lysosomal abnormalities, thereby closely mimicking the human LSD disease state. Here we report a chronology of molecular and pathologic phenotypes that emerge with age in PGRN deficient mice. As previously shown, accumulation of lipofuscin becomes apparent in discreet brain regions by 6.5 months in *Grn* knockout mice. This corresponds with dysregulation of expression of several lysosomal proteins in the brain including; v-ATPase, Cathepsin D, and other lysosomal proteins. However, lipid profiling of mouse brain revealed little significant change between genotypes. Mass spectrometry analysis of cerebrospinal fluid from PGRN deficient mice revealed alterations in the lysosomal protein, SPARCL1. PGRN has been shown to act as an immunomodulatory molecule and PGRN deficient mice have increased gliosis with age. Associated with increased gliosis, the inflammatory protein osteoactivin/GPNMB was found to be upregulated in the brain of aged animals. All of these abnormalities can be linked to lysosomal dysfunction and are characteristic of LSDs. These results provide a series of disease biomarkers that can be evaluated for reversal by restoring expression of progranulin.

Disclosures: **L.H. Martens:** None. **F. Albayya:** None. **J.H. Soper:** None. **V. King:** None. **D.E. Sleat:** None. **P. Lobel:** None. **M. Townsend:** None. **L. Leventhal:** None. **G. Koenig:** None. **H. Patzke:** None.

Poster

046. Modulating and Tracking Neuroinflammation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 46.06/P4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: DTRA

Title: The therapeutic role of recombinant IL-1 signaling inhibition on behavioral functioning of nerve agent soman-exposed mice in the shuttle box apparatus

Authors: *D. D. PALMER, J. K. CHANDLER, T. M. FERRARA-BOWENS, J. F. IRWIN, K. LATIPAYA, J. L. LANGSTON, T. M. MYERS, E. A. JOHNSON;
US Army Med. Res. Inst. of Chem. Def., Gunpowder, MD

Abstract: Exposure to soman (GD), a chemical warfare nerve agent that acts as a potent acetylcholinesterase inhibitor, causes status epilepticus (SE), leading to impaired behavioral functioning and widespread neuronal death throughout several regions of the brain. The activation of multiple neuro-inflammatory cytokines, including the pro-inflammatory cytokine IL-1, initiates a detrimental neurodegenerative cascade exacerbating neuropathology. While most

treatments following GD exposure serve to ameliorate SE, currently no approved treatment counters the neurodegenerative process. Previous research has identified the pro-inflammatory cytokine interleukin-1 (IL-1) as a key component of the neurodegeneration and inflammation that occurs after exposure to GD. Therefore, inhibition of IL-1 may be therapeutically beneficial to preserve neuronal tissue and improve behavioral functioning after exposure to GD. Kineret (anakinra), a recombinant form of the natural IL-1R inhibitor IL-1Ra, was used in this study as a possible neuroprotective treatment for GD exposure. WT mice were treated with HI-6 dichloride 5 minutes prior to a 1.6 LD_{50} subcutaneous administration of GD. Treatment via repeated subcutaneous administration of anakinra began one hour after seizure onset and concluded 12 hours later. One week after exposure, behavioral experimentation was conducted using the shuttle box apparatus to evaluate learned avoidance behavior, anxiety levels, mobility and overall cognitive functioning. Additionally, neuropathology was evaluated. This study investigated the overall therapeutic role of recombinant IL-1 inhibition in reversing behavioral deficits and improving pathology after nerve agent exposure. The views expressed in this talk are those of the author(s) and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. These studies were funded by the Defense Threat Reduction Agency (DTRA).

Disclosures: **D.D. Palmer:** None. **J.K. Chandler:** None. **T.M. Ferrara-Bowens:** None. **J.F. Irwin:** None. **K. Latipaya:** None. **J.L. Langston:** None. **T.M. Myers:** None. **E.A. Johnson:** None.

Poster

046. Modulating and Tracking Neuroinflammation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 46.07/P5

Topic: C.03. Parkinson's Disease

Support: NIH CTSC Grant UL1-RR024996 (pilot award to JB & MEFP)

NIH Grant GM060665 (RISE Training Program at Hunter College from NIGMS to MNS

NIMHD Grant D007599 to Hunter College

NSF Grant 0965983 (IGERT Training Program at Hunter College to CC)

The Graduate Center, City University of New York

Title: Rat model of neuroinflammation exhibiting parkinsonian-like pathology induced by prostaglandin J2 a product of the cyclooxygenase pathway

Authors: C. CORWIN[1,3], *M. NUNEZ SANTOS[2], A. NIKOLOPOULOU[4], Y. KANG[4], S. VALLABHAJOSULA[4], P. SERRANO[1], J. BABICH[4], M. FIGUEIREDO PEREIRA[1]; [2]Biol. Sci., [1]Hunter Col., New York, NY; [3]The Grad. Center, CUNY, New York, NY; [4]Dept. of Radiology, Weill Cornell Med., New York, NY

Abstract: Upregulation of cyclooxygenase-2 has emerged as an important determinant of the cytotoxicity associated with neuroinflammation in Parkinson's Disease (PD). Moreover, epidemiological studies strongly support that chronic treatment with low levels of cyclooxygenase inhibitors (i.e., NSAIDs), lowers the risk for PD. Thus, NSAIDs are so far the only approved clinical drugs that prevent or delay the onset of PD. Prostaglandins (PGs) are major products of cyclooxygenases, but their role in neurodegeneration is poorly understood. PGD2 is the most abundant prostaglandin in the brain, increases the most under pathological conditions, and leads to the highly neurotoxic prostaglandin J2 (PGJ2) by spontaneous dehydration. To study the *in vivo* effects of PGJ2 we established a rat model of neuroinflammation. PGJ2 (or vehicle) was unilaterally injected into the right *substantia nigra* (SN) of adult Sprague Dawley male rats for two and four weeks (once per week). The rats were analyzed for motor deficits (cylinder test and open field), dopaminergic neuronal loss (tyrosine hydroxylase immunostaining quantified by stereology), and microglia activation (Iba1 immunostaining and μPET/CT imaging with the TSPO radioligand [^{11}C]PK11195). The rats that received four PGJ2-injections were analyzed four weeks after the last injection. Compared to vehicle controls, the PGJ2-treated rats exhibited significant motor deficits concomitant with dopaminergic neuronal loss in the impaired SN. The rats that received two PGJ2 injections were analyzed four and eight weeks after the last injection to determine the progression of neuronal damage. Compared to vehicle controls, these rats showed increased microglial activation (higher [^{11}C]PK11195 uptake) accompanied by significant loss of dopaminergic neurons in the impaired SN, as well as motor deficits. To determine the efficacy of NSAID therapeutic intervention in our model, a group of rats that received two PGJ2 injections were given 40mg/kg body weight/day of ibuprofen in their food, from the day after the first surgery until 4 weeks post-injection. The ibuprofen treatment appears to prevent the motor impairment developed by the PGJ2-treated rats. In conclusion, our newly developed PGJ2-induced rat model of neuroinflammation exhibits parkinsonian-like pathology correlating with in vivo PET imaging of neuroinflammation. We propose that this pre-clinical rat model is highly valuable to identify and optimize therapeutics that suppress the neurotoxic effects of inflammation as a strategy to prevent or delay the progression of PD. This model can also aid in testing novel PET ligands of neuroinflammation.

Disclosures: **C. Corwin:** None. **M. Nunez Santos:** None. **A. Nikolopoulou:** None. **Y. Kang:** None. **S. Vallabhajosula:** None. **P. Serrano:** None. **J. Babich:** None. **M. Figueiredo Pereira:** None.

Poster

046. Modulating and Tracking Neuroinflammation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 46.08/P6

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: DTRA

Title: Inhibition of IL-1 signaling provides neuroprotection following seizurogenic exposure to the nerve agent soman (GD) in mice.

Authors: *J. IRWIN[1], T. M. FERRARA-BOWENS[2], J. K. CHANDLER[2], D. D. PALMER[2], K. LAITIPAYA[2], M. WEGNER[3], E. A. JOHNSON[2];
[2]Res. Div., [3]Res. Support Div., [1]USAMRICD, Gunpowder, MD

Abstract: Exposure to organophosphates, such as soman (GD), causes cholinergic crisis from the accumulation of acetylcholine (ACh) at the synapse. Without intervention, GD rapidly and irreversibly binds to acetylcholinesterase (AChE) and will result in status epilepticus (SE), which can lead to severe neuropathology. Interleukin 1 (IL-1) signaling has a known proinflammatory role in the CNS and is involved in seizure development and maintenance. IL-1 signaling upregulates multiple proinflammatory cytokines and is naturally attenuated by the IL-1 antagonist IL-1Ra. A GD model was developed using wild type and IL-1 signaling knockout mouse strains (i.e., IL-1R1 and IL-1Ra) to validate the role of IL-1 signaling in brain damage after exposure to a seizurogenic dose of GD. In addition, the anti-IL-1 signaling drug anakinra was used as a treatment in wild type mice. Molecular and histological approaches were used to investigate the role of IL-1 in seizure propagation, neuroinflammation, and neuropathology. Multiplex bead assays allowed for the quantification of inflammatory markers and furthermore for the examination of the relationship between IL-1 signaling and the propagation of the neuroinflammatory cascade. H&E staining of the brains and physiological measurements obtained during the exposure allowed for evaluation of neuropathology, sickness, and the rates of convulsion and mortality. Neuroprotection was observed in strains with impaired IL-1 signaling, while a significant increase in multiple proinflammatory markers and neuropathology was observed in strains with enhanced IL-1 signaling. These results show that IL-1 signaling plays an acute role the progression of neuropathology and physiological outcomes and that regulation of this pathway may be beneficial in recovery after GD exposure.

Disclosures: J. Irwin: None. **T.M. Ferrara-Bowens:** None. **J.K. Chandler:** None. **D.D. Palmer:** None. **K. Laitipaya:** None. **M. Wegner:** None. **E.A. Johnson:** None.

Poster

046. Modulating and Tracking Neuroinflammation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 46.09/P7

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: 5T32NS045540-12

Title: Suppression of seizure-induced glial activation in the hippocampus using a novel N-acylethanolamine acid amidase inhibitor

Authors: *C. B. MERRILL[1], T. BANDIERA[2], F. BERTOZZI[2], D. PIOMELLI[3];
[1]Anat. and Neurobio., Univ. of California Irvine Dept. of Anat. and Neurobio., Irvine, CA; [2]Drug Discovery and Develop., Inst. Italiano di Tecnologia, Genoa, Italy; [3]Univ. of California-Irvine, Irvine, CA

Abstract: Palmitoylethanolamide (PEA) is one of several biologically active fatty acid ethanolamides in the brain. These molecules, which include the endocannabinoid anandamide, have demonstrated marked anti-inflammatory activity in animal models of neurological diseases such as Alzheimer's, multiple sclerosis, and epilepsy (Centonze D, et al., Trends Pharmacol Sci. 2007 Apr; 28(4): 180-7). PEA activates the nuclear receptor PPARα, which modulates in turn the activity of pro-inflammatory regulators such as NF-κB (D'Agostino G, et al., Eur J Pharmacol. 2009 Jun 24; 613(1-3): 54-9). Therefore, enhancement of PEA levels has recently been proposed as a potential anti-inflammatory target, due to its ability to modulate pro-inflammatory cascades in the brain. We have recently developed a novel inhibitor directed toward *N*-acylethanolamine acid amidase (NAAA), the lysosomal cysteine hydrolase that degrades PEA. The compound, called ARN16186, inhibits human NAAA with an IC_{50} of 83 nM and has adequate pharmacokinetic properties and brain penetration in mice.
In the present study, we used kainic acid-induced seizures to initiate pro-inflammatory cascades in the brain and tested the ability of ARN16186 to prevent glial activation and neuroinflammation. The NAAA inhibitor was administered by intraperitoneal administration (10 mg/kg) prior to kainic acid injection and twice daily for the four following days. We measured fatty acid ethanolamide levels by liquid chromatography/mass spectrometry, and examined astrocyte and microglial activation by immunostaining GFAP and IBA_1, respectively, within the hippocampus, a focus of several neurological diseases. We found that blocking NAAA activity decreases glial activation within the hippocampus four days after i.p. injection of kainic acid. These results suggest that PEA plays a significant anti-inflammatory role within the central nervous system and that enhancing PEA levels through NAAA blockade may represent a potential therapy to ameliorate neuroinflammation, which may exacerbate progression of neurological disease.

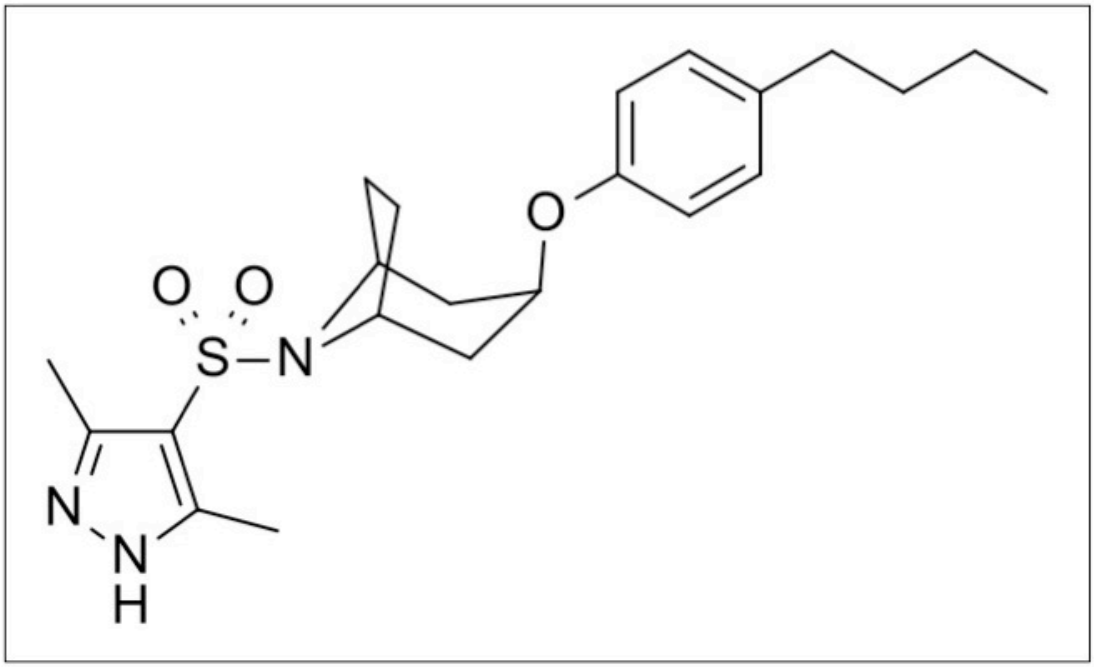

Fig. 1: Chemical structure of ARN16186

Disclosures: **C.B. Merrill:** None. **T. Bandiera:** None. **F. Bertozzi:** None. **D. Piomelli:** None.

Poster

046. Modulating and Tracking Neuroinflammation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 46.10/P8

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CIHR grant

Title: Interactions of peripherally derived macrophages and microglia after spinal cord injury

Authors: *A. D. GREENHALGH[1], S. DAVID[2];
[1]McGill Univ., Montreal, QC, Canada; [2]Res. Inst. of McGill Univ. Hlth. Ctr., Montreal, QC, Canada

Abstract: Microglia and infiltrating monocyte derived macrophages (MDMs) play pivotal roles in inflammation during CNS injury and disease. Microglia and MDMs are distinct, based on their ontogeny and transcriptional profiles; however, it is not known whether these two cell types influence each other's functions in areas of CNS damage. We have previously reported that after traumatic spinal cord injury, phagocytosis of tissue debris by microglia is reduced after macrophage infiltration. Therefore, we hypothesised that MDMs enter the injured CNS and signal to microglial cells to stop phagocytosis. To test this, we cultured adult primary microglia under conditions in which they retain a similar transcriptional profile to freshly isolated adult microglia, together with bone marrow derived macrophages, in a tiered Banker system. We assessed the expression of several microglial 'signature' genes and inflammatory cytokines with RT-qPCR. Under inflammatory conditions (LPS), the presence of macrophages did not affect the expression of a panel of microglial signature genes in microglia. However, the presence of macrophages significantly suppressed the pro-inflammatory cytokine, TNF, in microglia, while the presence of microglia significantly increased the pro-inflammatory cytokine, interleukin-1β, in macrophages. We also assessed the ability of microglia and macrophages to influence each other to phagocytose myelin using pHrodo-labelled myelin. Flow cytometry revealed that macrophages suppressed microglial phagocytosis of myelin while the presence of microglia increased macrophage phagocytosis of myelin, as compared to cells in isolation. These studies reveal that microglia and macrophages differentially affect each others' inflammatory cytokine expression and phagocytic function. Studies are now underway to identify the soluble mediators responsible for these interactions and assess these mechanisms in *in vivo* models of CNS injury.

Disclosures: A.D. Greenhalgh: None. **S. David:** None.

Poster

046. Modulating and Tracking Neuroinflammation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 46.11/P9

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: DTRA

Title: Gene expression profiling of the interlukin-1 inflammatory pathway in knockout mice following soman exposure

Authors: *J. K. CHANDLER, T. M. FERRARA-BOWENS, J. F. IRWIN, K. LAITIPAYA, D. D. PALMER, H. M. HOARD-FRUCHEY, E. A. JOHNSON;
US Army Med. Res. Inst. of Chem. Def, Aberdeen Proving Ground, MD

Abstract: Soman, an organophosphorus (OP) compound, is a potent acetylcholinesterase inhibitor. The accumulation of acetylcholinesterase leads to convulsions, prolonged seizures, and neuropathology which can result in death. Previous studies have shown that changes in inflammatory signaling pathways, such as the IL-1 pathway, exacerbate neuronal damage and elicit long term cognitive deficits after OP poisoning. Using microarray, we analyzed gene expression changes across multiple mouse strains to further understand the pathways involved in neuroinflammation and their relationship to OP poisoning. Piriform cortex samples were collected at 1, 3, 6, 12, 24, and 48 hours following soman exposure in IL-1R1 knockout mice, IL-1Ra knockout mice, and background matched wild-type mice (C57BL/6J). Analysis of the individual gene expression profiles of each strain identified similar changes in canonical pathways and gene networks across all three mouse strains. Further investigation into these pathways will help identify potential therapeutic targets for effective mitigation of OP poisoning effects.

Disclosures: **J.K. Chandler:** None. **T.M. Ferrara-Bowens:** None. **J.F. Irwin:** None. **K. Laitipaya:** None. **D.D. Palmer:** None. **H.M. Hoard-Fruchey:** None. **E.A. Johnson:** None.

Poster

046. Modulating and Tracking Neuroinflammation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 46.12/P10

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Consortium for Frontotemporal Dementia Research

Title: Is progranulin deficiency in microglia alone sufficient to cause microglia activation and neurodegeneration?

Authors: *M. CAHILL[1], J. ZHANG[1], H. LUI[1,2], E. J. HUANG[1];
[1]Pathology, Univ. of California San Francisco, San Francisco, CA; [2]Sch. of Publ. Hlth., Univ. of California, Berkeley, Berkeley, CA

Abstract: Global progranulin (PGRN [protein], *Grn* [gene]) deficiency leads to an age-dependent activation of microglia, which promotes complement-mediated synaptic pruning that acts as a major driver of neurodegeneration in frontotemporal dementia. Given the abundant PGRN expression in microglia, these results lead to the hypothesis that PGRN deficiency in microglia alone should be sufficient to cause aberrant microglia activation and consequently neurodegeneration. To test this hypothesis, we generated the *Cx3cr1-Cre;Grn*$^{fl/fl}$ mice, in which *Grn* is selectively deleted in microglia. Surprisingly, unlike *Grn*$^{-/-}$ mice, *Cx3cr1-Cre;Grn*$^{fl/fl}$ mice

only show very modest age-dependent microglial infiltration in the ventral thalamus and no detectable increase of astrogliosis or loss of synapses at 12-month-old. Consistent with these results, *Cx3cr1-Cre;Grn*$^{fl/fl}$ mice also do not exhibit OCD-like excessive grooming behavior. These results indicate that loss of PGRN in microglia alone is not sufficient to recapitulate the global loss of PGRN phenotypes and further suggest that cross talk between microglia, astrocytes and neurons is needed in order to fully activate *Grn* deficient microglia. In support of this idea, *CaMKII-Cre;Grn*$^{fl/fl}$ mice also show no evidence of microglial infiltration, astrogliosis, excessive synaptic pruning or OCD-like grooming behaviors during the aging process, further supporting that loss of PGRN in microglia, astrocytes and neurons is required to fully recapitulate global PGRN deficiency phenotype. These unexpected findings represent an important conceptual advance that a homeostatic loop exists between neurons and glia and that dysfunction of this loop only occurs when PGRN is absent from both neurons and glia. We are in the process of developing microglia-astrocyte-neuron co-cultures to further test this hypothesis.

Disclosures: M. Cahill: None. **J. Zhang:** None. **H. Lui:** None. **E.J. Huang:** None.

Poster

046. Modulating and Tracking Neuroinflammation

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Program#/Poster#: 46.13/P11

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01AG041944

Title: An immunohistochemical analysis of inflammation-driven changes in synaptic plasticity: the combined effects of aging and an immune challenge

Authors: V. G. ROMETT, C. W. MOODY, N. TANAKA, L. R. PEACH, *S. L. PATTERSON;
Biol., Temple Univ., Philadelphia, PA

Abstract: Mounting evidence suggests that the peripheral immune system not only has extensive communication with the central nervous system (CNS), but also has a significant effect on behavior and cognition. Interestingly, cognitive vulnerabilities to immune challenges (e.g. injury, illness, surgery) seem to increase with age. Even cognitively sound elderly individuals who experience a significant immune challenge often experience an abrupt cognitive dysfunction, termed delirium, which is associated with increased probabilities of eventually developing dementia, even when the delirium is transient. In rodent models of this phenomenon, it has been shown that a peripheral immune challenge produces a more robust and longer lasting response in

the CNS of older rats. This exaggerated CNS immune response correlates strongly with deficits in hippocampal-dependent learning and memory. Investigations by our lab have found that immune challenged older rats have deficits in Brain-derived neurotrophic factor (BDNF) signaling, with less mature BDNF in hippocampal synapses; they also have deficits in hippocampal late-phase long-term potentiation (L-LTP). As L-LTP requires intricately orchestrated reciprocal communication between neuronal processes and the nucleus, an understanding of where and how this dialogue is disrupted is integral for understanding the detrimental effects of dysregulated immune responses on memory. To investigate these questions, immunohistochemistry was used to look at the association between inflammatory markers, such as activated microglia and complement system components, and the distribution of molecules essential for intracellular communication and synaptic remodeling, such as immediate-early gene products and proteins involved in signal transduction. By looking at different age groups of rats at different time points of immune response (following *E. coli* injection) and the responses of these groups to pharmacological LTP stimulation, we have disentangled some of the complex effects of ageing and neuroinflammation on synaptic plasticity. This study may provide important insights for understanding the genesis of early failures of synaptic plasticity in neuroinflammatory conditions such as Alzheimer's disease (AD), multiple sclerosis (MS), and traumatic brain injuries (TBIs), and may aid the development of novel treatment options.

Disclosures: V.G. Romett: None. **C.W. Moody:** None. **N. Tanaka:** None. **L.R. Peach:** None. **S.L. Patterson:** None.

Poster

046. Modulating and Tracking Neuroinflammation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 46.14/P12

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant DA07304

Title: Inhibition of monoacylglycerol lipase prevents HIV gp120-induced synapse loss by decreasing prostaglandin production

Authors: *X. ZHANG[1], S. THAYER[2];
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Abstract: About half of HIV infected patients develop HIV-associated neurocognitive disorder (HAND). Cognitive decline in HAND patients correlates with deficits in synaptic structure and

function. Previous studies from our lab showed that treating rat hippocampal cultures with 600 pM HIV envelope protein gp120, a potent neurotoxin, induced synapse loss through an interleukin-1β (IL-1β) mediated neuroinflammtory pathway. Activation of cannabinoid type 2 (CB2) receptors on microglia prevented gp120-induced synapse loss by blocking IL-1β release. Monoacylglycerol lipase (MAGL) degrades the endocannabinoid 2 arachidonoylglycerol (2-AG) to arachidonic acid. Inhibition of MAGL with the selective inhibitor, JZL 184, increases 2-AG levels and decreases arachidonic acid, the precursor for brain prostaglandins. Thus, MAGL inhibition might reduce neuroinflammation and prevent synapse loss induced by gp120. Here, we tested the effect of MAGL inhibition on gp120-induced synaptic changes using confocal imaging of an enhanced green fluorescent protein fused to an intrabody that binds to endogenous postsynaptic density protein 95. Treatment with JZL 184 completely blocked synapse loss induced by gp120. Synapse loss results from the potentiation of N-methyl-D-aspartate (NMDA) receptors. The potentiation of NMDA-evoked Ca^{2+} influx induced by gp120 was also blocked by JZL 184. Quantitative real-time PCR measurement of IL-1β mRNA indicated that JZL 184 blocked gp120 triggered production of IL-1β. The CB2 receptor antagonist, AM630, failed to attenuate the inhibition of IL-1β production caused by JZL 184, suggesting that elevated 2-AG levels were not activating CB2 receptors on microglia. In contrast, dimethyl-prostaglandin E2 (dmPGE2), an analog of prostaglandin E2 increased gp120-induced IL-1β production in the presence of JZL 184. These data suggest that the protective effect of JZL 184 is likely through decreased prostaglandin production. MAGL is a promising therapeutic target for neuroinflammatory conditions such as HAND. While modulation of endocannabinoid and prostaglandin levels could both potentially contribute to the neuroprotective effect of JZL 184, in this in vitro model of gp120 evoked synaptic injury, reduction of prostaglandins is the predominant effect.

Disclosures: X. Zhang: None. **S. thayer:** None.

Poster

046. Modulating and Tracking Neuroinflammation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 46.15/Q1

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Department of Defense Grant ID # W81XWH-11-1-0816

Title: Immune sensitization in sequential stem cell transplantation in the spinal cord

Authors: *M. S. TORA[1,3], J. J. LAMANNA[1], J. GUTIERREZ[1], L. N. URQUIA[2], C. MORETON[1], J. L. WAGNER[1], T. FEDERICI[1], N. M. BOULIS[1];

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Abstract: Stem cell transplantation is under investigation in both animal models and clinical trials for the treatment of spinal cord disease such as Amyotrophic Lateral Sclerosis (ALS). However, there is a poor understanding of the immunological barriers to successful stem cell engraftment. Importantly, it is not known if multiple transplants over time will sensitize the immune system and impair stem cell survival. In a Phase I/II clinical trial conducted by our group, ALS patients received stem cell transplantation into the lumbar spine and later received transplantation to the cervical spine. The effect of multiple transplantations on cell survival is unknown as there is currently no method to quantify stem cell graft survival in these patients. We hypothesized that the initial stem cell transplantation would sensitize the immune response and lead to decreased stem cell survival in subsequent transplantation.

To assess this in an animal model, female Göttingen minipigs (n=5/group) first received ten thoracolumbar spinal cord injections of either porcine neural progenitor cells (pNPC) or vehicle (Sham). Three weeks after initial transplantation, both groups received pNPC transplants into the cervical spine. Three weeks after the second transplantation, the pigs were sacrificed and the spinal cords were sectioned and stained for pNPCs, Iba-1 (activated microglia), CD8, and CD4. The pigs were maintained on IV tacrolimus (0.005 mg/kg/day) for the duration of the study. pNPC Grafts were quantified for cell survival and microglial activation, summarized in Table 1. Interestingly, there was no statistically significant difference in survival between the sensitized group (Graft + Graft) and non-sensitized group (Sham + Graft). However, there is a statistically significant increase in Iba-1 staining in the sensitized group compared to the non-sensitized ($p < .05$), indicating increased microglial activation with previous cell transplantation. Quantifications of CD8+ and CD4+ infiltrating cells are ongoing. These data suggest that, while there does not seem to be a change in graft survival at three weeks, there may be an increased immune response which may impact long term cell graft survival and therefore therapeutic efficacy.

Table 1. Summary of Stem Cell Graft Survival and Microglial Response

Group	% Graft Survival		Iba-1 Staining [a]	
	Mean (STDEV)	Range	Mean (STDEV)	Range
Sham	0 (±0)	0	6.98 (±3.82)	2.67-10.93
Sham + Graft	1.22 (±0.52)	0.03-3.37	7.16 (±8.18)	0.64-20.59
Graft	1.04 (±0.68)	0.06-2.63	7.95 (±4.14)	1.88-12.60
Graft + Graft	1.54 (±0.49)	0.13-5.19	21.47 (±11.91)	8.78-40.87

a – Units in ten-thousands

Disclosures: **M.S. Tora:** None. **J.J. Lamanna:** None. **J. Gutierrez:** None. **L.N. Urquia:** None. **C. Moreton:** None. **J.L. Wagner:** None. **T. Federici:** None. **N.M. Boulis:** None.

Poster

046. Modulating and Tracking Neuroinflammation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 46.16/Q2

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: Peter Deane Trust

Title: APOBEC1-mediated RNA-editing of LAMP2 in microglia is linked to lysosomal accumulation in hippocampus and impaired cognitive decline in middle-aged Apobec1$^{-/-}$ mouse

Authors: K. GAGNIDZE[1], D. COLE[1], K. HAJDAROVIC[1], F. N. PAPAVASILIOU[3], T. A. MILNER[4], V. RAYON-ESTRADA[5], *K. BULLOCH[2];
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Abstract: Microglia (MG), the resident immune macrophages in the CNS, play important roles in brain homeostasis and neuronal plasticity. Under steady state conditions, MG aid synaptic stripping and clearing of cellular debris, while supporting myelination, and promote growth, survival and maintenance of neurons. MG also serve as immune sentinels that quickly become activated during brain pathogenic or traumatic insult, produce pro-inflammatory cytokines and recruit circulating monocytes(dendritic cells and macrophage) to the site of damage. Here we report that mice lacking the RNA editing enzyme, APOBEC1, in MG have marked pathophysiology. This is characterized in the male middle-aged (9-12 mo) APOBE1$^{-/-}$ mouse at the organismal level by aberrant behaviors and at the light and ultrastructural level by clustering of activated MG, increased inflammation, demyelination and accumulation of lysosomes noted within hippocampal neurons and microglia. Conversely, we have demonstrated in the wildtype mouse brain that APOBEC1-mediated RNA editing occurs within microglia, in analogy with editing functions for APOBEC1 in bone marrow derived macrophages. To determine the impact of RNA editing may have on lysosomal function within MG we isolated two subsets defined as CD45^{int}CD11b^{hi}CD11c^{neg} (*CD11c^{neg} MG)* and CD45^{int}CD11b^{hi}CD11c^{+} (*CD11c^{+} MG)*, from wildtype and knockout middle aged mouse brains and performed RNAseq followed by an analysis of RNA editing events. These data show that the lysosomal-associated membrane protein (L*AMP2)* is edited in wildtype microglia, and the loss of its editing reduces the abundance of this protein. Thus, loss of *LAMP2* editing in the APOBEC1$^{-/-}$ mouse brain may be

related to the accumulation of lysosomes and autophagic vesicles, as the lack of LAMP2 results in similar pathology. Since this neuropathology phenotype is common to lysosomal storage disease and age-related neurodegeneration these findings may open up new avenues of therapeutic strategies to address both heritable neurological diseases and those of previously unknown etiology.

Disclosures: K. Gagnidze: None. **D. Cole:** None. **K. Hajdarovic:** None. **F.N. Papavasiliou:** None. **T.A. Milner:** None. **V. Rayon-Estrada:** None. **K. Bulloch:** None.

Poster

046. Modulating and Tracking Neuroinflammation

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 46.17/Q3

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CDMRP Grant W81XWH-09-2-0098

Title: A mouse model of Gulf War Illness reveals a primed neuroinflammatory response to subsequent systemic inflammatory challenge.

Authors: *K. A. KELLY[1], A. R. LOCKER[1], L. T. MICHALOVICZ[1], J. A. VRANA[1], S. VASHISHTHA[2], G. BRODERICK[2,3,4], D. B. MILLER[1], J. P. O'CALLAGHAN[1]; [1]CDC-NIOSH, Morgantown, WV; [2]Univ. of Alberta, Edmonton, AB, Canada; [3]Dept. of Med., Univ. of Miami, Miami, FL; [4]Inst. for Neuro Immune Med., NOVA Southeastern Univ., Fort Lauderdale, FL

Abstract: Gulf War Illness (GWI) is a multi-symptom disorder diagnosed by a phenotype which includes persistent headaches, chronic fatigue, memory loss, confusion, skin and gastrointestinal problems. These features are characteristic of persistent sickness behavior, which is known to result from underlying neuroinflammation. Chronic exposure to corticosterone (CORT), at levels associated with high physiological stress, can prime the CNS to mount an exacerbated neuroinflammatory response, indicated by an increase in proinflammatory cytokines/chemokines, following systemic exposure to neurotoxicants and inflammagens. By mimicking the stresses of war with exogenous CORT (200 mg/L 0.6% EtOH in drinking water) for 7 days prior to exposure to sarin surrogate acetylcholinesterase inhibitor (AChEI), diisopropyl fluorophosphate (DFP; 4 mg/kg, i.p.), heightened neuroinflammatory responses were observed without astrogliosis or neurodegeneration from 6 to 72 hours after DFP exposure. While these observations recapitulated early symptoms of GWI, the essential pathobiology of this illness is persistence of heightened responses to external stimuli for 20+ years. Here, we

employed episodic exposure to CORT in drinking water of male C57Bl6/J mice for up to 90 days (CORT drinking water for 4 or 7 days every other week) to emulate episodic stress incurred by ill veterans following their exposures to multiple AChEI agents in theater. Since the GWI phenotype is punctuated by symptom flare-ups, systemic exposure to lipopolysaccharide (LPS - a bacterial mimic), at sub- and neuroinflammatory doses (0.5 or 2 mg/kg, s.c., respectively), was used to challenge the GWI phenotype. While CORT pretreatment primes the neuroinflammatory response to produce augmented LPS-induced inflammation, a single dose of DFP significantly exacerbated this effect. Using a comprehensive survey of molecular markers, blood cytokine expression profiles of ill veterans compared to our GWI mouse model revealed a significant overlap of the phenotype between man and mouse. This correlation supports the hypothesis that exposure to sarin, or a similar AChEI, in theater served as a major precipitating factor for the development of GWI. Overall, we have demonstrated that a paradigm of CORT in the drinking water with a single DFP exposure followed by episodic CORT instigates a primed neuroinflammatory phenotype similar to that of Gulf War Illness. An LPS challenge in our model results in an exacerbated inflammatory response, recapitulating the flare-up of symptoms seen in affected patients. Together, these data suggest that GWI is a chronic, stressor primed, neuroinflammatory condition.

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Poster

046. Modulating and Tracking Neuroinflammation

Location: Halls B-H

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Program#/Poster#: 46.18/Q4

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH K08NS052550

NIH R01 NS072446

Title: Complement activation due to HexB deficient microglia targets neurons: implications for Sandhoff and Tay-Sachs disease

Authors: *N. SASIDHARAN, B. JUN, Y. GONG, F. EICHLER;
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Abstract: G_{M2} gangliosidoses (Tay-Sachs Disease, TSD; Sandhoff Disease, SD) are caused by an inherited deficiency of the lysosomal enzyme β-hexosaminidase. In humans, mutations in the

α-subunit (HexA) and the β-subunit (HexB) lead to TSD and SD respectively. Microglial cells have been shown to express high levels of both HexA and HexB, with expression of HexB to be 6 fold higher than HexA. Despite marked microglial activation in mouse models and human patients, the role of these resident macrophages during neurodegeneration remains poorly understood. The complement immune response, largely elicited by glial cells in the CNS, has been identified as putative contributor of neuronal loss. To determine the role of the complement system in G_{M2} gangliosidoses, we examined a $HexB^{-/-}$ mouse model, human post-mortem SD brain tissue and a CRISPR/CAS9 edited HexB (-/-) microglial cell line. In $HexB^{-/-}$ mouse brain, mRNA expression of complement factors—C1q, C3a, and C5a—was significantly increased. Primary cells isolated from these mice, revealed that microglia were a major source of those complement factors, which were pathologically upregulated in primary $HexB^{-/-}$ microglia. Furthermore, C1q expression was localized to apoptotic and G_{M2}-bearing neurons in the $HexB^{-/-}$ mouse suggesting that neurons are the primary target of complement activation. Examination of post-mortem SD and TSD patient brain tissue confirmed elevated expression of C1q in human cells. Analysis of CRISPR/CAS9 edited HexB (-/-) cell lines, showed changes in microglial morphology suggesting a primed state. These cells displayed an increase in the expression of C3 receptor, but little or no change in inflammatory and other complement markers. Interestingly, co-culture experiments of CRISPR/CAS9 edited HexB (-/-) microglia with differentiated neurons reveal a significant increase in inflammatory marker IBA1. Furthermore, GM2 staining in CRISPR/CAS9 edited HexB (-/-) microglia revealed no changes in ganglioside levels *in vitro*. Taken together these results suggest that complement upregulation is a cell non-autonomous response that only occurs *in viv*o due to contact with other cell types in the brain. This study implies a novel role of complement activation during microglia-neuron interactions in the pathology of G_{M2} gangliosidoses.

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Poster

046. Modulating and Tracking Neuroinflammation

Location: Halls B-H

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

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Title: Neuroinflammation in vervet brains following chronic dietary exposures to a cyanobacterial toxin

Authors: *D. A. DAVIS[1], A. DELLE DONNE[2], S. A. BANACK[3], R. PALMOUR[4], W. G. BRADLEY[2], P. A. COX[3], D. C. MASH[2];
[1]Dept. of Neurol., [2]Neurol., Univ. of Miami, Miami, FL; [3]Neurochemistry, Inst. of Ethnomedicine, Jackson Hole, WY; [4]McGill Univ., Montreal, QC, Canada

Abstract: Alzheimer's disease and amyotrophic lateral sclerosis (ALS) are characterized by the appearance of reactive microglial and astroglial cells. Astrocytes and microglial are highly reactive to environmental insults. We have recently reported that dietary exposure to beta-methylamino-alanine (L-BMAA), produced by cyanobacterial blooms, triggers AT8-positive neurofibrillary tangles (NFT) and sparse β-amyloid inclusions in vervet brain (Cox et al., 2016). To investigate the effects of BMAA on microglia and astrocytes, we examined the brain and spinal cord from vervet monkeys fed oral doses of L-BMAA (210 mg/kg/day for 140 days; N=12). Formalin fixed brain sections were stained with antibodies to glial fibrillary acidic protein (GFAP) and microglial/macrophage membrane protein (Iba1) (Neuroscience Associates, Knoxville, TN). The distribution of immunostained microglia and astrocytes was mapped using a Huron Digital Pathology Scanner and nonbiased image analysis software (ImageJ; NIH). Chronic dietary exposure to L-BMAA induced AT8-immunopositive glial lesions similar to those reported in the brain of Guamanian ALS PDC patients, including plaques, tufts, granular hazy astrocytic inclusions, coiled bodies and crescent inclusions. GFAP-positive astrocytes were observed in the amygdala, entorhinal, insular and motor cortices where AT8-positive NFTs were abundant. BMAA is known to target motor neurons and GFAP reactivity was robust in primary motor cortex and in the vicinity of the anterior horn cells of the lumbar spinal cord. Activated microglia were observed within the midbrain and brainstem at the level of corticospinal tract. These observations suggest that BMAA causes early microglia activation in upper motor neurons in the absence of cell loss. The neuroinflammatory reaction may represent an early stage of BMAA pathology in vervets following chronic dietary exposure.

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Poster

046. Modulating and Tracking Neuroinflammation

Location: Halls B-H

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Program#/Poster#: 46.20/Q6

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Type I interferon response in alzheimer's disease pathology

Authors: A. CARRANO[1], C. VERBEECK[2], *P. DAS[1];
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Abstract: Type I interferon (IFN-I) responses are an early immune-mediated defense mechanism against microbes. Although the IFN-I associated anti-microbial activities are well characterized, the IFN-I responses in the aging brain and in correlation with neurodegeneration is not well still not elucidated. Recent studies have shown that Type I interferon responses are up-regulated in the aged brain and in the Alzheimer disease (AD) brains, although the consequence of this up-regulation is not clear. In this study we have tested the effects of Abeta and tau on the activation of IFN-I responses in neuronal and microglial cell lines in vitro. In response to pathological Aβ and tau, we observed activation of the IFN-1 signaling pathways in both cell lines, as measured by an activation of transcription factor IRF7. In addition, activation of the IFN-I pathway affected cell susceptibility to Abeta and tau exposure. Our next aim is to determine whether blocking IFN-1 signaling can lead to beneficial outcomes in AD pathologies using IFN-1 knock out cell lines and siRNA techniques. These studies have revealed that the inflammatory Type I interferon responses may be detrimental in AD pathogenesis and blocking this pathway could lead to novel therapeutic options for the treatment of Alzheimer’s disease.

Disclosures: **A. Carrano:** None. **C. Verbeeck:** None. **P. Das:** None.

Poster

046. Modulating and Tracking Neuroinflammation

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CIHR

NSERC

CFI

CCNA

Title: Detecting gangliosides expression profile changes during microglial activation

Authors: *M. M. ALSHAIKH[1], G. LAJOIE[2], S. WHITEHEAD[1];
[1]Anat. and Cell Biol., [2]Biochem., Western Univ., London, ON, Canada

Abstract: With aging, our brains become more susceptible to diseases and injuries. Different brain regions have differing levels of vulnerability to stress and injury, and this brain region dependent variability to vulnerability could be partly explained by the existence of glycosphingolipids within the cell's plasma membrane called gangliosides. Gangliosides are expressed predominantly within the brain and play various roles within the central nervous system including development, differentiation and neural regeneration. Moreover, it has been shown that different species of gangliosides are associated with specific roles within the brain. For instance, several studies demonstrated the importance of GM1 gangliosides in neural repair and cell survival. Simple gangliosides GM2 and GM3, on the other hand, have been associated with neurodegeneration in animal models of stroke and Alzheimer's disease. Our lab has demonstrated that gangliosides can shift their composition from GM1 back to GM2 and GM3 following stroke in rats indicating a role for simple gangliosides in the neurodegenerative process. Interestingly, the transient increase in GM2 and GM3 occurred within 3 and 7 d post stroke-reperfusion indicating that these changes may be part of a neuroinflammatory cascade. It is therefore possible that the observed increases in GM2 and GM3 in the mouse stroke model may have occurred in glial cells; in particular, microglia. Microglia are the innate immune cells of the brain. In response to injury, microglia exhibit functional and morphological changes, and transform into either the classical M1 phenotype or the anti-inflammatory M2 phenotype.
Based on the literature and preliminary studies conducted in our lab, we hypothesize that GM2 and GM3 gangliosides will increase following microglial activation, while GM1 ganglioside will decrease. BV2 microglia were cultured and activation were induced by either LPS for 24 and 48 h or IL-4 for 24 h to induce M1 and M2, respectievly . Immunofluorescence was used to stain for GM1, GM2 and GM3 species. To identify different microglial activation states, OX-6 and CD206 antibodies were used as markers for M1 and M2, respectively. electrospray ionization mass spectrometry (ESI-MS) was used to quantify the gangliosides expressed within these activated microglia.
Both MS and immunofluorescence results revealed that GM2 and GM3 increased during microglial activation while there were no significant changes for GM1 expression. This increase in GM2 and GM3 following activation *in vitro* support the idea that microglia can be the source of the increase in GM2 and GM3 found in animal models of neurodegeneration.

Disclosures: M.M. Alshaikh: None. **G. Lajoie:** None. **S. Whitehead:** None.

Poster

046. Modulating and Tracking Neuroinflammation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 46.22/Q8

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Title: Toxoplasma gondii-infected monocytes can disrupt outer blood retinal barrier by paracrinely activating FAK signaling pathway

Authors: *H. SONG[1,2], H. JUN[2], J. KIM[2], S.-M. LEE[4], M.-H. CHOI[5], J. KIM[1,2,3];
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Abstract: In patients with ocular toxoplasmosis, disruption of retinal pigment epithelium is frequently observed. The retinal pigment epithelial layer constitutes outer blood retinal barrier (BRB) that lies between retina and leaky choroidal vasculature. In this study, we investigated the effect of monocytes infected with *Toxoplasma gondii* on *in vitro* model of outer BRB. Retinal pigment epithelial cells, ARPE-19, were cultivated on transwell to form a confluent monolayer. Then, human monocytic cells, THP-1, infected with *Toxoplasma gondii* or their conditioned medium were treated and the barrier function was evaluated by measurement of transepithelial electrical resistance (TEER) and immunocytochemistry of tight junction proteins. Additional treatment with neutralizing antoby against CXCL8 or FAK inhibitor (PF-573228) was performed to investigate the associated signaling pathway. Six hours after the treatment with infected monocytes could disrupt tight junction protein. The disruption was not restricted to the area adjacent to the cells but was happening widely. To evaluate the paracrine effect of the infected monocytes, their conditioned media alone were treated and found to decrease TEER and disrupt tight junction protein as well. To figure out the factors in the conditioned media mediating the disruption, CXCL8 was selected based on the microarray data regarding gene expression of *Toxoplasma gondii*-infected monocytes. Although the effect of neutralizing antibody against CXCL8 was nor remarkable to restore disrupted barrier but inhibition of downstream signaling pathway by additional treatment with FAK inhibitor could attenuate the decreased TEER and disrupted tight junction protein. In conclusion, monocytes infected with *Toxoplasma gondii* can impair outer BRB paracrinely. The paracrine effect of *Toxoplasma gondii*-infected monocytes is mediated by FAK signaling partly activated by CXCL8.

Disclosures: **H. Song:** None. **H. Jun:** None. **J. Kim:** None. **S. Lee:** None. **M. Choi:** None. **J. Kim:** None.

Poster

046. Modulating and Tracking Neuroinflammation

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: LIPI-NAT-VIEP-BUAP 2015-2016

CONACyT 169023

Title: The galectin-1 and -3 participate in the neuroinflammation-induced by the administration amyloid-β (25-35) into hippocampus of rats

Authors: *E. RAMIREZ[1], L. MENDIETA[2], M. A. MAYORAL[3], I. MARTINEZ[4], F. LUNA[5], I. D. LIMÓN[6];
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Abstract: The galectins are animal lectins that bind to β-galactosides, such as lactose and N-acetyllactosamine, contained in glycoproteins or glycolipids. Galectin 1 (Gal-1) and Galectin 3 (Gal-3) are involved in different pathologies associated to inflammatory process as well as in cell proliferation, production of cytokines, cell adhesion, migration and apoptosis. Amyloid-β 25-35 ($A\beta_{25-35}$) is able to cause memory impairment and neurodegenerative events. Recent evidences have shown that the administration of $A\beta_{25-35}$ into the hippocampus of rats increases the inflammatory response. However, the relation how the galectins are participating in the neuroinflammation process and that could be involved in the progression of $A\beta_{25-35}$ toxicity it is unclear. Our aim was to evaluate the expression of Gal-1 and Gal-3 in the neuroinflammation after administration of $A\beta_{25-35}$ into the hippocampus. We examined the spatial memory in the Morris water maze. After behavioral test in the day 32 the hippocampus was assessed for astrocytes (GFAP), microglia (Iba1), Galectin-1 (Gal-1) and Galectin-3 (Gal-3) by immunohistochemical analysis. The administration of $A\beta_{25-35}$ in the day 31 the animal was tested for spatial memory in the Morris Water Maze. Behavioral performance showed that inflammation evoked by $A\beta_{25-35}$ impaired spatial memory, because animal showed a major time to find the platform during the task that the control groups. Our results showed a significant increase of reactive gliosis (GFAP and Iba1) in the hippocampus (CA1, CA3 and DG) of $A\beta_{25-35}$ treated rats. The expression of Gal-1 and Gal-3 was detected predominantly in most of the Iba1-positive microglia and in GFAP-positive astrocytes of the hippocampus. Therefore, we suggest that Gal-1 and Gal-3 is involved in the inflammatory process of neurodegenerative disorder induced by administration of the $A\beta_{25-35}$.

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Poster

046. Modulating and Tracking Neuroinflammation

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Program#/Poster#: 46.24/Q10

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: QA was supported by The Higher Committee for Education Development in Iraq (HCED)

Title: Cofilin mediates LPS-induced microglial cell activation & associated neurotoxicity through NF-κB & JAK-STAT pathways

Authors: ***Q. M. ALHADIDI**[1], Z. A. SHAH[2];
[1]Medicinal and Bioloogical Chem., [2]Medicinal and Biol. Chem., Univ. of Toledo, Toledo, OH

Abstract: Microglial cells are activated in response to different types of injuries or stress in the CNS. Such activation is necessary to get rid of the injurious agents and restore tissue homeostasis. However, excessive activation of microglial cells is harmful and contributes to secondary injury. Pertinently, microglial cell activity was targeted in many preclinical and clinical studies but such strategy failed in clinical trials. The main reason behind the failed attempts is the complexity of the injury mechanisms which needs either a combination therapy or targeting a process that is involved in multiple pathways. Cofilin is a cytoskeleton associated protein involved in actin dynamics. In our previous study, we demonstrated the role of cofilin in mediating neuronal apoptosis during OGD conditions. Previous studies on microglia have shown the involvement of cofilin in ROS formation and phagocytosis. However, additional studies are needed to delineate the role of cofilin in microglial cell activation. Therefore, in the current study, we investigated the role of cofilin in LPS-induced microglial cell activation using cofilin siRNA knockdown paradigms. The viability of differentiated PC12 cells was used as a measure of the neurotoxic potential of conditioned medium derived from cofilin siRNA-transfected and LPS-activated microglial cells. Cofilin knockdown significantly inhibited LPS-induced microglial cell activation through NF-κB and JAK-STAT pathways. The release of proinflammatory substances (NO, TNF-α, IL-1β, iNOS and COX2) as well as microglial proliferation and migration rates were significantly reduced by cofilin knockdown. Furthermore, differentiated PC12 cells were protected from the neurotoxicity induced by conditioned medium derived from cofilin-transfected and LPS-activated microglial cells. In conclusion, we demonstrated that cofilin is involved in the cascade of secondary injury in microglia and further

validates our previous study on cofilin's role in mediating neuronal apoptosis. Together, our results suggest that cofilin could present a common target in neurons and microglial cells during stroke and might prove to be a promising therapy. Keywords: Cofilin; Microglia; LPS, Inflammation

Disclosures: Q.M. Alhadidi: None. **Z.A. Shah:** None.

Poster

046. Modulating and Tracking Neuroinflammation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 46.25/Q11

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH grant RL56M118990

NIH grant 1UL16M18991

NIH grant TL4GM118992

Title: Inflammation induces a biphasic nuclear Nrf2 translocation activation in neuronal cells

Authors: S. KREPEL, *T. B. KUHN;
Dept Chem. & Biochem., Univ. Alaska Fairbanks, Fairbanks, AK

Abstract: Persistent inflammatory and oxidative stress (IOS) is largely responsible for the progressive loss of neuronal integrity and connectivity underlying the continuous decline of cognitive function associated with most chronic CNS disorders as well as normal aging. The transcription factor Nrf2 plays a pivotal role in the antioxidant defense of non-neuronal and neuronal cells. Nrf2 remains sequestered in the cytosol of cells under basal conditions tightly bound to its repressor Keap1. Upon oxidative stress, Nrf2 dissociates from Keap1 and translocates into the nucleus to stimulate transcription of antioxidant defense mechanisms. It is not yet clear how duration and extent of IOS impact Nrf2 activation and nuclear translocation in neuronal cells. Exposure of SH-SY5Y human neuroblastoma to 200 ng/ml TNFα caused a time dependent Nrf2 translocation (2 fold increase) into the nucleus peaking 4 h after addition. However, nuclear Nrf2 decreased and remained at 50% of peak maximum for a prolonged time period. A presence of 50 ng/ml TNFα resulted in the highest accumulation of Nrf2 in the nucleus compared to higher concentrations (100 to 400 ng/ml). Immunocytochemistry against Nrf2 corroborated these findings. Additionally, prolonged exposure of SH-SY5Y cells to TNFα (24 h) at concentrations 200ng/mL or higher resulted in significant cell death due to oxidative stress. Together these findings suggest that Nrf2-mediated antioxidant defenses in response to TNFα

were insufficient. One of the many health benefits of nutrition rests on the capacity of distinct botanicals, in particular polyphenols, to directly act on biochemical mechanisms rather than through passive antioxidant capacities. We demonstrated that supplementation of SH-SY5Y neuroblastoma cells with extracts obtained from Alaska wild bog blueberries (5 μg/ml) increased neuronal viability in the prolonged presence of 200 ng/mL TNFα. Surprisingly, blueberry extracts prevent Nrf2 nuclear accumulation upon exposure to TNFα. Understanding the regulation of Nrf2 through botanicals could provide vital insight for developing neuroprotective strategies applicable to an array of neurodegenerative diseases.

Disclosures: S. Krepel: None. **T.B. Kuhn:** None.

Poster

046. Modulating and Tracking Neuroinflammation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 46.26/Q12

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CDMRP W81XWH-09-2-0098

CDMRP W81XWH-09-2-0085

CDC-NIOSH NORA Intramural Grant

Title: Quantification of brain acetylcholine in a mouse model of Gulf War Illness using HILIC-UPLC-MS/MS

Authors: *J. A. VRANA[1], A. R. LOCKER[1], K. A. KELLY[1], L. T. MICHALOVICZ[1], R. F. LEBOUF[2], J. P. O'CALLAGHAN[1], D. B. MILLER[1];
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Abstract: Approximately 30% of veterans from the 1991 Persian Gulf War suffer from an exaggerated and persistent form of sickness behavior, classified as Gulf War Illness (GWI). Numerous studies on GWI have suggested that exposure to cholinergic-acting organophosphates (OP) in-theater, such as the irreversible acetylcholinesterase (AChE) inhibitor and chemical warfare agent, sarin, as well as other pesticides and insecticides, may have contributed to GWI symptomatology. Additionally, concomitant exposure to physiological stressors in-theater may have been involved in the initiation of the GWI phenotype. Traditionally, inhibition of AChE and the subsequent accumulation of acetylcholine (ACh) result in the activation of the cholinergic anti-inflammatory pathway. However, the association of GWI with neuroinflammation appears to contradict this effect of AChE inhibitors. Therefore, it is possible that exposure to OPs (e.g.,

sarin, chlorpyrifos) both alone and with corticosterone (CORT) pretreatment to mimic physiological stress targets biomolecules other than AChE to induce the neuroinflammatory effects seen in our mouse model of GWI. To further investigate this phenotype, adult male C57BL/6J mice were exposed to CORT (400mg/L in 1.2% ethanol) in the drinking water for four days. On the fifth day, mice were exposed to a single dose of a sarin surrogate, diisopropyl fluorophosphate (DFP; 4.0mg/kg, i.p.). To fully evaluate the impact of DFP on AChE, AChE activity and ACh concentrations were measured in the brain. Here, DFP decreased AChE activity, while CORT pretreatment ameliorated this effect. Quantification of ACh in brain homogenates has been difficult due to brain matrix effects, polarity of neurotransmitters, neurochemical derivatization requirements, and endogenous AChE activity occurring after euthanasia. To overcome these effects, we have developed a simple and high-throughput method for ACh quantification from brain homogenates using hydrophilic interaction liquid chromatography (HILIC) with ultra-performance liquid chromatography (UPLC)-tandem-mass spectrometry (MS/MS). Also, to ensure rapid inactivation of AChE, mice were euthanized using focused microwave irradiation. ACh concentrations were inversely related to AChE activity with the highest ACh concentration observed in the striatum, followed by the hippocampus and cortex. From these datasets, the alterations in AChE activity and ACh in the brain do not appear to correspond with the exacerbated neuroinflammation induced by CORT and DFP exposure, suggesting that the symptomatology of GWI may be due to the organophosphorylation of currently unidentified biomolecular targets.

Disclosures: **J.A. Vrana:** None. **A.R. Locker:** None. **K.A. Kelly:** None. **L.T. Michalovicz:** None. **R.F. LeBouf:** None. **J.P. O'Callaghan:** None. **D.B. Miller:** None.

Poster

046. Modulating and Tracking Neuroinflammation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 46.27/Q13

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CDMRP Grant W81XWH-09-2-0098

CDMRP Grant W81XWH-09-2-0085

CDC-NIOSH NORA Intramural Grant

Title: Using novel ALDH1L1 bacTRAP technology to evaluate the astrocyte-specific responses to Gulf War Illness-related exposures

Authors: *L. T. MICHALOVICZ[1], K. A. KELLY[1], A. R. LOCKER[1], J. A. VRANA[1], G. BRODERICK[2,3,4], D. B. MILLER[1], J. P. O'CALLAGHAN[1];
[1]CDC-NIOSH, Morgantown, WV; [2]Dept. of Med., Univ. of Miami, Miami, FL; [3]Inst. for Neuro Immune Med., Nova Southeastern Univ., Fort Lauderdale, FL; [4]Univ. of Alberta, Edmonton, AB, Canada

Abstract: A central problem in neurobiology is the ability to reliably assess cell-specific expression profiles in response to various conditions, such as development, disease, or exposure. Specifically, it is challenging to elucidate a particular cell's *in vivo* response without affecting it's interaction with other cells in the brain. To overcome this issue, Heintz and Greengard (2008) developed bacTRAP (translating ribosome affinity purification) technology which allows for the evaluation of the actively translating transcriptome of a particular cell type, as directed by a cell-specific gene sequence. Thus, using the astrocyte-specific housekeeping gene, Aldh1l1, it is possible to extract the astrocytic response to various conditions. Here, we have characterized the ALDH1L1 bacTRAP mouse using the dopaminergic neurotoxicant, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; 12.5 mg/kg, s.c.), and expanded upon these observations to evaluate the effects of Gulf War Illness (GWI)-related exposures on astrocytes. Using our published animal model of GWI, which models the exposures experienced by GWI suffering veterans during the 1990-1991 Persian Gulf War (O'Callaghan *et al.*, 2015), we evaluated the astrocyte-specific response of ALDH1L1 bacTRAP mice to corticosterone (CORT) in the drinking water (200 mg/L in 0.6% EtOH) for 4 days followed by a single exposure to the sarin surrogate, diisopropyl fluorophosphate (DFP; 3 mg/kg, s.c.). Overall, in comparison to C57BL/6J (wild-type) mice, ALDH1L1 bacTRAP mice had a significantly higher neuroinflammatory response to CORT DFP exposure. Illumina RNAseq analysis identified 703 unique, differentially expressed genes (DEGs, FC>1.5) in the cortical astrocytes of CORT DFP exposed mice, over all control conditions. Furthermore, 211 DEGs were found to be enriched in the astrocytes of CORT DFP exposed mice, displaying a 5-fold greater mRNA expression over total "unTRAPed" cortex. Subsequent gene ontology and pathway analysis of the two DEG sets indicated that most of the genes were related to inflammation and cell death. Specifically, cytokine-cytokine receptor interaction and the complement cascade pathways were identified in both gene sets. Interestingly, we have found that despite causing neuroinflammation and altering mRNA expression in astrocytes, exposure to these GWI conditions do not result in astrogliosis. By understanding cell-specific responses, especially in disease states, it is not only possible to identify the cell-types responsible for cellular and molecular features, but it also creates the potential to specifically target therapeutics to affected cells and avoid off-target effects.

Disclosures: L.T. Michalovicz: None. **K.A. Kelly:** None. **A.R. Locker:** None. **J.A. Vrana:** None. **G. Broderick:** None. **D.B. Miller:** None. **J.P. O'Callaghan:** None.

Poster

046. Modulating and Tracking Neuroinflammation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 46.28/Q14

Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: IIBIS Advanced Imaging Research Technology Development Grant

Title: Initial characterization of 11C-GSK1482160 as radioligand for P2X7 receptors.

Authors: J. S. PETERS, *J. A. MEYER, A. A. RILEY, B. P. MCCARTHY, M. GAO, M. WANG, Q.-H. ZHENG, G. D. HUTCHINS, P. R. TERRITO;
Radiology and Imaging Sci., Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Neuroinflammation is an essential step in the progression of brain diseases [1] in which pro-inflammatory cytokines play a central role [2]. Recent work has shown that the pathogenesis of neuroinflammation is mediated in part by the release of adenosine/uridine derivatives from the damage site, involving a family of ionotropic purinergic receptors including P2X7 with elevated receptor expression [3], microglial proliferation and phagocytosis of the injured site [4, 5]. This mechanism is common amongst a wide array of neurodegenerative inflammatory diseases including Alzheimer's, Parkinson's, and Huntington's Diseases, frontotemporal dementia, Atrophic Lateral Sclerosis, Multiple Sclerosis, and Traumatic Brain Injury [6-8]. The P2X7 receptor represents a novel molecular target for imaging neuroinflammation via PET. The compound GSK1482160 is an ideal starting point for evaluation of the P2X7 receptors, has high receptor affinity and ideal blood-brain barrier penetration [9]. Moreover, recent work from our institution has radiolabeled this compound [^{11}C]-GSK1482160 [10] yielding a potential biomarker for neuroinflammation, of which saturation kinetics revealed a B_{max} and K_d of 923fmol/mg and 1.1nm in hP2X7R-HEK cells, respectively. Association kinetic determination of K_{on}, K_{off}, and B_p in hP2X7R-HEK cells were 0.231/min*nM, 0.272/min, and 0.91, respectively. These results were confirmed via P2X7 binding and IFA in hP2X7R-HEK cell blocks with secondary and tertiary confirmation in western blot and IHC using a lipopolysaccharide (LPS, 5mg/kg IP) neuroinflammation mouse model. Biodistribution of [^{11}C]-GSK1482160 showed a time dependent tissue distribution in all tissues studied with the liver, intestines, and kidney, showing the highest uptake by 30min. The brain, blood, heart, lung, spleen and skeletal muscle all had uptake which showed similar temporal patterns and uptake levels. To characterize tracer kinetics, in vivo PET/CT [^{11}C]-GSK1482160 was performed in mice administered saline or LPS (5mg/kg IP) and kinetically modeled for 1 cortical and 1 hippocampal brain region. Total volumes of distribution (V_t) for 6 of 8 all brain regions were statically significantly elevated ($p<0.05$, n=3/treatment). We report the initial physical and biological characterization of this novel ligand, which shows high affinity

and favorable association/disassociation kinetics and was confirmed via multiple in vitro assays. In vivo biodistribution indicated that the liver, intestine and kidney uptake were maximal by 30min and in vivo PET/CT tracer kinetic modeling indicated that V_t followed expected trends for mice treated with LPS.

Disclosures: **J.S. Peters:** None. **J.A. Meyer:** None. **A.A. Riley:** None. **B.P. McCarthy:** None. **M. Gao:** None. **M. Wang:** None. **Q. Zheng:** None. **G.D. Hutchins:** None. **P.R. Territo:** None.

Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 47.01/R1

Topic: A.04. Transplantation and Regeneration

Title: Electrical stimulation enhances migratory ability of transplanted bone marrow stromal cells in stroke model of rats.

Authors: *J. MORIMOTO, T. YASUHARA, M. KAMEDA, M. UMAKOSHI, K. KIN, M. OKAZAKI, H. TAKEUCHI, T. SASAKI, A. TOYOSHIMA, T. AGARI, I. DATE;
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Abstract: Objective Bone marrow stromal cells (BMSCs) transplantation is an important strategy for treatment of ischemic stroke. But there are no crucial method to guide BMSCs toward the lesion site. In this study, we investigated the effect of electrical stimulation on BMSCs migration in ischemic model of rats. **Methods** Adult male Wistar rats weighing 200 to 250g received right middle cerebral artery occlusion (MCAO) for 90 minutes. BMSCs (2.5×10^5cells/ 4μl PBS) were stereotaxically injected into the left corpus callosum at 1day after MCAO. After BMSCs injection, plate electrode with diameter 3mm which connected to the implantable electrical stimulator was placed on the right frontal epidural space and then counter electrode was placed extra-cranial space. Electrical stimulation, at preset current (100μA) and frequency (100Hz), was performed for two weeks. Behavioral tests were performed at1, 4, 8, 15 days after MCAO using the Modified Neurological Severity Score (mNSS) and cylinder test. Rats were euthanized at 15 days after MCAO for evaluation of infarct volumes and the migration distance and area of BMSCs found in the brain tissue. **Results** Behavioral test at 4, 8, and 15days after MCAO revealed that stimulation group displayed the significant improves in mNSS compared to control group ($p<0.05$). Similarly, the infarct volumes of stroke rats in stimulation group were significantly decreased compared to control group ($p<0.05$). Migration distance and area of transplanted BMSCs were significantly longer and wider respectively in stimulation

group. **Conclusions** These results suggest that electrical stimulation enhances migratory ability of transplanted BMSCs in stroke model of rats. If we can direct the implanted BMSCs to the site of interest, it may have a greater therapeutic effect.

Disclosures: J. Morimoto: None. **T. Yasuhara:** None. **M. Kameda:** None. **M. Umakoshi:** None. **K. Kin:** None. **M. Okazaki:** None. **H. Takeuchi:** None. **T. Sasaki:** None. **A. Toyoshima:** None. **T. Agari:** None. **I. Date:** None.

Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 47.02/R2

Topic: A.04. Transplantation and Regeneration

Support: ERC Consolidator grant

Title: Development of optimized hematopoietic stem cell gene-therapy approaches for treatment of infantile neuronal ceroid lipofuscinosis.

Authors: *M. PEVIANI[1], U. CAPASSO[2], R. MILAZZO[3], V. CIPOLLA[1], S. GATTI[2], D. MOSCATELLI[2,4], A. BIFFI[1];
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Abstract: Infantile neuronal ceroid lipofuscinosis (INCL), caused by defects in CLN1 gene coding for palmitoyl protein-thioesterase-1 (PPT1), is one of the most severe forms of NCLs, leading to progressive vision loss, dementia, epileptic seizures and loss of motor coordination, culminating in premature death. Although gene-therapy is a promising therapeutic option for these pathologies, insufficient delivery of PPT1 activity to the central nervous system (CNS) remains an issue that hindered successful clinical application of the approaches tested by now. Exploiting the reconstitution of brain-resident microglia as local source of the wild type hydrolase, by transplantation of gene-corrected hematopoietic stem cells (HSCs) in myeloablated recipients, may represent an alternative strategy to obtain widespread distribution of wild type PPT1 in the CNS. We highlighted that systemic treatment with busulfan, an alkylating agent capable of ablating brain resident microglia progenitors, is instrumental to foster efficient turnover with donor-derived progenitor cells administered systemically. We hypothesized that CNS-directed delivery of the chemotherapeutic drug and injection of HSCs in the cerebrospinal

fluid may work as optimized approach to favor the engraftment of donor-derived gene-corrected microglia progenitors in the brain, potentially speeding up enzyme activity reconstitution within the CNS without exposure to the side effects associated with the myeloablative chemotherapy administered systemically. As first steps to reach this goal, here we present the development and characterization of a new drug delivery biodegradable and biocompatible nanocarrier for busulfan, based on polymeric nanoparticles (NPs), able to selectively target CNS microglia/macrophages. By analyzing the biodistribution of microglia-targeted NPs after intra-cerebroventricular administration in mice, we observed prominent localization in proliferating cells localized in brain regions lining the lateral ventricles and CNS stem/progenitor cells niches. These regions are also the first to be colonized by donor-derived HSCs soon after transplant, which suggests that targeting these specific CNS niches with cell-selective chemotherapy may create space for efficient engraftment of donor derived HSCs. Modification of the NPs core with functional groups selected according to their affinity for busulfan, led to improved drug-loading, within a range of biologically active concentrations. Experiments are now on-going to optimize NPs dosing and timing/route of HSC administration to obtain improved engraftment in healthy and INCL mouse brain.

Disclosures: **M. Peviani:** None. **U. Capasso:** None. **R. Milazzo:** None. **V. Cipolla:** None. **S. Gatti:** None. **D. Moscatelli:** None. **A. Biffi:** None.

Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 47.03/R3

Topic: A.04. Transplantation and Regeneration

Support: NIH PO1 NS055976

Shriners Hospital for Children

Christopher L. Moseley Foundation

Title: Transplanting human NPCs derived from iPSC into the rodent spinal cord

Authors: ***Y. JIN**[1], J. BOUYER[1], K. HAYAKAWA[1], C. HAAS[1], J.-P. RICHARD[2], N. MARAGAKIS[2], I. FISCHER[1];
[1]Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA; [2]Dept. of Neurol., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Transplantation of neural progenitor cells (NPCs) is a promising strategy to replace, repair and bridge the injured spinal cord. Transplants of human iPSC derived NPCs (iPSC/NPCs) are clinically relevant because of the potential for using patients own cells. In the present study, we examined the survival and differentiation of transplanted human iPSC/NPCs (Haidet-Phillips et al., Stem Cells Transl Med, 2014 3(5):575-585) following grafting into the intact and injured spinal cord of rats. We initially examined the survivability and phenotypic repertoire of transplanted cells in the intact spinal cord, derived either directly from frozen stock or after culturing. Cells harvested from frozen stocks demonstrated few surviving cells at 1 week, and no survival at 3 and 5 weeks post-transplantation. In contrast, transplants survived much better when the cells were cultured for 2 days. The cells differentiated into neurons and astrocytes by 3 and 5 weeks post-transplantation. Next, we examined the fate of cells transplanted into the injured spinal cord using two distinct models: C4 dorsal column and lateral funicular lesions. Transplanted cells from a frozen stock into an acute lesion did not survive at the lesion site, but survived near the lesion area. In contrast, cultured cells survived within the lesion as well as around lesion, but failed to fill the entire lesion area even at 5 weeks post-transplantation. In both cases, the transplanted cells differentiated into neurons and astrocytes. Human iPSC/NPCs from a frozen stock transplanted after a 2 week delay survived within and near the lesion but did not fill the entire lesion area. In a separate experiment, human iPSC/NPCs from a frozen stock were enriched with GRP-derived astrocytes (prepared from the same iPSC cell line) and transplanted after a 2-week delay. Similar to grafts of human iPSC/ NPCs alone, these grafts survived within the lesion, but again failed to fill the entire lesion area. Transplanted cells also differentiated into neurons and astrocytes 5 weeks post-transplantation within the lesion site. These results indicate that transplants of human iPSC/NPCs can generate neurons and therefore be used to form relays to reconnect the injured spinal cord, but they require additional care to improve survival for efficient replacement of lost tissue.

Disclosures: **Y. Jin:** None. **J. Bouyer:** None. **K. Hayakawa:** None. **C. Haas:** None. **J. Richard:** None. **N. Maragakis:** None. **I. Fischer:** None.

Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 47.04/R4

Topic: A.04. Transplantation and Regeneration

Title: Survival of iPSC-derived grafts within the striatum of immunodeficient mice: importance of developmental stage of both transplant and host

Authors: *C. TOM, S. YOUNESI, E. MEER, V. B. MATTIS;
Cedars-Sinai Med. Ctr., Los Angeles, CA

Abstract: Degeneration of the striatum can occur in multiple disorders and these lesions have devastating consequences for the patients. For example, infantile infections with streptococcus, measles or herpes cause striatal necrosis associated with dystonia or dyskinesia. Additionally, in Huntington's disease the striatum undergoes massive degeneration and presents with behavioral, psychological and movement issues, ultimately resulting in death. Currently, only supportive therapies are available for striatal degeneration. Clinical trials have shown some efficacy using transplantation of fetal-derived primary striatal progenitors. For transplantation therapy to be a reality, large banks of fetal progenitors that give rise to the primary neuron of the striatum (medium spiny neuron (MSN)) are needed. However, fetal tissue is of limited supply, has ethical concerns and requires patient use of immunosuppressive drugs, due to the risk of rejection. Another potential source of MSNs is from induced pluripotent stem cells (iPSCs), adult somatic tissues reprogrammed back to a stem cell fate. Multiple publications have demonstrated the ability to differentiate MSNs from iPSCs.
Previous publications reveal that the graft survival of fetal progenitors is critically dependent upon the age of the donor embryo. With the advent of iPSC technology, the question regarding "age" of the graft is even more essential, since transplanting pluripotent cells has an inherent risk of overproliferation and teratoma formation, but has gone largely unanswered. iPSCs were therefore transplanted at different stages along a paradigm of striatal differentiation into the striatum of both the neonatal and adult immunodeficient mice. This study aims to examine the importance of both the developmental age of the transplant and recipient for graft survival, migration and immunohistochemical expression of cell markers in iPSC-derived striatal cell transplants.

Disclosures: C. Tom: None. **S. Younesi:** None. **E. Meer:** None. **V.B. Mattis:** None.

Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 47.05/R5

Topic: A.04. Transplantation and Regeneration

Support: Neuroscience program, the College of Medicine, the Field Neurosciences Institute, and the John G. Kulhavi Professorship in Neuroscience at CMU

Title: Donor age and passage number of Mesenchymal stem cells have an effect on alleviating the motor deficits in R6/2 mouse model of Huntington's disease

Authors: *B. SRINAGESHWAR[1,2,3], A. ANTCLIFF[3,5,2], A. CHRISTIANSEN[5], A. STONER[5], M. DAM[5], A. YALAMARTHY[5], A. CRANE[3,2], N. KOLLI[3,2], A. AL-GHARAIBEH[3,2], R. CULVER[3,2], D. STORY[3,2,4], O. V. LOSSIA[5,3,2], A. MOORE[3,2], G. L. DUNBAR[3,2,4,6], J. ROSSIGNOL[5,3,2];
[1]NEUROSCIENCE, CENTRAL MICHIGAN UNIVERSITY, CHENNAI, India; [2]Field Neurosciences Inst. Lab. for Restorative Neurol., Mt Pleasant, MI; [3]Neurosci., [4]Psychology, Central Michigan Univ., Mt Pleasant, MI; [5]Col. of Medicine, Central Michigan Univ., Mt Pleasant, MI; [6]Field Neurosciences Inst., Saginaw, MI

Abstract: Huntington disease (HD) is a fatal late-onset neurodegenerative disorder caused by the degeneration of medium spiny neurons in the striatum region of the brain. The main cause of the disease is the CAG repeat expansion on the Huntingtin gene (HTT) leading to poly glutamine tract which is toxic to the cells. To date, there is no cure or effective treatment for this disease. Previous studies have shown the therapeutic effects of bone marrow and umbilical cord derived mesenchymal stem cells (MSC) on the motor deficits of HD. However, a very recent study has indicated that the therapeutic effect of MSCs for HD depends on how many times the cells have been passaged prior to transplantation. It was shown that higher passaged MSCs (P40 to P50) delayed the onset of motor, cognitive, and neuropathological loss in HD mouse models, most likely through the release of neurotrophic factors, specifically the brain derived neurotrophic factor (BDNF). However, there is also evidence showing that the higher passaged MSCs may not demonstrate an acceptable safety profile, due to accumulation of chromosomal abnormalities that may occur over several passages. Hence, higher passage MSCs may not confer optimal clinical utility. Previous studies have revealed synergistic effects of donor age and passage number. Given this, the present study examined these two critical aspects - the donor age and the passage number of the bone marrow derived MSCs in the context of alleviating motor deficits in R6/2 mouse model of HD. Our findings indicates that donor ages of 5 weeks, 6 months and 10 months of the MSCs and the number of passages prior to transplantation, ranging from lower passage (P3 to P8), intermediate passage (P20 to P30) and high passage (P40 to P50) can affect therapeutic efficacy. *Support for this study was provided by the Neuroscience program, the College of Medicine, the Field Neurosciences Institute, and the John G. Kulhavi Professorship in Neuroscience at CMU*

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Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 47.06/R6

Topic: A.04. Transplantation and Regeneration

Title: The significance of HLA matching in allogeneic human iPS cell-derived neural stem/progenitor cell transplantation for spinal cord injury

Authors: *M. OZAKI[1], A. IWANAMI[1], J. KOHYAMA[2], N. NAGOSHI[1], G. ITAKURA[2], H. IWAI[1], M. MATSUMOTO[1], H. OKANO[2], M. NAKAMURA[1];
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Abstract: Background
Aiming at a first-in-human trial of human iPS cell-derived neural stem/progenitor cell (iPSC-NS/PC) transplantation for spinal cord injury, it would be realistic to use allogeneic human leukocyte antigen (HLA)-matched iPSC-NS/PCs from the viewpoint of quality control and cost performance. The purpose of this study is to determine the clinical significance of HLA matching on post-transplant immunoreaction using mixed lymphocyte reaction (MLR).
Materials and Methods
1×10^5 peripheral blood mononuclear cells (PBMCs) from volunteers were co-cultured with 1×10^5 irradiated iPSC-NS/PCs from a HLA-A, -B, and -DRB1 loci homozygous donor. The proliferative activities of PBMCs were measured by incorporation of ^{3}H-thymidine represented as counts per minute (CPM). HLA genotyping of iPSC-NS/PCs and their PBMCs was performed prior to the mixed culture. Stimulation index (SI) was calculated as the CPM of stimulated PBMCs divided by the CPM of the unstimulated PBMCs. Cut off value for immune rejection was defined as SI = 2.
To assess the immune suppressive effect of iPSC-NS/PCs on PBMCs proliferation, 5×10^4, 1×10^5 or 2×10^5 HLA homozygous iPSC-NS/PCs were co-cultured with 1×10^5 allogeneic PBMCs stimulated by allogeneic antigens.
Subsequently, the expression of immune-related antigens on iPSC-NS/PCs was analyzed using flow cytometry. To mimic an inflammatory environment at the injured spinal cord, iPSC-NS/PCs were exposed to IFN-γ and/or TNF-α for 48 hours prior to the analysis.
Results
Allogeneic HLA-A, -B, -DRB1-mismatched response was equivalently low as allogeneic HLA-A, -B, -DRB1-matched response (mean SI: 1.14 ± 0.19 vs. 1.20 ± 0.14 p=0.802). Furthermore, allogeneic response was also similar to autologous response (mean SI: 0.96 ± 0.15 vs. 0.85 ± 0.23 p=0.807). 2×10^5 iPSC-NS/PCs significantly suppressed the proliferation of stimulated allogeneic PBMCs compared to 5×10^4 (p=0.001) or 1×10^5 iPSC-NS/PCs (p=0.017). The

expression of HLA-DR, CD40, CD80 and CD86 on iPSC-NS/PCs was not elevated even in the presence of IFN-γ and/or TNF-α.

Conclusions

Immunological examination using MLR revealed no significant differences in immune response between HLA-matched and -mismatched conditions. Furthermore, the stimulation indexes in both two conditions were less than 2, suggesting that the immure response in iPSC-NS/PC transplantation could be unexpectedly low. Taken together, the immune suppressive effect and the low antigen presenting function of iPSC-NS/PCs could affect low immune response even in allogeneic HLA-mismatched setting.

In the near future, it is crucial to further verify whether these results could be reproduced in the clinical setting.

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Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 47.07/R7

Topic: A.04. Transplantation and Regeneration

Support: Swiss National Foundation Grant 31003A_140940/1

SPUM Grant 33CM30-124101

Title: Grafted glutamatergic neuronal precursors integrate into the postnatal cerebral cortex and promote functional recovery after targeted neuronal apoptosis

Authors: J. MIHHAILOVA, V. PETRENKO, P. CONSTANTHIN, A. CONTESTABILE, R. BOCCHI, K. EGERVARI, P. SALMON, *J. Z. KISS;
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Abstract: Transplantation of appropriate neuronal precursors after neuronal injury holds promises for reconstruction of cortical circuits. However, the efficiency of these approaches for neuronal replacement *in vivo* remains yet very limited. Here we studied the sequential events that characterize the integration of grafted neuronal precursors into the injured neocortex. We took advantage of a new, highly reproducible model of neuronal ablation utilizing a diphtheria toxin/diphtheria toxin receptor system to induce synchronized apoptotic death of the layer II/III neurons in the rat somatosensory cortex at postnatal day 16 (P16). Transplantation of embryonic

progenitors was carried out 4 days after the lesion that was followed by the analysis of transplanted grafts and somatosensory behavior tests at 7, 14 and 30 days after transplantation. Our results indicate long-term survival (up to 180 days) and good tissue integration of donor cells. Transplanted precursors differentiated into glutamatergic neurons, sent long distance projections into the recipient cortex and received thalamocortical as well as GABAergic innervation. Moreover, neurons in the graft were engaged in synaptically interconnected networks with the host circuits. Compared to transplants into the intact cortex, grafts in the environment of the apoptotic injury showed earlier maturation, increased dendritic tree complexity and accelerated development of GABAergic innervation. Finally, transplantation of neuronal precursors significantly attenuated the apoptotic injury-induced functional deficits in behavior tests. The model provides new possibilities for exploring the synaptic integration of transplanted cells and their impact on functional recovery.

Disclosures: **J. Mihhailova:** None. **V. Petrenko:** None. **P. Constanthin:** None. **A. Contestabile:** None. **R. Bocchi:** None. **K. Egervari:** None. **P. Salmon:** None. **J.Z. Kiss:** None.

Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 47.08/R8

Topic: A.04. Transplantation and Regeneration

Support: NIH T90 DE022732

Title: Human-porcine chimerism and the use of blastocyst complementation to generate a source of nigral dopamine precursors suitable for transplantation

Authors: ***A. CRANE**[1,2], P. SWAMINATHAN[1], H. HEWITT[1,3], F. XIAO[1,3], V. SAVANUR[1], J. VOTH[1], Z. SCHULTZ[1], D. CARLSON[4], S. FAHRENKRUG[1,4], J. DUTTON[1], W. LOW[1,3]; [1]Stem Cell Inst., [2]MinnCResT Program, [3]Neurosurg., Univ. of Minnesota, Minneapolis, MN; [4]Recombinetics Inc., St. Paul, MN

Abstract: Ethical considerations and limited tissue availability represent a significant hurdle in transplantation of human fetal midbrain dopamine (mDA) progenitors for patients with Parkinson's disease. Blastocyst complementation can provide an alternative source of tissue, a process in which human pluripotent stem cells (PSCs) are introduced into genetically engineered organogenesis-disabled porcine blastocysts. Through normal development, injected PSCs can potentially fill the niche and fully develop into a human organ. The goal of our research is to obtain genuine human fetal mDA progenitors grown within the porcine midbrain. In the current

study, our first experiment represents a proof-of-concept for human-porcine chimerism. *In vitro* observations of human induced pluripotent stem cells (hiPSCs) and human cord blood stem cells (hUCBSCs) injected into wild-type porcine blastocysts indicate human cells can incorporate and proliferate within the porcine inner cell mass. Furthermore, human cells were widely present in E30 porcine fetuses derived from hUCBSC injected blastocysts, following transfer to surrogate sows. Subsequent experiments examined the ability to complement *PITX3* knockout porcine embryos with hUBSCS or hiPSCs to generate a human substantia nigra within the porcine midbrain. Examination of *PITX3*-knockout fetuses at E62 demonstrate a reduction in tyrosine hydroxylase (TH) immunoreactivity within the developing substantia nigra as well as a closed-eye phenotype, which were attenuated through blastocyst complementation. Interrogation of fetuses complemented with either hiPSCs or hUCBSCs failed to identify any human cells present throughout the fetus. In a subset of complemented animals, intermediate phenotypes were observed in the morphology of the retina or an increase in TH immunoreactivity within the substantia nigra. As no human cells were observed in the target tissue, further studies will interrogate porcine-porcine chimerism as well as the mutation of additional genes earlier in the development of the ventral mesencephalon. This study represents the first attempt in human-porcine chimerism, providing the groundwork for generating exogenic human mDA progenitors in swine.

Disclosures: **A. Crane:** None. **P. Swaminathan:** None. **H. Hewitt:** None. **F. Xiao:** None. **V. Savanur:** None. **J. Voth:** None. **Z. Schultz:** None. **D. Carlson:** A. Employment/Salary (full or part-time): Recombinetics Inc. **S. Fahrenkrug:** A. Employment/Salary (full or part-time): Recombinetics Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Recombinetics Inc.. **J. Dutton:** None. **W. Low:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Recombinetics Inc..

Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 47.09/R9

Topic: A.04. Transplantation and Regeneration

Title: hESC-derived dopaminergic grafts improve L-DOPA induced dyskinesia in a rat model of Parkinson’s disease

Authors: *O. F. ELABI[1], A. KIRKEBY[2], M. PARMAR[2], E. LANE[1];
[1]Sch. of Pharm. and Pharmaceut. Sci., Cardiff Univ., Cardiff, United Kingdom; [2]Dept. of experimental Med. Sci., Lund stem cell centre, Lund, Sweden

Abstract: Human embryonic stem cell derived dopaminergic neurons are currently the best candidates to supersede primary dopaminergic neurons as a source of cells for transplantation in the treatment of Parkinson's disease. However, although studies have now demonstrated survival of these cells in rodent models of the disease, there has been debate about the interaction of anti-parkinsonian drugs with the grafts. This is critical, given that patients will be taking dopaminergic medication (L-DOPA or dopamine agonists) prior to, during and following transplantation. Furthermore, long term treatment provokes L-DOPA induced dyskinesia, and may precipitate graft-induced dyskinesia (a side effect of the transplantation procedure). It is therefore essential to determine how grafts respond in the presence of these medications. Five groups of female Sprague Dawley rats (n=8-9) received unilateral infusions of 6-hydroxydopamine (6OHDA) into the median forebrain bundle to selectively kill the nigro-striatal dopaminergic neurons. hESC-derived dopaminergic neurons were transplanted into the dopamine depleted striatum of 4 groups while one group was a lesion only control. Two transplanted groups received chronic L-DOPA treatment for 4 weeks before transplantation and 16 weeks after transplantation. One L-DOPA and one non-L-DOPA treated groups were administered exendin-4 (0.5ug/kg twice daily) starting immediately after transplantation. Behavioural assessments were performed prior to transplantation and at repeated intervals post-transplantation: amphetamine induced rotations test and other simple hand motor tests (stepping, whisker and cylinder) were performed. L-DOPA induced dyskinesia side effect were measured weekly from starting L-DOPA treatment by using the AIMs rating scale as described by Breger,L. et al 2013 . Brains were then be harvested for histological analysis. All transplanted groups showed a significant improvement in amphetamine rotation test at week 12 of transplantation. Exendin-4 with and without L-DOPA significant improved rats' performances in whisker test since week 8 post transplantation compared to the lesion control group. AIMs scoring and L-DOPA induced rotations were significantly reduced from 6 weeks post transplantation. This study demonstrates for the first time that hESC derived dopaminergic neurons are able to ameliorate L-DOPA induced dyskinesia. We also found that L-DOPA had no detrimental effect on the ability of the graft to improve motor performance and that exendin-4 may support graft function and integration. Histological analysis will further these findings.

Disclosures: O.F. Elabi: None. **A. Kirkeby:** None. **M. Parmar:** None. **E. Lane:** None.

Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 47.10/R10

Topic: A.04. Transplantation and Regeneration

Support: SFB 870 A06

Title: Transplanted embryonic neurons integrate adequately into adult neocortical circuits

Authors: *S. GRADE[1,4], L. DIMOU[1,4,2], K. CONZELMANN[3], M. GÖTZ[1,4,2];
[1]Physiological Genomics, [2]SYNERGY, Excellence Cluster of Systems Neurol., [3]Max von Pettenkofer Inst. and Gene Ctr., Ludwig-Maximilians Univ., Muenchen, Germany; [4]Inst. of Stem Cell Res., Helmholtz Ctr. Munich, Neuherberg, Germany

Abstract: Neuronal connectivity forms the structural foundation underlying neural function, as brain circuits restrain or even define the functional neuronal networks that act together in a sophisticated and highly coordinated fashion to express motor, sensory or cognitive behavior. Transplantation strategies aiming at replacing neurons lost upon brain injury or disease have been thriving in the last decades, however, it remains elusive whether new neurons can faithfully integrate and wire into the existing circuits. Herein, we induced cell death of upper layer projection neurons in the primary visual cortex (V1) of adult mice and transplanted neurons from the embryonic mouse neocortex. Our data show that the new neurons display the appropriate upper layer neuron identity, acquire mature morphologies by extending axons and dendrites through the host brain parenchyma, and develop synaptic specializations on those arbors. Using monosynaptic tracing based on a modified rabies virus and brain-wide analysis, we show here that transplanted neurons receive area-specific, afferent projections matching those of endogenous pyramidal neurons in the primary visual cortex. These comprise a number of sensory and associative cortices, as well as subcortical regions including the dorsal lateral geniculate nucleus (dLGN), the relay nucleus of the retino-geniculate-cortical pathway. Interestingly, connectivity ratios are comparable to the ones found endogenously, and the new geniculate-cortical connections respect the topographic maps that are established during development of the mouse visual system, revealing accurate pairwise connections and maintenance of neighbor to neighbor relationships between subregions of the visual thalamus and the V1. Excitingly, transsynaptic tracing after transplantation in a highly inflammatory injury as a stab wound, reveals as well a broad and V1-specific connectome, raising prospect for the success of neuronal replacement in conditions where environment is highly changed by reactive glia and infiltrating immune cells. Similarly to what we observed in both lesion models, neurons transplanted in the intact brain establish mostly adequate connections, however considerably fewer connections per cell, far below the connectivity ratio existing endogenously. Altogether our data indicate that

transplanted neurons can integrate with great specificity into already existing neocortical circuits, which normally do not harbor new neurons, a critical question for functional reconstruction in the adult brain.

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Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 47.11/R11

Topic: A.04. Transplantation and Regeneration

Support: California Institute of Regenerative Medicine (CIRM)

Title: A novel strategy for developing a robust stem cell based therapeutic for Parkinson's disease using the transcription factor MEF2C

Authors: *R. AMBASUDHAN[1], N. DOLATABADI[1], D. POLIOUDAKIS[2], A. SULTAN[1], K. LOPEZ[1], A. NUTTER[1], J. PARKER[1], M. TALANTOVA[1], S. MCKERCHER[1], N. NAKANISHI[1], E. MASLIAH[3], D. GESCHWIND[2], D. E. REDMOND, Jr.[4], S. A. LIPTON[1]; [1]Neurodegenerative Dis. Ctr., Scintillon Inst., San Diego, CA; [2]Ctr. for Neurobehavioral Genet., Univ. of California Los Angeles, Los Angeles, CA; [3]Neurosci., Univ. of California San Diego, La Jolla, CA; [4]Yale Stem Cell Ctr., Yale Univ. Sch. of Med., New Haven, CT

Abstract: The hallmark motor symptoms of Parkinson’s disease (PD) are attributable to the loss of midbrain dopaminergic (DA) neurons projecting from the substantia nigra pars compacta (SNpc) to the striatum. Replacing these neurons by cell transplantation is an attractive strategy for restoring dopaminergic dysfunction and modifying disease progression in patients. Recent advances in developing robust cell culture protocols for directed differentiation of human pluripotent stem cells (hESCs or hiPSCs) to near pure populations of SNpc (A9-type) DA neurons has heightened the prospects for PD cell therapy. However, some of the remaining challenges include: risk of hyperproliferation/tumor formation of the grafts, generation of unwanted side populations (e.g., 5-HT neurons that may contribute to graft-induced dyskinesia), and limited graft survival. Directly addressing these issues, we report a novel genetic-programming strategy in hESCs using a constitutively-active form of the transcription factor MEF2C, combined with the floor plate DA neuronal differentiation protocol of Lorenz Studer’s group, in order to yield cultures that contain a nearly pure population of DA neurons expressing A9-type markers and electrophysiological properties by patch-clamp recording and without 5HT expression. Additionally, we have shown that expression of MEF2C, which is known to be

dysfunctional in at least some forms of PD (Ryan et al. *Cell*, 2013), is anti-apoptotic and drives BDNF expression. Direct comparison of gene expression profiles by RNAseq of these MEF2C-engineered cells with non-engineered cells reveal that the MEF2C-cells are substantially more similar to human brain-derived A9 DA neurons. Transplantation of the engineered cells into the striatum of the 6 hydroxydopamine-lesioned rat and MPTP monkey models of PD showed significant motor behavioral improvement. This was accompanied by long-term (>1 year) survival of the grafts containing DA neurons, as analyzed by SPECT scan, robust innervation of A9 targets, and complete absence of hyperproliferation. These results support the robustness and translational readiness of our approach toward developing a human stem cell-based PD therapeutic.

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Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 47.12/R12

Topic: A.04. Transplantation and Regeneration

Support: Funded by VID Contingency grant, 2010 (U. Chile)

Title: An immortalized cell line from the motor cortex of an adult rat as a model of cell transplant therapy in rats subjected to cortical ischemia. Survival, phenotype and functionality post grafting

Authors: A. ARMIJO[1], *C. F. ARRIAGADA[2,4], R. CAVIEDES[3], P. CAVIEDES[3]; [1]Program of Anat. & Devel. Biol., [3]Program of Molec. & Clin. Pharmacol., [2]ICBM, Fac. Med, Univ. Chile, Santiago, Chile; [4]Program of Anat. & Devel. Biol., ICBM, Fac. of Medicine, Univ. of Chile, S, Chile

Abstract: Transplant models using stem cells have resulted in survival and differentiation of engrafted cells in models of cerebral ischemia. Therapeutic developments have been limited due to reduced quantities of procurable cells, variable phenotypes, potential tumorogenesis in cells transfected with oncogenes, and the ethical dilemma on the origin of the stem cells. An alternative source are immortalized cell lines, per the experience with the implant of a human immortalized cell line, hNT, in the cerebral cortex of patients with permanent ischemic damage.

We present our work evaluating the engraftment of an immortalized cell line, named RCCCM, derived from the motor cortex of an adult Fisher 344 rat, in the brain of rats subjected to temporary brain ischemia. Four Fisher 344 rats previously subjected to brain ischemic lesions by occlusion of the middle cerebral artery, were transplanted with the RCCCM cell line transfected with enhanced green fluorescent protein (RCCCMt) along three sites of stereotactic coordinates in the primary motor cortex, in a density of 100.000 cells/µl. 12 weeks post-surgery, the animals were euthanized and the brains fixated. Engrafted RCCCMt cells were immunodetected with either diaminobenzidine (DAB) against EGFP or immunofluorescence. Maturation and phenotype of grafted RCCCMt cells were evidenced by colocalization experiments with immunofluorescent labeling against Ki67, βIII-tubulin, MAP-2, NT3 and GFAP. DAB labelling was visualized through light microscopy and double labeling was confirmed using confocal microscopy. RCCCMt cells were found at the bottom of the implantation sites, where they exhibited a round, immature shape with signs of migration through the ipsilateral corpus callosum. RCCCMt cells were also encountered in Layers 4 and 5 of the cerebral cortex, where they exhibited a rounded and pyramidal shape, with long processes. βIII-tubulin (7,72%), MAP-2 (18,12%) and NT3 (17,79%) were present in RCCCMt cells, whereas labeling against Ki67 and GFAP was absent. Hence, RCCCMt cells survived in the host brain tissue even in the proximity of a damaged cerebral cortex, and they could migrate from this site or the end of the needle tract and localize in layers 4 and 5. Immunofluorescent colocalization analysis confirmed that RCCCMt cells maintained their neuronal phenotype and developed two stages of differentiation in the host tissue, immature and mature, as expressed by the presence of βIII-tubulin and MAP-2 and NT3, respectively Further, no cells in undifferentiated/proliferative stages were found The results strongly suggest that RCCCMt cells can survive after grafting in the brain, migrate and maintain neuronal traits.

Disclosures: **A. Armijo:** None. **C.F. Arriagada:** None. **R. Caviedes:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); RC declares patent protection on RCCCM cell line. **P. Caviedes:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PC declares patent protection on RCCCM cell line.

Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 47.13/R13

Topic: A.04. Transplantation and Regeneration

Support: William and Ella Owens Medical Research Foundation

Title: Embryonic stem cell transplants as a therapeutic strategy in a rodent model of autism

Authors: *J. J. DONEGAN[1], A. M. BOLEY[2], D. J. LODGE[2];
[1]Pharmacol., Univ. of Texas Hlth. Sci. Ctr. At San Antonio, San Antonio, TX; [2]Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX

Abstract: Autism is a neurodevelopmental disorder characterized by disruptions in three core behavioral domains: deficits in social interaction, impairments in communication, and repetitive and stereotyped patterns of behavior or thought. There are currently no drugs available for the treatment of the core symptoms of ASD and drugs that target comorbid symptoms often have serious adverse side effects, suggesting an urgent need for new therapeutic strategies. The neurobiology of autism is complex, but converging evidence suggests that ASD involves disruptions in the inhibitory GABAergic neurotransmitter system. Autistic patients show reductions in GABA itself, in the GABA synthesizing enzymes, GAD65 and GAD67, and in subunits of the $GABA_A$ receptor. Post mortem studies demonstrate that these reductions may be due to an actual loss of GABAergic interneurons, specifically the parvalbumin (PV)-positive subtype. PV-positive interneurons play a critical role in regulating pyramidal cell excitability and synchronizing coordinated network activity. Autistic patients show hyperexcitability and reduced coordinated activity in the prefrontal cortex (PFC) and this dysregulated PFC activity has been associated with autism-like behaviors, including reduced social interaction and cognitive inflexibility. Therefore, we hypothesize that restoring PV interneuron function will normalize aberrant cortical activity and ultimately abolish the behavioral deficits associated with autism. To induce an autism-like phenotype, pregnant rats were injected with the viral mimetic Poly I:C (7.5 mg/kg) on gestational day 12. To restore interneuron function, we used a mouse embryonic stem cell line containing dual reporters (Lhx6::GFP and Nkx2.1::mCherry) to grow PV-positive interneurons. Cells were sorted using flow cytometry, then injected into the mPFC of Poly I:C- or saline-treated offspring. After a 30 day recovery period, during which time the transplanted cells migrate and integrate into the mPFC circuitry, we measured social interaction, ultrasonic vocalizations, attentional set-shifting, and pyramidal cell activity in the mPFC. PV-positive transplants into the mPFC of Poly I:C-treated rats increased social interaction time, improved cognitive flexibility, and normalized pyramidal cell function, suggesting that targeting PV-positive interneurons in the mPFC may be a novel and effective treatment strategy to reduce the core symptoms of autism.

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Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 47.14/R14

Topic: A.04. Transplantation and Regeneration

Support: College of Medicine, the Field Neurosciences Institute, and the John G. Kulhavi Professorship in Neuroscience at CMU.

Title: Potential of induced pluripotent stem cells (iPSCs) and iPSC-derived neural stem cells (iNSCs) for treatment of the behavioral and neuropathological deficits in the YAC128 mouse model of Huntington's disease

Authors: *A. AL-GHARAIBEH[1,5,2], R. CULVER[2,5], A. CRANE[2,5], R. WYSE[2,5,3], A. ANTCLIFF[2,5,3], B. SRINAGESHWAR[2,5], N. KOLLI[2,5], L. FROLLO[2,5], D. STORY[4,5,2], G. PATRICIA[2,5], S. MOORE[2,5], O. LOSSIA[2,3], A. EICKHOLT[2], G. DUNBAR[2,6,5,4], J. ROSSIGNOL[2,5,3];
[1]Central Michigan Univ., Amman, Jordan; [2]Neurosci., [3]Col. of Med., [4]Psychology, Central Michigan Univ., Mt.Pleasant, MI; [5]Field Neurosciences Inst. Lab. for Restorative Neurol., Mt.Pleasant, MI; [6]Field Neurosciences Inst., Saginaw, MI

Abstract: There is no cure or effective therapy for Huntington's disease (HD), and the only FDA-approved treatment for this disorder has only palliative effects. However, stem cell therapy holds significant promise for HD patients, but more research is needed to test the safety and efficacy of this approach in order to determine the optimal stem cell type for transplantation. The goal of the current study was to assess the efficacy of intra-striatal transplantation of induced pluripotent stem cells (iPSCs) and iPSC- derived neural stem cells (iNSCs) as a treatment for HD. For this purpose, we developed mouse adenovirus-generated iPSCs and transplanted them into striata of wild-type (WT) and HD YAC128 mice. Also, we differentiated the iPSCs into neural stem cells *in vitro*, and then transplanted them into striata of WT and HD YAC128 mice. We tested the efficacy of these transplanted iPSCs or iNSCs for their ability to survive and differentiate into neuronal phenotypes, and their ability to reduce the significant behavioral deficits observed in this mouse model of HD. To analyze the functional efficacy of iPSC and iNSC transplantation, 10-month old male and female WT and HD YAC128 mice were given bilateral intra-striatal transplants of iPSCs or iNSCs, pre-labelled with Hoechst, or given injections of vehicle control. Measures of accelerod, open field, and clasping behaviors were made one day before the transplantation, and then weekly for 10 weeks. Our results revealed an amelioration of locomotor deficits for the HD groups that received iPSC or iNSCs transplantation, but no between-group differences were observed on measures of open-field activity levels or the amount of clasping at baseline and week 10. Ten weeks following

transplantation, the mice were perfused, and their brains were frozen, sectioned and analyzed by immunohistochemistry. The results from our histological examinations revealed that iPSCs in both WT and HD mice had survived and showed evidence of differentiation into neuronal phenotypes, with co-labelling of Hoechst with Tuj1 and NeuN. Further analyses of this tissue is aimed at measuring the extent of potential differentiation of the transplanted cells into region-specific (e.g., DARPP-32) neurons, detection of any early signs of tumor formation, markers for inflammation and mutant *huntingtin* aggregate formation. Collectively, our present histological and behavioral data suggest that adenovirus-generated iPSCs or iNSCs may provide a safe and effective option for neuronal replacement therapy.

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Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

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Program#/Poster#: 47.15/R15

Topic: A.04. Transplantation and Regeneration

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Simons Center for the Social Brain/SFARI

NIH grant MH085802

HFSP Long-Term Fellowship (LT001068/2014-L)

NARSAD young investigator award

FRQS and NSERC Postdoctoral training grants

Title: Growth, differentiation and connectivity of implanted human neuronal precursor cells in the mouse visual cortex

Authors: ***J. BENOIT**[1], H. WU[2], V. BRETON-PROVENCHER[1], J. P. K. IP[1], D. FELDMAN[1], S. CHOU[1], R. JAENISCH[2], M. SUR[1];
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Abstract: The use of induced pluripotent stem cells (iPSCs) to recapitulate the effect of human genetic diseases in an experimentally tractable system requires both a rich genomic as well as cellular context. Current models for neurological diseases generally consist of human induced-neuronal (iN) cells engineered with the specific mutations of interest, or derived from patient cells with those same mutations, and grown *in vitro* in a 2D culture. In order to create a more natural and 3D environment in which to grow and assess human iNs, we differentiated "wild-type" AAVS1-CAG-tdTomato human neuronal precursor cells (NPCs) which were then transplanted into the mouse cortex to form a "humanized" functional network in a model system. We injected approximately 10,000 NPCs into the primary visual cortex (V1) of SCID immunodeficient mice at P21 and then using a craniotomy, examined their morphological development *in vivo* from 6 weeks post-injection onwards. Of the injected NPCs, several hundred survived and were localized mainly to the injection tract although some cells in deeper layers (~300 um from pia) were well-intercalated between endogenous mouse cells within several hundred um of the injection. NPCs sent wide-ranging projections, some of which reached to adjacent cortical areas and extended beyond the craniotomy (1.5 mm lateral distance) in some cases. We found evidence of filopodia and potential immature spines on human dendrites which suggests that the NPCs have differentiated into a neuronal phenotype and may be forming synapses with endogenous mouse neurons. We are currently examining calcium responses in these cells to determine if they possess functional contacts with the mouse cortical circuit and are therefore responsive to visual input. We posit that this system represents a more realistic environment with superior experimental validity in which the development of normal and patient-derived human neurons can be studied.

Disclosures: J. Benoit: None. **H. Wu:** None. **V. Breton-Provencher:** None. **J.P.K. Ip:** None. **D. Feldman:** None. **S. Chou:** None. **R. Jaenisch:** None. **M. Sur:** None.

Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 47.16/R16

Topic: I.04. Physiological Methods

Support: NIH Grants NS091585, NS062097, NS085568

AHA Grant 12GRNT12060222

VA National Merit Grant RX000666

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AHA Postdoctoral Fellowship 15POST25710112

National Natural Science Foundation of China 81500989

Title: Optogenetics stimulation improves stem cell engraftment after transplantation into neonatal rats with focal ischemic stroke

Authors: *Z. WEI[1], A. GANGAL[1], X. GU[1], D. CHEN[1], C. LIU[1], Z. LI[1], K. BERGLUND[2], S. YU[1], L. WEI[1];
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Abstract: Neonatal ischemic stroke is a devastating disorder affecting 1/4000 infants worldwide and clinically applicable treatment is very limited. Currently, there is no effective acute treatment or regenerative therapy to repair the damaged brain tissue following ischemic stroke in neonates. In animal models, intracranial delivery of neuronal precursors has been tested and shown beneficial effects of brain repair and stimulating endogenous neurogenesis. However, whether transplanted cells can reconnect to the surviving or new generating neurons through synaptic formation following ischemia has rarely been verified. Utilizing a neonatal stroke model in the present study, we investigated intracranial delivery of neural progenitor cells derived from mouse induced pluripotent stem cells (iPSCs). Before transplantation, iPSCs were genetically modified to express ChR2 or VChR1 channels that can be selectively activated by optogenetics stimulation using blue laser light or luciferin-luciferase. Neonatal P7 rats were subjected to a focal ischemia targeting the right sensorimotor cortex. Neural progenitor cell transplantation was performed at 7 days after stroke; optogenetics stimulation (15 min/day) was carried out from 14 days after stroke until sacrifice. This transplantation and stimulation paradigm increased the expression of synaptic proteins including PSD-95, SNAP-25, SYN-1 and synaptophysin in immunohistochemical assays and Western blot analysis. In functional assessments, cell transplantation and optogenetics stimulation improved the performance of stroke mice in whisker-touching test, sensorimotor function evaluation, computer-based automated homecage behavioral analyses, and neurocognitive tests. Optogenetics stimulation also showed significant increases in MBP expression, DCX-expressing neuroblasts and Glut-1+/BrdU+-colabeled small vessels in the penumbra region. Our finding demonstrates that optogenetics activation and transplantation of iPSC-derived neural progenitor cells act synergistically to augment neural network repair and provides an exciting opportunity to improve cell therapies for neonatal ischemic stroke.

Disclosures: **Z. Wei:** None. **A. Gangal:** None. **X. Gu:** None. **D. Chen:** None. **C. Liu:** None. **Z. Li:** None. **K. Berglund:** None. **S. Yu:** None. **L. Wei:** None.

Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 47.17/R17

Topic: C.09. Brain Injury and Trauma

Title: Chronically reduced adult neurogenesis in the mouse hippocampus contributes to functional deficits following soman-induced status epilepticus

Authors: K. M. HOFFMAN[1], K. L. SCHULZ[1], E. K. JOHNSON[1], J. K. CHANDLER[1], *P. M. MCNUTT[2];
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Abstract: *Status epilepticus* (SE) resulting from central exposure to organophosphorus nerve agents causes devastating damage to the brain, including acute neurotoxicity and impaired brain function. In most cases cognitive and behavioral deficits do not resolve, and the only treatment option is supportive care. The hippocampal formation is a primary target of nerve agents, and the acute loss of pyramidal CA neurons is a well-described outcome of nerve agent-induced SE (NASE). However the contributions of other hippocampal pathologies to aberrant cognition have yet to be described. Here we evaluated the effects of NASE on functional neurogenesis in the subgranular zone (SGZ) of the hippocampus. SGZ-derived newborn neurons integrate into the dentate gyrus within 2-3 wk and are functionally important for certain types of memory. In preliminary studies, immunohistochemistry revealed a 90% reduction in doublecortin-expressing immature neurons at 21 and 56 d (but not at 1 d) after soman exposure as compared to age-matched, vehicle-treated controls. To evaluate the functional consequences of reduced neurogenesis on hippocampal function, high frequency stimulation (HFS) of the medial perforant pathway was used to induce long-term potentiation (LTP) in DG neurons. While robust LTP was observed in all vehicle control mice and in soman-exposed mice at 1 d, LTP was completely eliminated in soman-exposed mice at 21 and 56 d. These data suggest that impaired hippocampal neurogenesis has both delayed and chronic effects on DG function in NASE survivors. Ongoing studies are focused on determining the precise mechanism(s) and timing of impaired neurogenesis.

Disclosures: K.M. Hoffman: None. **K.L. Schulz:** None. **E.K. Johnson:** None. **J.K. Chandler:** None. **P.M. McNutt:** None.

Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 47.18/S1

Topic: A.04. Transplantation and Regeneration

Support: CIRM grant TR4-06648

NEI grant 1R01EY024045

Title: Histological characterization of stem-cell derived 3D retinal progenitor sheet transplants in an immunodeficient retinal degenerate rat model

Authors: A. MATHUR[1], B. T. MCLELLAND[1], M. SARY[1], J. WAN[1], G. NISTOR[3], I. NASONKIN[4], R. ARAMANT[1], H. S. KEIRSTEAD[3], *M. J. SEILER[2];
[1]Sue & Bill Gross Stem Cell Res. Ctr., [2]Phys.Med.&Rehab../Sue & Bill Gross Stem Cell Res. Ctr., UC Irvine, Sch. of Med., Irvine, CA; [3]AiVita Biomed., Irvine, CA; [4]Bio Time Inc., Alameda, CA

Abstract: Incurable eye diseases such as age related macular degeneration (AMD) and retinitis pigmentosa (RP) are characterized by retinal degeneration (RD). While current therapies aim to delay photoreceptor death, replacement with retinal progenitor sheets has the potential to restore vision. The current study investigated whether transplants of human stem cell (hESC) derived retinal progenitor sheets could replace the degenerating cells of the host retina and make functionally relevant connections (see abstract Thomas et al.). Our RD model is an immunodeficient (nude) rho SS34ter-line3 rat (will not reject human cells) in which most of the outer nuclear layer (ONL) is absent by P30. hESCs were differentiated into retinal organoids (retinoids) following a protocol similar to Zhong et al. 2014 (Nature Communications 5:4047). One set of transplants was differentiated after Singh et al. 2015 (Stem Cells & Development 24:2778). These 3D retinoids (differentiation day 30-63) were dissected into sheets and then transplanted into the host subretinal space (P25-30). Cryostat sections through the transplants 50-300 days post-surgery (DPS) were stained with hematoxylin and eosin (H&E), or processed for immunohistochemistry to label human donor, retinal cells and synaptic markers using light and confocal microscopy. RD sham surgery eyes (150+ DPS) had a complete loss of ONL, however the inner nuclear (INL), plexiform (IPL) and ganglion cell layers (GCL) appeared intact. Transplanted progenitor sheets developed a rosetted morphology in vivo, with a distinct ONL and photoreceptor inner and outer segments located in the center of the rosettes. Transplants (stained with human marker SC-121) extended processes into the host IPL and GCL. Outer segments clearly stained for rhodopsin within the rosettes. The mature retinal markers PKCα (rod bipolar), recoverin (rods and cones, cone bipolar) or rhodopsin (rod outer segments) showed less expression in younger (<100 DPS) than in older (150+ DPS) transplants (labeled with either

SC-121 or Ku80). Neuronal processes of human donor cells (human neurofilament) were dense within the transplant area and extended into the host IPL in all transplants. The labeling of synaptic markers (bassoon and synaptophysin) indicated the presence of functional connections, possibly between the transplant and host retina. In summary, hESC-derived retinal progenitor sheet transplants develop mature retinal cell subtypes including photoreceptors and integrate with the RD host retina.

Disclosures: **A. Mathur:** None. **B.T. Mclelland:** None. **M. Sary:** None. **J. Wan:** None. **G. Nistor:** None. **I. Nasonkin:** None. **R. Aramant:** None. **H.S. Keirstead:** None. **M.J. Seiler:** None.

Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 47.19/S2

Topic: A.04. Transplantation and Regeneration

Support: California Institute for Regenerative Medicine Grant TG2-01153 to AA-B

California Institute for Regenerative Medicine Grant R01EY025174 (to A.A.-B.)

NIH R01EY025174 (to A.A.-B. and M.P.S.)

NIH R01EY02874 (to M.P.S.)

NIH R01DC014101 (to A.H.)

T32GM007618 (to Y.T.)

R25NS070680 (to P.L.)

Title: Heterochronic transplants of caudal ganglionic eminence precursors disperse into host visual cortex and adapt lineage-appropriate lamination and neurochemical identity

Authors: J. SPATAZZA[1], P. LARIMER[2], S. ESPINOSA[3], Y. TANG[4], *A. R. HASENSTAUB[5,6], M. P. STRYKER[3], A. ALVAREZ-BUYLLA[1];
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Abstract: Cortical interneurons originate from either the medial ganglionic eminence (MGE) or from the caudal ganglionic eminence (CGE). Previous studies have shown that transplanted MGE precursors can migrate and functionally integrate into a variety of heterochronic environments. It is not known whether heterochronic transplantation of CGE precursors can induce similar gains, or whether CGE precursors can survive, disperse, and laminate appropriately. We show that CGE cells transplanted into the postnatal visual cortex survive, disperse widely, and adopt laminar locations and overall dendritic morphology appropriate for their lineage. In addition, the mature cells demonstrate immunocytological markers characteristic of their pretransplant fate. We further show that anatomically isolated CGE transplants contain a subpopulation of MGE cells. We used a genetic approach for the elimination of MGE-derived interneurons from CGE transplants by expressing a diphtheria toxin alpha subunit in *Nkx2.1*-expressing neurons. These genetically purified CGE transplants disperse and laminate nearly identically to the anatomically isolated CGE transplants, without the PV- and SST-expressing interneurons ordinarily seen following transplantation of anatomically isolated CGE. This work shows that heterochronically transplanted CGE neurons can integrate appropriately into visual cortex and that they retain the lamination and immunocytological fates of their lineage.

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Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

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Topic: A.04. Transplantation and Regeneration

Support: NIH Grant R01EY025174 (to A.A.-B.)

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NIH Grant K22NS089799 (to J.S.E.)

NIH Grant T32GM007618 (to Y.T.)

Title: Heterochronic transplants of caudal ganglionic eminence precursors are functionally integrated into visual cortex but do not induce ocular dominance plasticity

Authors: *P. LARIMER[1,5,6], J. SPATAZZA[7,9], J. S. ESPINOSA[8,5,2], Y. TANG[7,9], M. KANEKO[8], A. ALVAREZ-BUYLLA[7,9], M. P. STRYKER[8,3,10], A. R. HASENSTAUB[8,4,5]; [1]Neurol., [2]Dept. of Physiol., [3]Dept. of Physioglogy, [4]Dept. of Otolaryngology, Univ. of California San Francisco, San Francisco, CA; [5]Kavli Inst. for Fundamental Neurosci., San Francisco, CA; [6]Ctr. for Integrative Neurosci., San Francisco, CA; [7]Dept. of Neurolog. Surgery, [8]Ctr. for Integrative Neurosci., UCSF, San Francisco, CA; [9]Eli and Edythe Broad Ctr. for Regenerative Med., San Francisco, CA; [10]Kavli Ctr. for Fundamental Neurosci., San Francisco, CA

Abstract: Maturation of inhibitory GABAergic cortical circuits regulates experience-dependent plasticity. We recently showed that the heterochronic transplantation of interneurons from the medial ganglionic eminence (MGE) reactivates ocular dominance plasticity (ODP) in the postnatal mouse visual cortex. Genetic deletion of parvalbumin (PV) or somatostatin (SST) expressing interneurons from those transplants demonstrated that PV and SST cells each have the capacity to reactivate ODP. Might other types of interneurons similarly induce cortical plasticity? Here we establish that although heterochronic transplantation of anatomically isolated caudal ganglionic eminence reactivates ODP, genetic elimination of the small fraction of MGE cells (which are normally present in anatomically isolated CGE, as they migrate through the CGE en route to caudal cortex) eliminates the ability of CGE transplants to induce ODP. We use brain slice recording techniques to demonstrate that CGE transplants develop diverse active and passive physiological properties consistent with CGE lineage interneurons. In addition, we demonstrate that interneurons derived from hetereochronic CGE transplantation demonstrate orientation selective visual responses and are synaptically coupled to local excitatory and inhibitory neurons, consistent with their CGE lineage fate. These findings demonstrate that genetically purified heterochronically transplanted CGE cells can functionally integrate into host visual cortical circuits without allowing heterochronic ODP, thus illustrating the unique role of MGE lineage neurons in transplant induced ODP.

Disclosures: **P. Larimer:** None. **J. Spatazza:** None. **J.S. Espinosa:** None. **Y. Tang:** None. **M. Kaneko:** None. **A. Alvarez-Buylla:** None. **M.P. Stryker:** None. **A.R. Hasenstaub:** None.

Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 47.21/S4

Topic: A.04. Transplantation and Regeneration

Support: CIRM TR4-06648 (MS)

NIH R01EY024890

Title: Restoration of visual cortical responses after retinal sheet transplantation in rats with retinal degeneration

Authors: *A. T. FOIK[1], G. A. LEAN[1], B. T. MCLELLAND[2], A. MATHUR[2], R. B. ARAMANT[2], M. SEILER[2], D. C. LYON[1];
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Abstract: Age-related macular degeneration and retinitis pigmentosa lead to a profound loss of vision in millions of people worldwide. Many of these patients lose both retinal pigment epithelium (RPE) and photoreceptors. Fetal-derived retinal progenitor sheets have been successfully transplanted into both rodents (*review: Seiler and Aramant, 2012 Prog Retin Eye Res, 31:661-87*) and humans (*Radtke et al, 2008 Am J Ophthalmol, 146:172-182*). In several models of retinal degeneration (RD), transplants restore rudimentary responses to flashes of light in a region of the superior colliculus (SC) corresponding to the location of the transplant in the host retina; and synaptic connectivity between transplant and RD host retina has been confirmed. However, in order to determine the quality and accuracy of visual information provided by the transplant, here we study visual responsivity at the level of visual cortex where higher visual perception is processed. Specifically, we used the transgenic *Rho S334ter-3* RD rat, which begins to lose photoreceptors at an early age, becoming blind shortly after 1 month post-natal. Between 24-40 days of age, RD rats received fetal rat retinal sheet transplants in one eye. Donors were rats expressing human placental alkaline phosphatase in all cells. Three to ten months following surgery, we found several neurons in the region of primary visual cortex (V1) matching the transplanted portion of the retina that were well tuned to stimulus orientation, size, contrast, and spatial and temporal frequency. Each of these response features are considered fundamental properties of V1 neurons that are necessary building blocks for higher level visual processing, such as shape and motion perception. In addition, we find that these response properties are absent in non-transplanted and sham-transplanted RD rats, but are on par with normal age-matched controls that do not suffer from RD. Moreover, in rats with normal retinas and RD rats with retinal transplants spontaneous firing rates were low, whereas in the RD rats without transplants, spontaneous firing rates were often higher, indicating abnormal function in the absence of visual input. In conclusion, our data thus far indicate that fetal rat retinal sheet transplants can restore visual cortical responses in transgenic *Rho-S334ter-3* RD rats. This restoration of 'normal' cortical physiology in a rat model represents a critical step towards developing an effective remedy for the visually impaired human population.

Disclosures: **A.T. Foik:** None. **G.A. Lean:** None. **B.T. McLelland:** None. **A. Mathur:** None. **R.B. Aramant:** None. **M. Seiler:** None. **D.C. Lyon:** None.

Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

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Topic: A.04. Transplantation and Regeneration

Support: CIRM grant TR4-06648

NEI grant 1R01EY024045

Title: *In vivo* imaging and functionality of stem-cell derived 3D retinal organoid (retinoid) transplants assessed in an immunodeficient rat disease model

Authors: *B. B. THOMAS[1], B. T. MCLELLAND[2], A. MATHUR[3], B. LIN[2], J. SIGMAN[2], P. N. PATEL[2], G. NISTOR[4], L. M. KITZES[2], I. NASONKIN[5], R. B. ARAMANT[2], H. J. KEIRSTEAD[4], M. J. SEILER[2];
[1]Ophthalmology, USC, Los Angeles, CA; [2]UC Irvine Sch. of Med., Irvine, CA; [3]UC Irvine, Sch. of Med., Irvine, CA; [4]AiVita Biomed. Inc., Irvine, CA; [5]Biotime Inc, Alameda, CA

Abstract: Retinal degeneration (RD) diseases such as Age-related Macular Degeneration (AMD) and Retinitis Pigmentosa (RP) affect millions of people worldwide. Most treatment strategies can only delay the progression of the diseases. Our goal is to repair the damaged retina and restore the lost vision by transplanting stem-cell derived, 3D retinal organoid (retinoid) sheets. Based on qPCR and immunohistochemistry, we have shown previously that the human embryonic stem cell (hESC) derived retinoids develop retinal markers and lamination over time (McLelland et al Neuroscience abstract 2015). The data suggested a gene expression pattern similar to the developing human fetal eye. We hypothesize that after transplantation into immunodeficient rho S334ter line-3 RD rats (nude RD rats) - that do not reject human cells - the hESC derived retinal progenitor tissue will create new photoreceptors for the host and generate new synaptic connections for phototransduction processing leading to visual function. To test this, stem-cell derived 3D retinoids, created based on a protocol modeled after Zhong et al. 2014 (Nature Communications 5:4047), were transplanted unilaterally into the subretinal space of 24-30 day old RD nude rats. One set of transplants was differentiated after Singh et al. 2015 (Stem Cells & Development 24:2778). Transplants were routinely imaged by optical coherence tomography (OCT) at 2-4 weeks after transplantation and then in 2-month intervals, and were selected for functional testing based on OCT. Optokinetic testing of visual acuity (= OKN) and superior colliculus (SC) electrophysiology was performed at 4 - 9 months after transplantation. In OCT imaging, transplants developed layers, integrated with the host and photoreceptor rosettes were recognizable in transplants 4-6 months after transplantation. Histological analysis showed that transplants developed photoreceptors in rosettes (see abstract by Mathur et al.). OKN testing demonstrated significant improvement in visual acuity of the hESC-derived retinoid

transplanted rats ($P<0.05$ for months 3-5, paired t test, transplanted eye vs non-surgery eye). Electrophysiological recordings from the SC showed that visual function was observed in the majority of the RD nude rats after hESC-derived retinoid transplantation (4/6) whereas age-matched RD controls had no visual responses. Based on these data, it may be concluded that hESC-derived retinoid transplants in rho S334ter line-3 nude rats can integrate with the host retina and support improved visual function.

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Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 47.23/S6

Topic: A.04. Transplantation and Regeneration

Support: 2014CB942800

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31421091

91332110

31271157

31228012

NCET-12-0128

Title: Fear erasure facilitated by immature inhibitory neuron transplantation

Authors: ***Y.-C. YU**, W.-Z. YANG, T.-T. LIU, J.-W. CAO, L.-Y. LIU;
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Abstract: Transplantation of embryonic GABAergic interneurons has been demonstrated to modify disease phenotypes in rodent models of neurologic and psychiatric disorders. However, whether transplanted interneurons can modulate fear memory remains largely unclear. Here, we show that transplantation of embryonic interneurons into the amygdala does not alter host fear memory formation. However, approximately two weeks after transplantation, but not earlier or later, extinction training produces a marked reduction in spontaneous recovery and renewal of fear response. Further analyses reveal that transplanted interneurons robustly form functional synapses with neurons of the host amygdala and exhibit similar developmental progresses as endogenous amygdala interneurons. Importantly, transplanted immature interneurons reduce the expression of perineuronal nets, promote long-term synaptic plasticity, and modulate amygdala circuit dynamics. Our findings demonstrate that transplanted immature interneurons modify amygdala circuitry and suggest a previously unknown strategy for the prevention of extinction-resistant pathological fear.

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Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

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Program#/Poster#: 47.24/S7

Topic: A.04. Transplantation and Regeneration

Support: NHMRC Grant #1083569

Australian Postgraduate Award

Title: Spontaneous neuronal repopulation within the dorsal hippocampus following an acute kainic acid-mediated excitotoxic injury

Authors: *Y. YIN[1,2], L. M. KONEN[2], C. W. VAUGHAN[1], B. VISSEL[2,3,4];
[1]Kolling Inst. of Med. Res., Univ. of Sydney, Sydney, Australia; [2]Dept. of Neurosci., Garvan Inst. of Med. Res., Sydney, Australia; [3]Fac. of Sci., Univ. of Technol., Sydney, Australia; [4]Fac. of Med., UNSW Australia, Sydney, Australia

Abstract: Excitotoxicity is a contributing factor to a variety of neurodegenerative disorders and acute brain injuries. Additionally, neuronal repopulation outside of well-established neurogenic regions is highly controversial. The assumption that the central nervous system (CNS) has a poor capacity for recovery after injury has led to the view that only exogenous interventions can drive CNS repair. The present study aims to demonstrate the extent to which spontaneous neuron

repopulation of the CA1/CA3 dorsal hippocampal regions is possible following an acute excitotoxic injury, based on varying degrees of induced injury.
Excitotoxic neurodegeneration was induced by stereotaxic injection of kainic acid (KA; 1μg/μl) in one of several discrete locations (intrahippocampal-dorsal, intrahippocampal-ventral, intracerebroventricular (ICV) and amygdalar) in male C57BL/6 mice. Vehicle controls were injected with sterile phosphate buffered saline (PBS) at the same coordinates. Mice were perfused with 4% paraformaldehyde at 2 and 8 weeks post-injection (wpi), and brains were removed. The level of neuronal injury and regeneration was assessed in the CA1 and CA3 by immunohistochemistry, using NeuN as a marker of mature neurons. Non-fluorescent quantification of neuron populations was performed using StereoInvestigator7.
ICV injection of KA produced profound loss of neurons in the CA1 ($p<0.001$) and CA3 ($p<0.05$) regions, ipsilateral to the site of injury at 2 wpi, as compared to vehicle treated controls. However, by 8 wpi, the CA1 neuronal population was significantly increased in KA treated mice compared to 2 wpi, and was not significantly different to vehicle treated controls. Furthermore, both the CA1 and CA3 neuronal populations quantified at 8 wpi were comparable to PBS-injected animals, suggesting repopulation of the CA1 and CA3 regions. KA injection into the other three locations resulted in varying degrees of observed damage with subsequent effects on the magnitude of neuronal repopulation within the CA1 and CA3 regions. The results from the present study suggest the existence of intrinsic mechanisms for spontaneous repair and recovery within the brain in the absence of exogenous interventions. By providing the framework for understanding the extent to which spontaneous neurogenesis can occur in the unperturbed brain, we hope to inform current and future therapies for treatment of acute brain injuries and disorders.

Disclosures: **Y. Yin:** None. **L.M. Konen:** None. **C.W. Vaughan:** None. **B. Vissel:** None.

Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

Location: Halls B-H

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Program#/Poster#: 47.25/S8

Topic: A.04. Transplantation and Regeneration

Support: Stem Cell Research Fund

Department of Genetics, Development and Cell Biology

Title: Characterization of neural progenitor cell differentiation on poly (ε-caprolactone) microfibers

Authors: *B. B. PATEL[1,2], F. SHARIFI[3], D. P. STROUD[1], N. HASHEMI[3], D. S. SAKAGUCHI[1,2];
[1]Genetics, Develop. and Cell Biol., [2]Neurosci. Program, [3]Dept. of Mechanical Engin., Iowa State Univ., Ames, IA

Abstract: Biomaterials have become increasingly important for development of novel biomedical and therapeutic applications. Poly (ε-caprolactone) (PCL) is a biodegradable synthetic polymer used for fabricating microfibers using a microfluidic approach. PCL fibers help align cells and may be used for applications in axon regeneration and directed cell migration. These microfibers may mimic the microenvironment and provide a more complex 3-D platform in which cells can differentiate and proliferate. Multipotent adult hippocampal progenitor cells (AHPCs) have the ability to differentiate into neurons, oligodendrocytes and astrocytes. In this project our goal is to investigate the adhesion, proliferation, and differentiation of rat AHPCs *in vitro* growing on PCL microfibers. A panel of cell type specific antibodies was used to identify neural progenitors, neurons and glial cells. Proliferating cells were immunolabeled with Ki67 antibody, neural progenitor/stem cells were detected with Sox2 and Nestin antibodies, neuronal cell differentiation characterized by TuJ1 and MAP2ab antibodies and glial cell differentiation characterized by GFAP antibody. Preliminary results have indicated that the PCL microfibers support cell adhesion, survival, proliferation and differentiation of the AHPCs based on immunolabeling experiments. AHPCs play an important role in adult neurogenesis and due to the limited capacity of CNS to regenerate, they may become an important cell population for transplantation and the development of brain repair strategies. Transplantation of neural progenitor/stem cells within a PCL microfiber scaffolding construct may provide important biological and topographic cues that facilitate the survival, differentiation and integration of transplanted cells.

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Poster

047. Transplantation and Integration of Neural Precursors in the Adult Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 47.26/S9

Topic: I.04. Physiological Methods

Title: Combining optogenetics and *In vivo* two photon calcium imaging to explore the functional impact of newborn neurons in the mouse olfactory bulb

Authors: *C. FOIS[1], S. PÉRON[2], N. MARICHAL[2], P.-H. PROUVOT[1], B. BERNINGER[2], A. STROH[1];
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Abstract: In the mammalian brain, adult neurogenesis is maintained within two neurogenic niches located in the subgranular zone (SGZ) of the hippocampus and in the subventricular zone (SVZ) of the lateral ventricles. Neuroblasts migrate from the SVZ via the rostral migratory stream (RMS) towards the olfactory bulb where they integrate into the pre-exisiting neuronal circuitry. However, the functional impact of the newborn neurons - mainly GABAergic interneurons - to the OB microcircuitry of the mitral/tufted (M/T cells) cells layer, representing the main output of the OB, remains poorly understood. Here, we establish the combination of optogenetics, two-photon calcium imaging, immunohistochemistry and electrophysiology for a causal understanding of how the spontaneous activity and the odor processing of the M/T cells are modulated by the mature newly integrated neurons in the main OB. To specifically and precisely control adult-born OB neurons activity, an adeno-associated viral vector (AAV) encoding the hyperpolarizing opsin ArchT is delivered to the OB neuron progenitors that are located in the SVZ. To monitor M/T cells activity, a second AAV vector coding for the genetic calcium indicator (GECI) GCaMP6f is injected locally in the dorsal OB at the depth of 350-400 µm. Subsequently, a chronic optical window is implanted at the level of the dorsal OB to allow for repeated 2P imaging. Six weeks post-injections, the M/T cells strongly express GCaMP6f and the ArchT-expressing newborn neurons have migrated to the OB where they are becoming fully integrated and mature. A custom-made 2P microscope is then used to record spontaneous and odor-evoked activity of the M/T cells (30.5 Hz) with and without the functional contribution of the newborn neurons. A mix of several odorant molecules known to activate the dorsal glomeruli are presented to the mouse's snout to elicit odor-evoked responses in the M/T cells. To transiently optogenetically inhibit newborn neurons 552 nm light (green) is delivered by an optic fiber (200 µm diameter) to the ArchT-expressing interneurons during the odor processing. The challenging combination of these techniques will provide unprecedented levels of understanding of the functional role that a complex process such as neurogenesis plays in OB microcircuitry.

Disclosures: C. Fois: None. **S. Péron:** None. **N. Marichal:** None. **P. Prouvot:** None. **B. Berninger:** None. **A. Stroh:** None.

Poster

048. Disorders of the Auditory or Visual System

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 48.01/S10

Topic: D.01. Sensory Disorders

Support: NSF BCS-0821855

Title: Reduced binocular summation of fMRI responses to visual stimulation in ventral extrastriate cortex in anisometropic amblyopia is related to visual cortical GABA concentration

Authors: *K. N. BYRNE[1,2,3], E. YANG[2,3], L. LI[4], D. M. LEVI[1,2,5], M. A. SILVER[1,2,3,5]; [1]Vision Sci. Grad. Group, [2]Sch. of Optometry, [3]Henry H. Wheeler Jr. Brain Imaging Ctr., [4]Cognitive Sci. Program, [5]Helen Wills Neurosci. Inst., Univ. of California Berkeley, Berkeley, CA

Abstract: Background: Amblyopia is a neurodevelopmental disorder characterized by reduced visual acuity due to abnormal early visual experience. Previous brain imaging studies have yielded mixed results regarding visual cortical abnormalities in amblyopia, and none have compared responses to monocular, binocular (identical stimuli in the two eyes), and dichoptic (different stimulus in each eye) stimuli. Recent studies have linked occipital GABA levels with fMRI response amplitude in the visual cortex of healthy individuals. Here, we measured fMRI responses to binocular, monocular, and dichoptic stimulation to measure binocular summation in anisometropic amblyopes and healthy controls, and we correlated these measures with visual cortical GABA levels.
Methods: We recorded fMRI activity in retinotopically-defined early visual cortical areas in twelve subjects with anisometropic amblyopia and fifteen visually normal control subjects for four stimulus conditions: binocular, dichoptic (orthogonal orientations in the two eyes), monocular stimulation of the amblyopic eye (for controls, the non-dominant eye) (monocularAE), and monocular stimulation of the fellow non-amblyopic eye (for controls, the dominant eye) (monocularFE). In addition, magnetic resonance spectroscopy was performed in the absence of visual stimulation to measure resting GABA concentration in a 3 cm^3 voxel centered on the calcarine sulcus.
Results: We quantified binocular summation of visual cortical responses by comparing the amplitude of fMRI responses to binocular stimulation to the responses to dichoptic and to monocular stimulation. In cortical area V2v, we found significant differences between amblyopic and control subjects for binocular-monocularAE ($p=0.048$), binocular-monocularFE ($p=0.026$), and binocular-dichoptic ($p=0.005$), with amblyopes showing significantly less binocular summation than controls. Similar group differences were obtained in area V3v for binocular-dichoptic ($p=0.016$). In addition, visual cortical GABA concentration was inversely correlated with binocular-monocularAE in V1 ($p=0.005$) and V2v ($p=0.035$) and with binocular-dichoptic in V2v ($p=0.024$) in the combined group of subjects.
Discussion: Our results demonstrate reduced binocular summation of fMRI responses in ventral (V2v and V3v) but not dorsal (V2d and V3d) extrastriate cortex in anisometropic amblyopia. This finding may be related to the previously reported dorsoventral asymmetry in $GABA_A$ receptor density in V2 and V3 in healthy subjects. In addition, binocular summation was inversely correlated with visual cortical GABA levels across all subjects.

Disclosures: **K.N. Byrne:** None. **E. Yang:** None. **L. Li:** None. **D.M. Levi:** None. **M.A. Silver:** None.

Poster

048. Disorders of the Auditory or Visual System

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 48.02/S11

Topic: D.01. Sensory Disorders

Support: Grant-in-Aid for Challenging Exploratory Research 25560197

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Grant-in-Aid for JSPS Fellows 13J01314

Title: Spatiotemporal excitation of the primary visual cortex induced by intracortical microstimulation in the Royal College of Surgeons rats

Authors: ***S. MIYAMOTO**, N. SUEMATSU, Y. UMEHIRA, Y. HAYASHIDA, T. YAGI; Grad. Sch. of Engin., Osaka Univ., Suita-Shi, Japan

Abstract: Visual prostheses by means of intracortical microstimulation has a potential to restore a certain degree of visual function in the patients suffering from acquired vision loss. However, there have been reports that the visual cortical excitability is likely to be reduced in the blind patients. Therefore, in order to design the best suitable stimulation for the prostheses, it is desirable to understand the quantitative relationship between the stimulus parameters and the induced responses in relevant animal models. Although the Royal College of Surgeons (RCS) rat has been known for several decades as an animal model with inherited retinal degeneration, little has been known about the neural responses to microstimulation in their visual cortex. Thus, in the present, we examined neural excitability of the visual cortex in the RCS rats by using the voltage-sensitive dye (VSD) imaging technique. The RCS rats used here were grouped into early (3-5 week-old, $n = 5$), middle (9-10 week-old, $n = 6$) and end (88-100 week-old, $n = 5$) stages. The neural responses to single-pulse current stimuli (cathodic-first biphasic, 0.2 msec/phase, 0.675-5.33 nC/phase) delivered through a metal-based intracortical electrode were recorded by means of the VSD fluorescence. As results, we found no statistically significant difference among the three groups in terms of the intensity-response (I-R) profiles measured in the electrode vicinity; namely 1) the threshold stimulus charges (in nC/phase) were 0.47/0-0.63 (median/1st-3rd quartiles) in the early, 0.62/0-0.71 in the middle, and 0.22/0-0.60 in the end stages, 2) the Hill coefficients of the I-R curves were 2.00/1.97-2.30 in the early, 1.55/1.34-2.00

in the middle, 1.75/1.00-2.08 in the end stages. On the other hand, spatial extents of the trans-synaptic responses changed in their shapes more elliptical with age; namely the spatial aspect ratios of the responding areas were 1.41/1.33-1.58 in the early, 1.52/1.38-1.62 in the middle, 1.62/1.51-1.94 in the end stages. These results suggested that although the synaptic connections could be remodeled with age in the RCS rats, yet the intracortical microstimulation is valid to induce the neural excitations even in the end stage of the retinal degeneration.

Disclosures: S. Miyamoto: None. **N. Suematsu:** None. **Y. Umehira:** None. **Y. Hayashida:** None. **T. Yagi:** None.

Poster

048. Disorders of the Auditory or Visual System

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 48.03/S12

Topic: D.01. Sensory Disorders

Title: A case report of visual and motor recovery after cognitive sensorimotor rehabilitation in a patient with cortical blindness

Authors: D. DE PATRE[1], C. PERFETTI[1], F. PANTE[1], C. RIZZELLO[1], M. ZERNITZ[1], *A. VAN DE WINCKEL[2];
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Abstract: Background and purpose: Spontaneous partial visual recovery happens 1-6 months after onset of cortical blindness. Further recovery can occur with visual rehabilitation. However, currently there is no gold standard therapy; clinical outcomes are variable and rarely translate into improvements in daily life activities (ADL).
The purpose of this study was to demonstrate feasibility and potential value of cognitive sensorimotor rehabilitation in a patient with cortical blindness.
Case description: A 48 year old female patient, with severe cortical blindness and tetraplegia caused by hypoxia after cardiac arrest, was dependent in ADL and distinguished shapes and colors after 1.5 year of standard visual rehabilitation. She then started cognitive sensorimotor rehabilitation for 8 months, 5 days/week, 3 hours/day, consisting of discrimination exercises correlating sensory and visual information to reconstruct vision and improve daily life motor performance. Clinical assessments and PET imaging were performed before and after rehabilitation.
Outcomes: Visual performance significantly improved: Her field of view increased to 15*10cm; she recognized and described objects; watched television; and used her cell phone. She improved

45 points (65/100) on the “Barthel ADL index”, reflecting independence in self-care and improved walking. She increased 23 points (48/58) on the “Motor Evaluation of Upper Extremity in Stroke Patients” (MESUPES), i.e. she moved her arm and hand accurately. She improved 23 points (57/70) on the “Warwick-Edinburgh Mental Well-being Scale” (WEMWBS), i.e. she felt self-reliant.
Before rehabilitation, PET imaging confirmed reduced glucose metabolism in the visual cortex. After rehabilitation, glucose metabolism increased in the occipital, frontal and parietal cortex. Correlating sensory and visual information during rehabilitation possibly provides an alternative route to reactivate preserved visual areas.
Conclusions: This study demonstrates feasibility of cognitive sensorimotor rehabilitation in a patient with cortical blindness, who experienced an impressive clinical visual and motor recovery with marked ADL improvement, more than 2 years after onset.

Disclosures: D. De Patre: None. **C. Perfetti:** None. **F. Pante:** None. **C. Rizzello:** None. **M. Zernitz:** None. **A. Van De Winckel:** None.

Poster

048. Disorders of the Auditory or Visual System

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 48.04/S13

Topic: D.01. Sensory Disorders

Support: NIH Grant EY022358

Title: Sodium channel redistribution in the DBA/2J mouse model of glaucoma

Authors: *M. A. SMITH, G. N. WILSON, C. M. DENGLER-CRISH, J. R. RICHARDSON, S. D. CRISH;
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Abstract: Axonopathy is an early pathology common to many chronic neurodegenerations. We have previously reported that semifunctional retinal ganglion cell (RGC) axons persist after anterograde axonal transport deficits in two mouse models of glaucoma, a group of optic neuropathies that make up the leading cause of irreversible blindness worldwide. In other neurodegenerative conditions that exhibit persistent axons, there are multiple alterations in axonal structure and physiology that can be protective or detrimental. The best characterized of these changes is in multiple sclerosis (MS) and its animal models, experimental autoimmune encephalomyelitis (EAE). In these disorders, there is extensive redistribution and differential expression of sodium channels (Nav) are thought to play a major role, both protective and

detrimental, in the course of the disease. Regarding glaucoma, Nav blockade has been reported to be neuroprotective in one model, but little is known about distribution of sodium channels in this disorder. Using tract tracing, immunofluorescence, and capillary-based electrophoresis we examined the nodes of Ranvier in a common mouse model of chronic glaucoma, the DBA/2J mouse. In agreement with previous work (and contrasting with MS/EAE) we did not find overt demyelination; however, RGC axons in pathological animals exhibited substantial expansion of the nodal region. Most intriguingly, as in MA/EAE, we found redistribution of the "mature node" sodium channel Nav1.6 and massive increases in the "premyelination channel" Nav1.2. These changes suggest that these persisting axons are actively undergoing compensatory processes that may lead to impaired neuronal signaling.

Disclosures: M.A. Smith: None. **G.N. Wilson:** None. **C.M. Dengler-Crish:** None. **J.R. Richardson:** None. **S.D. Crish:** None.

Poster

048. Disorders of the Auditory or Visual System

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 48.05/S14

Topic: D.01. Sensory Disorders

Support: EY026662

Title: Bioenergetic analysis of optic nerve mitochondria in the DBA/2J model of glaucoma

Authors: *L. L. COUGHLIN[1,3], P. T. KANG[2], D. M. INMAN[1];
[1]Dept. of Pharmaceut. Sci., [2]Integrative Med. Sci., Northeast Ohio Med. Univ., Rootstown, OH; [3]Sch. of Biomed. Sci., Kent State Univ., Kent, OH

Abstract: In glaucoma, retinal ganglion cell (RGC) axons that make up the optic nerve degenerate, leading to irreversible blindness. As with other neurodegenerative diseases, alterations in mitochondrial morphology, autophagy/mitophagy, and ATP production have been observed in glaucoma. Specifically, in the DBA/2J (D2) mouse glaucoma model, it has been observed that mitochondria in the optic nerve are both smaller in size and more numerous than in age-matched controls. In addition to the altered appearance of optic nerve mitochondria, optic nerves from aged mice with increased intraocular pressure (IOP) produce less ATP. Despite the changes in mitochondrial morphology and energy levels reported in aged D2 optic nerves, the functional capacity of optic nerve mitochondria has yet to be fully characterized. The purpose of this study is to determine when mitochondrial respiration differences in glaucoma occur, and how they relate to other known events in the development of glaucoma pathology. Visual

acuities, IOP, and anterograde transport were measured in 3, 6, and 10 month D2 and control strain DBA/2-$^{wt\text{-}gpnmb}$ (D2G) mice. To measure mitochondrial respiration, optic nerves were analyzed using a Seahorse XF24 flux analyzer. Optic nerves were freshly dissected, sectioned, and placed in a 24 well islet capture plate. Optic nerve mitochondria were subjected to a stress test that included sequential exposure to oligomycin (10μg/ml), FCCP (4μM), and antimycin-A (1μM); extracellular acidification rate (ECAR) was also measured as a readout for glycolysis. Differences in OCR and ECAR were compared between strains, across age groups, and with regard to anterograde axon transport status; these comparisons were correlated with IOP and visual acuity. This work is designed to give a novel and detailed time course of mitochondrial respiration in glaucomatous optic nerve and confirm the role of mitochondrial failure in early glaucoma development.

Disclosures: L.L. Coughlin: None. **P.T. Kang:** None. **D.M. Inman:** None.

Poster

048. Disorders of the Auditory or Visual System

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 48.06/T1

Topic: D.01. Sensory Disorders

Support: EY002358

ES021800

Title: Effects of IL-4 on inflammation in the visual projection of DBA/2J glaucomatous mice

Authors: *G. N. WILSON[1,2], M. A. SMITH[2], C. M. DENGLER-CRISH[2], J. R. RICHARDSON[2], S. D. CRISH[2];
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Abstract: Glaucoma is a chronic neurodegeneration that is the leading cause of irreversible blindness worldwide. As with other neurodegenerations, neuroinflammation is a major component of the degeneration of retinal ganglion cells that characterize the disease. Indeed, we recently reported that neuroinflammation precedes overt degeneration in the DBA/2J mouse model of glaucoma, suggesting that anti-inflammatory treatment may be a viable target for intervention. Interleukin-4 (IL-4), an anti-inflammatory cytokine, has been shown to alter the microglial phenotype from a pro-inflammatory to anti-inflammatory state. Here, we injected recombinant IL-4 into the vitreal chamber of early glaucomatous (8-10 months old) and late glaucomatous (12-15 month old) DBA/2J mouse eyes. Forty-eight hours after IL-4 or vehicle

(1% BSA) injections, mice were anaesthetized, decapitated, and fresh retina, optic nerve (ON), and superior colliculus (SC) were dissected and immediately frozen on dry ice. Tissue was homogenized in T-Per buffer and cytokine levels were measured using bead-based multiplexing. We assessed levels of pro-inflammatory IL-1β, IL-6, TNF-α, and IL-12, as well as anti-inflammatory IL-10, and IL-4. Mirroring findings in other degenerative models, we found that IL-4 decreased pro-inflammatory cytokines within the treated projection (retina, optic nerve, and superior colliculus) when compared to vehicle injected projections. Anti-inflammatory IL-10 was significantly increased in treated projections. At older ages effects of IL-4 injection were not as prominent, suggesting that microglial phenotype may be less easily altered in aged animals or more effective in the earlier stages of degeneration. These findings suggest that exogenous application of IL-4 may be a viable mechanism to reduce inflammation and, potentially, neurodegeneration in glaucoma models.

Disclosures: G.N. Wilson: None. **M.A. Smith:** None. **C.M. Dengler-Crish:** None. **J.R. Richardson:** None. **S.D. Crish:** None.

Poster

048. Disorders of the Auditory or Visual System

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 48.07/T2

Topic: D.01. Sensory Disorders

Support: Call Retinitis Pigmentosa, Fondazione Roma

Title: Cortical plasticity in retinitis pigmentosa

Authors: *L. BARONCELLI, T. BEGENISIC, M. CENNI, A. SALE, L. GALLI;
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Abstract: Retinitis Pigmentosa (RP) is a family of inherited disorders caused by the progressive loss of retinal photoreceptors. There is no cure for RP, but research aimed at preventing further photoreceptor loss, or substituting new light-responsive elements of biological or artificial nature, is generating hope for these patients. These strategies require that the visual system downstream of the photoreceptors is capable of elaborating visual signals. Anatomical and functional studies have shown that retinal and thalamic structure are well preserved with RP, but the effect of photoreceptor degeneration on the visual cortex is still unknown. Here, we studied how visual cortical processing changed during the course of progression of RP, and whether the visual cortex retained the capability of plastic remodelling. We performed in vitro electrophysiological recordings of field excitatory post-synaptic potentials in V1. Basic synaptic

transmission, as assessed by response vs stimulus amplitude, showed a significant shallower response in RP mice. Biochemical analysis suggests that this synaptic deficit could be related to the alteration of absolute levels of inhibition and excitation in the visual cortex, with an overexpression of inhibitory markers. These results suggest that cortical changes occur in the visual cortex that might further compromise vision by downregulating or suppressing visual processing, as the retinal input progressively deteriorates. This work is supported by Fondazione Roma.

Disclosures: **L. Baroncelli:** None. **T. Begenisic:** None. **M. Cenni:** None. **A. Sale:** None. **L. Galli:** None.

Poster

048. Disorders of the Auditory or Visual System

Location: Halls B-H

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Program#/Poster#: 48.08/T3

Topic: D.01. Sensory Disorders

Support: MOST 105-2923-B-009 -001 -MY3

103-2221-E-039 -007 -MY3

Title: Differential changes in distribution of nitric oxide synthase at the central auditory relays after chronic injections of salicylate in rats

Authors: *T.-W. CHIU[1,2], C.-R. SOO[2], S.-T. HSU[1];
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Abstract: Tinnitus is a phantom perception of sounds in the absence of external acoustic stimuli. The effective cure remains unavailable since it underlying neural mechanisms remains unclear. Previous studies demonstrated the elevations in spontaneous and sound evoked neural activities at the central auditory relays in salicylate induced tinnitus animals. The increased activities was related to the excitation-inhibition imbalances but the causal link between the tinnitus generation and excitation-inhibition imbalances still unavailable. Nitric oxide (NO), a gas neurotransmitter, might be the key in the molecular signaling in the SS-tinnitus model since (i) the NO is function as a retrograde neurotransmitter to backward modulate the release of inhibitory neurotransmitter in long term potentiation; (ii) the neuronal form of nitric oxide synthase (nNOS) was widely expressed in the central auditory relays, and (ii) the number of NOS positive cells increased at the ventral cochlear nucleus of rats at after the acute salicylate treatment. However, it remains

unclear if the expression profile also changed in other central auditory relays (e.g., the auditory cortex) in chronic salicylate treated animals. Therefore, the aim of this study is to determine the expression and distribution profiles of NOS positive cells in chronic salicylate induced tinnitus rats.
Animals were randomly classified into control and experimental groups. The experimental rats were administrated with sodium salicylate injection (250mg/kg, i.p.) for five consecutive day. The controls were injected with same volume of phosphate buffer saline. On day 6, both control and experimental animals were placed inside a sound room for 8 h before sacrifice. Immunohistochemistry showed a significant increase in the number of nNOS positive cells at the ventral cochlear nucleus (VCN), auditory cortex (AC) and amygdala in salicylate treated rats. No apparent differences in nNOS positive neurons at the dorsal cochlear nucleus. Our results provide evidence to show changes of NOS positive cells at the amygdala and AC also have implications in the salicylate-induced tinnitus generation.

Disclosures: **T. Chiu:** None. **C. Soo:** None. **S. Hsu:** None.

Poster

048. Disorders of the Auditory or Visual System

Location: Halls B-H

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Topic: D.01. Sensory Disorders

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Title: Topographical functional connectivity patterns exist in the congenitally, prelingually deaf

Authors: *E. STRIEM-AMIT[1], J. ALMEIDA[3], M. BELLEDONNE[2], Q. CHEN[4], Y. FANG[5], Z. HAN[5], A. CARAMAZZA[2,6], Y. BI[5];
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Neurosci. and Learning; IDG/McGovern Inst. for Brain Resear, [5]State Key Lab. of Cognitive Neurosci. and Learning & IDG/McGovern Inst. for Brain Resea, Beijing Normal Univ., Beijing, China; [6]Ctr. for Mind/Brain Sci., Univ. of Trento, Rovereto, Italy

Abstract: Congenital deafness causes large changes in the auditory cortex structure and function, such that without early childhood cochlear-implant, profoundly deaf children do not develop intact, high-level, auditory functions. But how is auditory cortex organization affected by congenital, prelingual, and long standing deafness? Does the large-scale topographical organization of the auditory cortex develop in people deaf from birth? And is it retained despite cross-modal plasticity? We identified, using fMRI, topographic tonotopy-based functional connectivity (FC) structure in humans in the core auditory cortex, its extending tonotopic gradients in the belt and even beyond that. These regions show similar FC structure in the congenitally deaf throughout the auditory cortex, including in the language areas. The topographic FC pattern can be identified reliably in the vast majority of the deaf, at the single subject level, despite the absence of hearing-aid use and poor oral language skills. These findings suggest that large-scale tonotopic-based FC does not require sensory experience to develop, and is retained despite life-long auditory deprivation and cross-modal plasticity. Furthermore, as the topographic FC is retained to varying degrees among the deaf subjects, it may serve to predict the potential for auditory rehabilitation using cochlear implants in individual subjects.

Disclosures: E. Striem-Amit: None. **J. Almeida:** None. **M. Belledonne:** None. **Q. Chen:** None. **Y. Fang:** None. **Z. Han:** None. **A. Caramazza:** None. **Y. Bi:** None.

Poster

048. Disorders of the Auditory or Visual System

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 48.10/T5

Topic: D.01. Sensory Disorders

Support: NIH Grant RO1-DC008858

NIH Grant P30-DC008369

NIH Grant UL1-TR000153

Title: Disrupted auditory nerve activity limits peripheral but not central temporal acuity

Authors: *C. Q. PHAM[1,2], F.-G. ZENG[1,2,3,4,5];
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Abstract: Introduction. Auditory neuropathy refers to hearing pathologies affecting neural synchrony in the auditory nerve but sparing amplification in the inner ear. Patients with auditory neuropathy lack speech understanding despite having audibility. Neural discharges synchronized to frequencies up to several thousand Hz preserve the relative timing of action potentials propagated through several synaptic stages which encode auditory features for speech recognition. Auditory neuropathy subjects poorly recognize speech due to impaired temporal processing. The present study probed gap duration between 2 identical frequencies stimulating peripheral, overlapping nerve fibers and the same perceptual channel (within-channel; WC) or different frequencies stimulating non-overlapping nerve fibers and different perceptual channels (between-channel; BC). We intend to determine the contribution of neural synchrony to peripheral and central temporal acuity.
Methods. Three subjects pre-diagnosed with auditory neuropathy and 1 with an acoustic neuroma participated in the experiments. The subjects with auditory neuropathy had normal inner ear amplification (present cochlear nerve and outer hair cell responses) but absent/ abnormal auditory brainstem responses. The auditory neuropathy group had elevated audibility of octave frequencies 0.125 to 12 kHz, especially low frequency hearing loss as acoustic neuroma. Six subjects with audibility ≤20 decibels Hearing Level served as healthy, age- and gender-matched controls. A 3-interval forced choice, adaptive procedure measured subjects' just-noticeable-differences in gap (gap threshold) between two identical tone bursts (0.5:0.5; 2:2; 4:4 kHz; WC) and two different tone bursts (4:2 kHz; BC).
Results. Auditory neuropathy produce significantly impaired WC gap thresholds approximately an order of magnitude longer than the normal control. WC gap thresholds increased with increasing frequency and correlated significantly with the degree of hearing loss. Auditory neuropathy, however, produce normal BC gap thresholds.
Discussion. The present results suggest neural desynchrony due to auditory neuropathy decreases WC temporal acuity but produce no effect on BC temporal acuity. Peripheral machinery seems to limit WC processing on the order of several ms whereas central computations limit BC processing on the order of hundreds of ms. Auditory neuropathy appears to affect peripheral mechanisms producing a temporal delay of tens of ms, but central mechanisms remain unaffected. Differences in WC and BC gap detection can differentiate peripherally- vs. centrally-based temporal processing disorders.

Disclosures: C.Q. Pham: None. **F. Zeng:** None.

Poster

048. Disorders of the Auditory or Visual System

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 48.11/T6

Topic: D.01. Sensory Disorders

Title: A simple method to detect noise-induced “hidden” hearing loss

Authors: *H.-B. ZHAO, L. MEI, Y. ZHU;
Dept of Otolaryngology, Univ. Kentucky Med. Sch., Lexington, KY

Abstract: Noise-induced hearing loss is a major form of hearing disorders. Long-term, intensive noise can produce permanent hearing loss causing permanent hearing-threshold shift (PTS), whereas short-term noise-exposure can lead to temporal hearing loss, i.e., temporal threshold shift (TTS). Such TTS is recoverable and has long-term been considered to be a safe procedure, since the regular audiogram examinations show normal after stop of noise exposure, even some patients may have complaints on difficulty in speech sensing in noisy environments. Recently, it has been found that even suffering from short-term noise exposure and there is no apparent hair cell loss, exposed animals could still have irreversible neural degeneration, in particular, losing of lower spontaneous rate (LSR) auditory nerves and their synapses with inner hair cells. This can eventually lead to difficulty hearing speech in noisy environments. Such ‘hidden’ hearing loss is not readily detectable by currently standard clinical auditory tests. In this study, we have developed a new, clinical-practicable method to detect such noise-induced “hidden” hearing loss. Auditory nerves can be divided into three groups based on spontaneous rate. Middle and higher spontaneous rate (M/HSR) fibers have low-threshold and their discharges are quickly saturated as sound intensity increases, whereas LSR fibers have high-threshold and their discharge rates can still increase at high intensity levels. In the study, we have used middle levels of white noise to saturate M/HSR fiber discharge and use another stimulus to examine the LSR fiber function. Auditory brainstem response (ABR) was recorded under both quiet and noisy environments. We found that ABR thresholds of noise-exposure group were significantly increased than that of normal control group without noise-exposure under noisy environment. However, distortion product acoustic emission (DPOAE), which mainly reflects outer hair cell function and active cochlear mechanics, in the noise-exposure group had no significant changes in comparison with that in the control group. These data suggest that our newly-developed method can detect the deficit of LSR fiber function and provide a practicable testing method in the clinic to detect noise-induced “hidden” hearing loss.

Disclosures: H. Zhao: None. **L. Mei:** None. **Y. Zhu:** None.

Poster

048. Disorders of the Auditory or Visual System

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

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Topic: D.01. Sensory Disorders

Support: Singapore Ministry of Education

National University of Singapore

Title: Pharmacological activation of sphingosine 1-phosphate receptor 2 protects neural-derived cells from cisplatin toxicity by attenuating generation of reactive oxygen species

Authors: *D. R. HERR[1,2], M. J. Y. REOLO[1], Y. X. PEH[1], W. WANG[1];
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Abstract: Ototoxic drugs, such as platinum-based chemotherapeutics, often lead to permanent hearing loss through apoptosis of neuroepithelial hair cells and afferent neurons of the cochlea. There is no approved therapy for preventing or reversing this process, but our data suggest that a G protein-coupled receptor, sphingosine 1-phosphate receptor 2 ($S1P_2$), may represent an effective drug target. We show that $S1P_2$ knockout mice uniformly display progressive degeneration of the cochlea which results in profound deafness by 4 weeks of age. This degeneration is preceded by accumulation of reactive oxygen species (ROS) and can be attenuated by administration of the antioxidant N-acetylcysteine. Since loss of $S1P_2$ leads to cochlear degeneration, we sought to determine whether activation of $S1P_2$ can protect against ototoxicity. We validated a chemical probe, CYM-5478, as an $S1P_2$-selective agonist and used it in a number of in vitro cell-based assays to evaluate cell viability, induction of apoptosis, and accumulation of ROS following activation of $S1P_2$ in the presence of cisplatin. We show that activation of $S1P_2$ increases cell viability and reduces cisplatin-mediated cell death in neural-derived cell lines by reducing ROS, but does not protect non-neural tumor-derived cell lines. Cumulatively, these results suggest that $S1P_2$ may serve as a therapeutic target for attenuating cisplatin-mediated ototoxicity without affecting the therapeutic efficacy of platinum-based chemotherapy.

Disclosures: **D.R. Herr:** None. **M.J.Y. Reolo:** None. **Y.X. Peh:** None. **W. Wang:** None.

Poster

048. Disorders of the Auditory or Visual System

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Claud D. Pepper Older Americans Independence Center Junior Scholar Award (S.S.)

Title: Effects of glutathione transferase alpha 4 deficiency on cochlear and auditory function.

Authors: *H.-J. PARK[1,2], C. HAN[1], M.-J. KIM[1], K. WHITE[1], M. TICSA[1], I. CAICEDO[1], S. MANOHAR[3], D. DING[3], P. LINSER[2], R. SALVI[3], S. SOMEYA[1];
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Abstract: The glutathione transferase (GST) detoxification system converts an exogenous/endogenous toxic compound into a less toxic form by conjugating the toxic compound to reduced glutathione by a variety of GST enzymes. Studies using C.elegans, D.melanogaster, and dwarf mice suggest that enhanced GST detoxification is associated with exceptional longevity or protection against aging. In humans, epidemiological studies have detected polymorphisms of several GST enzymes in individuals with noise-induced hearing loss, age-related hearing loss, or cisplatin-induced hearing loss, suggesting that GST detoxification has a key role in maintaining auditory function. The goal of this project is to investigate whether GSTA4 (glutathione transferase α4) plays an essential role in maintaining cochlear and auditory function during aging and/or in eliminating ototoxic compounds. To investigate whether deficiency of GSTA genes promotes oxidative stress-induced cell death, we conducted in vitro oxidative stress tests using hydrogen peroxide in *Gsta1*-, *Gsta3*-, or *Gsta4*-knockdown mouse inner ear cell lines (HEI-OC1, House Ear Institute-Organ of Corti 1). We found that downregulation of *Gsta1*, *Gsta3*, or *Gsta4* isoform increased susceptibility to oxidative stress-induced cell death. We also found that treatment with cisplatin, a well-known cancer drug that causes cochlear damage and hearing loss, significantly increased mRNA levels of *Gsta1*, *Gsta2*, *Gsta3*, and *Gsta4* isoforms in cochlear organotypic cultures from 3-days-old C57BL/6 mice, suggesting that *Gsta* family enzymes play a role in detoxifying hydrogen peroxide (reactive oxygen species) or cisplatin (ototoxic drug) in mouse cochlear cells. These results suggest that GSTAs play a critical role in removing hydrogen peroxide or cisplatin in mouse inner ear cell lines. Currently, we are investigating the effects of *Gsta4* deficiency on cochlear and auditory function during aging or under cisplatin treatment using *Gsta4* knockout mice in the CBA/CaJ background.

Disclosures: **H. Park:** None. **C. Han:** None. **M. Kim:** None. **K. White:** None. **M. Ticsa:** None. **I. Caicedo:** None. **S. Manohar:** None. **D. Ding:** None. **P. Linser:** None. **R. Salvi:** None. **S. Someya:** None.

Poster

048. Disorders of the Auditory or Visual System

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Topic: D.01. Sensory Disorders

Support: W81XWH-15-2-0024

Title: Auditory functional deficits < structures changes following blast shockwave exposure > mice

Authors: *Y. WANG[1], Y. WEI[1], S. VAN ALBERT[1], A. NORTHROP[2], R. URISTE[1], P. ARUN[1], D. WILDER[1], S. VENKATASIVASAISUJITH[1], I. GIST[1], S. MCLNTURFF[2], W. CHANG[2], T. FITZGERALD[2], M. KELLEY[2], J. LONG[1];
[1]Walter Reed Army Inst. of Res., Silver Spring, MD; [2]NIDCD/NIH, Bethesda, MD

Abstract: A high fidelity animal model is critical to define the mechanism(s) of blast-induced auditory injury and to develop therapeutic strategies. The Advanced Blast Simulator (ABS) incorporates design features which allow higher fidelity replication of the key features of blast wave flow conditions, including the negative phase and secondary shock. Using this device, the present research is aimed at producing a comprehensive characterization of auditory functional deficits and associated pathological changes in the central and peripheral auditory signal processing regions disrupted by exposure to blast shockwaves. isofluorane anesthetized CBA mice (male, 23 - 28 g) were exposed to blast overpressure (peak static pressure of 19 psi and 4 msec positive phase duration) generated by the ABS which consists of a 0.5 ft long compression chamber that is separated from a 21 ft long transition/expansion test section by rupturable Valmex membranes. A time-course of blast effects on auditory function was assessed by analyzing auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) under anesthesia. Data revealed that blast exposure caused significant elevations of ABR threshold, increased ABR wave latency, and reductions in ABR wave amplitude immediately following the blast shockwave insult. These changes were observed over the entire acoustic frequency spectrum and persisted over 14 days. DPOAE signals were undetectable immediately after blast exposure and their disappearance persisted over 14 days, suggesting significant damage to the inner ear. Immunostaining of Myo7a and Phalloidin in wholemount cochlea revealed appreciable damage to outer hair cells, inner hair cells, as well as to other structures in the inner ear. Silver staining of brain sections showed significant axonal degeneration in auditory and vestibular signal processing regions in brainstem and cerebellum. The results indicate that both peripheral and central auditory signal processing regions are vulnerable to blast overpressure exposure in the ABS. This mouse model of blast-induced auditory injury should provide a useful experimental tool for studying the mechanisms

underlying hearing impairment after blast exposure and for evaluating potential strategies for prevention and cure.

Disclosures: **Y. Wang:** None. **Y. Wei:** None. **S. Van Albert:** None. **A. Northrop:** None. **R. Uriste:** None. **P. Arun:** None. **D. Wilder:** None. **S. Venkatasivasaisujith:** None. **I. Gist:** None. **S. Mclnturff:** None. **W. Chang:** None. **T. Fitzgerald:** None. **M. Kelley:** None. **J. Long:** None.

Poster

049. Somatosensory Cortical Circuits

Location: Halls B-H

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Topic: D.03. Somatosensation: Touch

Support: NIH U01-NS090569

Title: Critical dynamics during processing of somatosensory input are maintained by interneuronal activity

Authors: *S. SESHADRI, D. PLENZ;
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Abstract: Ongoing neuronal activity in cortex, whether recorded *in vitro* in acute slices and slice cultures or *in vivo* in rodents, non-human primates and humans, has been shown to organize as neuronal avalanches. Avalanche sizes, durations, waveforms and their temporal occurrences are governed by power laws, supporting the view that ongoing cortical dynamics is poised at criticality. Theory and experiments have shown that numerous aspects of information processing are optimized at or near criticality. However, it is currently not clear whether avalanche dynamics is maintained upon the transition from ongoing to sensory evoked cortical activity. Recent experimental work suggests a fast recovery towards avalanche dynamics after sensory stimulation, such as flash stimuli in visual cortex of the turtle (Shew et. al., 2015) or sensory processing in humans (Arviv et. al., 2015). While these studies suggest a transient deviation from avalanches during evoked activity, they lack cellular resolution to monitor the precise network changes during these transitions. We recently demonstrated (Bellay et. al., 2015) that spontaneous spiking activity in local groups of pyramidal neurons organizes at criticality *in vivo* in the awake resting state using genetically encoded calcium indicators (GECIs). Here, we combine this cellular approach with interneuron specific optogenetic modulation to examine avalanche dynamics during resting and sensory evoked activity.
We performed *in vivo* 2-photon imaging of layer 2/3 pyramidal neurons in primary

somatosensory cortex, through chronic cranial windows in awake, head-fixed Thy1-GCaMP6s transgenic mice positioned on a running wheel. For optogenetic modulation of interneurons, we crossed Thy1-GCaMP6s with PV-Cre or SOM-Cre mice respectively, and injected AAVs to obtain Cre-dependent expression of excitatory (C1V1) or inhibitory (NpHR3.0) opsins. Our preliminary results show that spiking activity increases with self-initiated locomotion. Yet, power laws in avalanche size distributions are maintained at rest as well as during locomotion. Power law slopes were shallower during periods of running, as predicted by the observed increase in spiking activity. The role of critical dynamics during sensory processing has been an outstanding question in the field of population coding. Our results address this question experimentally and elucidate the role of interneurons in maintaining critical dynamics.

Disclosures: S. Seshadri: None. **D. Plenz:** None.

Poster

049. Somatosensory Cortical Circuits

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Topic: D.03. Somatosensation: Touch

Support: ISF

Minerva

DFG-SFB 1089

Title: Membrane potential correlates of increased SNR and asynchronous cortical activity following activation of the nucleus basalis

Authors: *I. MEIR, Y. KATZ, I. LAMPL;
Neurobio., Weizmann Inst. of Sci., Rehovot, Israel

Abstract: The basal forebrain (BF) plays a major role in arousal, attention, learning and plasticity. BF neurons, mainly from the nucleus basalis (NB), send cholinergic, GABAergic and glutamatergic projections to the cortex. NB activation shifts cortical dynamics from synchronized to asynchronized mode and exerts profound effects on neuronal activity. In sensory cortices, NB activation improves the signal to noise ratio (SNR) of sensory responses. The underlying mechanisms, especially at the subthreshold level, remain unclear. We studied the effects of NB electrical stimulation on ongoing and sensory evoked activities of cortical cells in the mouse barrel cortex of lightly anesthetized mice, using LFP, extracellular and intracellular recordings. In agreement with previous studies, NB stimulation reduced low frequency

components in the cortical local field potential (LFP). In extracellular recordings, a prominent increased SNR to whisker stimulation was observed, accompanied by an increased latency of sensory response. The increased SNR was mainly due to reduced background firing, while the response magnitude remained unchanged. We used whole cell patch recordings to reveal the underlying effects of NB stimulation and found that it eliminates the large spontaneous synaptic fluctuations and hyperpolarizes the resting membrane potential. However, NB stimulation had negligible effect on the magnitude of the sensory evoked synaptic response and on its trial to trial variability. Simultaneous Vm-LFP recordings indicate that NB stimulation reduced the cross-correlation between these signals during ongoing activity and lowered the trial to trial correlation of sensory response. Taken together, we suggest that activation of the NB induces a significant hyperpolarization of cortical cells which in turn reduces the amount of spontaneous activity. This filtering effect can account for the increased sensory evoked response SNR and the longer latency, and explains the reduced correlation between cells. The combined effects of filtering and decorrelation, can promote sensory processing and are in line with the effects of attention.

Disclosures: I. Meir: None. **Y. Katz:** None. **I. Lampl:** None.

Poster

049. Somatosensory Cortical Circuits

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Topic: D.03. Somatosensation: Touch

Support: BMBF Grant 01GQ1005B

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Title: Slow asynchronous synaptic noise broadens the dynamic range of spiny stellate neurons in layer 4 of the mouse barrel cortex

Authors: O. REVAH[1], A. BINSHTOK[2,3], F. WOLF[4,5], A. NEEF[4,5], *M. J. GUTNICK[1]; [1]Koret Sch. of Vet Med, Hebrew Univ. Jerusalem, Rehovot, Israel; [2]The Hebrew Univ. Med. Sch., Jerusalem, Israel; [3]The Edmond and Lily Safra Ctr. for Brain Sci., Jerusalem, Israel; [4]MPI For Dynamics and Self-Organisation, Goettingen, Germany; [5]Bernstein Ctr. for Comput Neurosci, Goettingen, Germany

Abstract: Spiny stellate (SpSt) neurons, which are the prevalent excitatory cells in layer 4 of the rodent somatosensory cortex, are organized in ”barrels”. Each receives nearly all its excitatory

input from the thalamus and from other SpSt neurons in the same barrel. Because it is a primary thalamo-cortical target, the layer 4 circuit comprises a key gateway into the cortical circuit. It is therefore imperative that the layer 4 circuit responds rapidly to input. However, SpSt cells are very small, and there are strong theoretical reasons to suspect that their compact morphology could impair their capacity to encode high input frequencies and thus hamper the temporal fidelity of cortical processing. Here, we used whole-cell patch clamp to measure the temporal properties of asynchronous noise in SpSt cells as compared with the much larger layer 5 pyramidal (Pyr) cells, and characterize the capabilities of both cell types to encode high frequencies in a synaptically active-like environment. We find that individual SpSt cells indeed have a much narrower dynamic range than Pyr cells when probed with fluctuating inputs with identical correlation time. However, we show that the synaptic dynamics in SpSt cells, as evidenced by the correlation time of asynchronous noise, is much slower than that of Pyr neurons, and that slower correlation time is associated with significant broadening of the dynamic range of SpSt cells. We further show that this improvement in encoding bandwidth of sensory input depends on activation of potassium conductance, as it disappears when potassium channels are pharmacologically blocked.

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Poster

049. Somatosensory Cortical Circuits

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Support: NIH Grant NS077989

Zilkha Family Discovery Fellowship in Neuroengineering

Title: Cholinergic control of cortical circuit dynamics

Authors: *R. DASGUPTA, F. M. SEIBT, M. BEIERLEIN;
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Abstract: Cholinergic neurons of the basal forebrain form extensive projections to neocortex and are critically involved in mediating numerous cognitive processes, including sensory processing, reward timing and fear learning. To understand how acetylcholine (ACh) shapes

cortical computations, it is critical to gain a better understanding of the underlying cellular and circuit mechanisms. While previous work has shown that cholinergic agonists can act on both nicotinic and muscarinic receptors expressed by distinct neurons and synapses throughout neocortex, how synaptically released ACh influences cellular targets and ultimately controls cortical circuit dynamics remains poorly understood. We addressed this question using a combination of *in vitro* electrophysiology and optogenetics in somatosensory (barrel) cortex of transgenic ChAT-ChR2-EYFP mice. We found that the activation of cholinergic afferents led to a strong reduction of evoked cortical activity, with nicotinic receptors (nAChRs) mediating fast and transient suppression, and muscarinic receptors (mAChRs) mediating delayed and sustained suppression. To investigate the underlying cellular mechanisms for ACh-mediated control of cortical activity, we characterized cholinergic synaptic inputs onto specific cell types in distinct layers. Postsynaptic cholinergic responses were prominent in layer 4 and were primarily mediated by mAChRs, with excitatory neurons showing long-lasting IPSCs and regular-spiking interneurons displaying EPSCs. In agreement, cholinergic suppression of cortical activity in the isolated layers 4-6 was entirely dependent on mAChR activation. In contrast, cholinergic responses in supragranular layers were predominantly mediated by nAChRs expressed by GABAergic interneurons. Taken together, our results show that cholinergic control of cortical network dynamics occurs over different time scales and is mediated by nAChR and mAChR-dependent mechanisms expressed in distinct cortical layers.

Disclosures: **R. Dasgupta:** None. **F.M. Seibt:** None. **M. Beierlein:** None.

Poster

049. Somatosensory Cortical Circuits

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Title: Complementary connectivity defines two distinct networks of cortical somatostatin-expressing interneurons

Authors: *A. S. NAKA, B. SHABABO, B. SNYDER, H. ADESNIK;
UC Berkeley, Berkeley, CA

Abstract: Cortical computation depends critically on a diverse array of GABAergic interneurons. Parsing this diversity is essential for understanding cortical operation, since evidence indicates that the various types of interneurons are specialized for distinct functions. One such specialization is target selection; for example, many somatostatin (SOM) expressing interneurons target the distal apical dendrites of pyramidal cells in layer 1 where they can powerfully influence neuronal computation through dendritic inhibition. Gain and loss of function studies using optogenetic manipulations in SOM-cre transgenic lines have pointed to possible functional roles for SOM-mediated inhibition, but have often necessarily treated SOM cells as a homogenous group. However, SOM cells are heterogeneous with respect to their morphology, intrinsic properties, and connectivity. Using paired whole cell recording, two photon imaging, and optogenetic circuit mapping, we provide evidence that SOM cells can be subdivided functionally into at least two distinct networks, which are spatially intermingled, but have complementary and non-overlapping patterns of laminar connectivity. These networks are likely to have divergent influences on cortical computation.

Disclosures: **A.S. Naka:** None. **B. Shababo:** None. **B. Snyder:** None. **H. Adesnik:** None.

Poster

049. Somatosensory Cortical Circuits

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Topic: D.03. Somatosensation: Touch

Support: NIH Grant P01NS074972

Title: Diversity and connectivity of somatostatin interneurons in layer V of the mouse barrel cortex

Authors: *M. J. NIGRO, Y. HASHIKAWA, R. TREMBLAY, B. RUDY;
Dept. of Physiol., New York Univ., New York, NY

Abstract: Inhibitory interneurons represent about 10-15% of the neuronal population in the somatosensory cortex, and their activity powerfully shapes sensory information processing in the cortex. Despite being a relatively small neuronal population, GABAergic interneurons are very diverse, and three main classes have being defined according to developmental, molecular, morphological, electrophysiological, and synaptic features. Among these classes the

somatosatin-expressing GABAergic interneurons are specialized in dendritic inhibition. Somatostatin-expressing interneurons represent about 30% of the GABAergic population in the somatosensory cortex, and are localized mainly in infragranular layers. We performed whole-cell patch clamp experiments from slices of transgenic mice expressing td-Tomato in somatostatin-expressing interneurons to study the diversity and connectivity of these cells. Our morphological and electrophysiological analysis shows three main types of somatostatin interneurons: type I Martinotti cells have an ascending axon travelling across the cortical layers and branching upon arrival to layer I; type II Martinotti cells have an ascending axon that starts branching at the border between layer IV and II/III; non-Martinotti cells have an ascending axon that specifically targets layer IV. Type I Martinotti represent about 10% of the recorded interneurons and show high input resistance and rebound bursting. Type II Martinotti neurons are about 40% of the recorded interneurons and show an adapting firing pattern. Non-Martinotti cells are about 40% of the recorded population and show a quasi fast-spiking firing pattern. Using dual and triple recordings in brain slices we show that these morphological diversity correlates with specific connectivity patterns.

Disclosures: **M.J. Nigro:** None. **Y. Hashikawa:** None. **R. Tremblay:** None. **B. Rudy:** None.

Poster

049. Somatosensory Cortical Circuits

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Topic: D.03. Somatosensation: Touch

Support: NIH Grant P01NS074972

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Title: Remodeling of dendritic inhibition during active wakefulness

Authors: ***W. MUNOZ**[1], R. TREMBLAY[1], D. LEVENSTEIN[2], B. RUDY[1];
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Abstract: How the brain processes information is contextually adjusted in order to optimize perception and behavioral performance. In neocortex, this is achieved by dynamically restructuring circuits. Recent experiments examining a variety of behavioral contingencies led to the discovery of a canonical disinhibitory circuit in which dendritic-targeting somatostatin-expressing (Sst) interneurons (INs) are suppressed during active wakefulness by the activation of VIP-expressing (Vip) INs. These observations have given rise to the notion that during active

states, the dendrites of pyramidal neurons are globally disinhibited, enhancing input responsiveness and serving important computational functions such as gating and gain modulation. Here, we challenge this concept by showing that during active whisking behavior, Sst IN-mediated dendritic inhibition is not globally suppressed throughout the cortical column, but rather remodeled. By examining the *in vivo* activity of Sst INs across the entirety of cortical columns in head-fixed mice, we discovered that while supragranular layer Sst INs are silenced, the majority but not all of granular and infragranular Sst INs are activated during whisking behavior depending on their subtype identity. These Sst IN subtypes with distinct axonal innervation domains and laminar distribution possess context-specific ability to differentially affect signaling in pyramidal neuron dendrites. Moreover, specific SST INs selectively receive Vip IN inhibitory inputs and cholinergic modulatory drive, which allow for their differential recruitment. Our study provides a novel framework for understanding the spatiotemporal control of neuronal excitability carried out by SST IN-mediated dendritic inhibition, emphasizing its potential interplay with the layer-specific organization of excitatory input lines and signaling zones in dendritic compartments of pyramidal cells in the mammalian neocortex.

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Poster

049. Somatosensory Cortical Circuits

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Support: DFG Grant EXC NeuroCure

DFG Grant LA 3442/3-1

Title: Dendritic dynamics in sensory perception

Authors: ***N. TAKAHASHI**, M. LARKUM;
Humboldt-Universität zu Berlin, Berlin, Germany

Abstract: Perception is a process that requires the matching of an internal representation of previous experience with real-world sensory data, for which the cellular mechanisms are poorly understood. There is abundant evidence that feedback information to primary sensory regions is integral to the perceptual process. Recent data suggest that feedback information activates dendritic processes in pyramidal neurons that are linked to cognitive function. Here, we show that calcium activity in the apical dendrites of a subset of layer 5 (L5) pyramidal neurons in

primary sensory cortex correlates with the threshold for perception of whisker stimulation in rodents. Another population of apical dendrites of L5 neurons is negatively correlated. The same positive and negative correlations are found for bursts of somatic spikes in L5 neurons that preceded the behavioral actions of the animals. Using pharmacological and optogenetic approaches, we show that the dendritic activity is causally linked to the animal's behavior, demonstrating that the perceptual process depends critically on activation of the apical dendrites of L5 pyramidal neurons in primary sensory cortex.

Disclosures: **N. Takahashi:** None. **M. Larkum:** None.

Poster

049. Somatosensory Cortical Circuits

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DFG FOR 2143

Helmholtz Society

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Title: *In vivo* monosynaptic excitatory transmission during active cortical states.

Authors: ***J. KREMKOW**[1,2,3], J.-S. JOUHANNEAU[2,1], A. L. DORRN[2,1], J. F. A. POULET[2,1]; [1]Cluster of Excellence NeuroCure, Neurosci. Res. Ctr., Charité-Universitätsmedizin Berlin, Berlin, Germany; [2]Dept. of Neurosci., Max-Delbrück Ctr. for Mol. Med. (MDC), Berlin, Germany; [3]Inst. for Theoretical Biol., Humboldt-Universität zu Berlin, Berlin, Germany

Abstract: In vivo, cortical neurons fire action potentials and perform synaptic transmission during network activity. While glutamatergic synaptic transmission has been studied in great detail in quiescent cortical slices, very little is known in active networks in vivo. Modeling and experimental studies have predicted that active cortical states can modulate subthreshold

computations underlying action potential generation to different extents. Some studies suggest that active states will cause a reduction in the amplitude of a unitary excitatory postsynaptic potential, whereas others suggest there will be no change. To understand how cortical neurons integrate synaptic input, it is therefore critical to investigate the properties of monosynaptic transmission in vivo, during active cortical states. We performed dual, triple and quadruple, two-photon targeted whole-cell patch-clamp recordings from monosynaptically connected somatosensory cortex layer 2/3 pyramidal neurons in anaesthetized mice. Under urethane anesthesia neurons oscillate between hyperpolarized Downstates with little network activity, and synaptically active, depolarized Upstates. We triggered single action potentials with brief current injection and identified a sparsely connected, structured network of small amplitude, reliable synaptic connections in the Downstate (Jouhanneau et al, 2015). We next compared the amplitude of connections between Up and Down states. Across the population, there was no overall change in amplitude, however, the ratio of Up to Down state unitary excitatory postsynaptic potential amplitude varied between individual connections. We provide a new approach to measure the impact of network activity on synaptic transmission and the processing of spontaneous and sensory input by connected, neighboring neurons in vivo.

Reference
Jouhanneau J-S. Kremkow, J. Dorrn, A. and Poulet J.F.A. (2015) In vivo monosynaptic excitatory transmission between layer 2 pyramidal neurons. *Cell Reports,* 13: 1-9.

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Poster

049. Somatosensory Cortical Circuits

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Topic: D.03. Somatosensation: Touch

Support: PSC-CUNY Awards

QCC MSEIP

John Jay College PRISM

Title: A quantitative morphological analysis of supragranular neurons of the barrel cortex and their connections

Authors: F. PALAGUACHI[1], M. C. ANAYA[2], C. TSE[3], S. SOSNOWIK[1], J. BRUMBERG[1], *A. TSIMOUNIS[2];
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Abstract: A 'bottom-up' approach to resolving neuronal circuits involves deciphering their fundamental building blocks, the individual neurons. While specific morphologies have been correlated with specific circuit functions the number of functional classes of neurons remains unknown. For example, there are pyramidal neurons in the supragranular layers of the mouse barrel cortex that originate connections to the opposite hemisphere (callosal cells), the primary motor cortex (M1) and the secondary somatosensory cortex (S2), but what remains unanswered is whether these cells have similar or different intrinsic properties. We have been taking a morphological approach to understanding neuronal circuits in the primary somatosensory cortex, more specifically those associated with supragranular neurons in the barrel field (S1BF) of the mouse. After defining groups of neurons that are distinguished from one another based on morphological characteristics, our aim is to determine if specific morphological groups are associated with particular cortical connections. Individual neurons in brain sections from CD-1 mice are labeled using Diolistics and reconstructed from confocal images. Morphological measurements are analyzed using principal component analysis followed by cluster analysis, revealing distinct groups of neurons. Cortical connections are revealed by in vivo injections of fluorescent beads in synaptic target areas of S1BF. We show the distribution of neurons projecting to three different synaptic targets of S1BF (contralateral S1, ipsilateral M1 and ipsilateral S2) within the overall morphological classification dendrogram. These experiments show the correlation between anatomical classes of neurons and specific roles within the cortical circuit.

Disclosures: F. Palaguachi: None. **M.C. Anaya:** None. **C. Tse:** None. **S. Sosnowik:** None. **J. Brumberg:** None. **A. Tsimounis:** None.

Poster

049. Somatosensory Cortical Circuits

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 49.11/U2

Topic: D.03. Somatosensation: Touch

Support: CONACYT Grant/ Retención

Title: Tactile noise improves somatosensory response in the injured rat brain

Authors: L. M. ANGUIANO-PACHECO[1], J. M. DUENAS-JIMENEZ[2], N. HUIDOBRO[4], P. LINARES[4], S. H. DUENAS-JIMENEZ[1], E. MANJARREZ[4], *B. DE LA TORRE[3]; [1]Neurosci., [2]Physiol., Univ. de Guadalajara, Guadalajara, Mexico; [3]CUCEI, Univ. de Guadalajara, GUADALAJARA, Mexico; [4]Integrative Physiol., Benemérita Univ. Autónoma de Puebla, Puebla, Mexico

Abstract: Previously we demonstrated that tamoxifen can restore the electrocorticographic activity in the somatosensory cortex of the injured rat. These findings suggest that tamoxifen can preserve or restore the injured neural circuits (Franco y cols. 2013). The purpose of the present study was to evaluate, in injured tamoxifen treated rats, whether an optimal level of tactile noise can increase the barrel cortex Evoked Field Potentials (EFP´s). We obtained EFP´s from the barrel cortex of 15 rats which were divided in three groups: 5 injured rats treated with tamoxifen, 5 injured rats without treatment and 5 intact rats. The injury was performed in the barrel cortex using a thin needle with the support of a stereotaxic frame. The EFP´s where elicited 30 days after the lesion by protracting the whiskers using a mechanical transductor (Chubbuck). Simultaneously we delivered three levels of mechanical noise applied in the whiskers by means of the Chubbuck. MATLAB software was used to evaluate EFP´s amplitudes. For all subjects, we found that the quantitative analyses of the amplitudes exhibited an inverted U-like form as a function of the applied noise, being the intermediate level of noise which produces the higher EFP´s amplitude. However, in tamoxifen treated rats, we observed that the EFP´s exhibited higher amplitudes compare with the not-treated group. In order to evaluated the amplitudes between the Zero Noise (ZN), Optimal Noise (ON) and High Noise (HN) we applied the one way ANOVA test and to compare the amplitudes between the three conditions. After comparing between the noises conditions, we applied the one way ANOVA test to compare differences of the EFP´s amplitude in ON condition between the experimental groups (treated untreated and intact rats). A Bonferroni post hoc was applied for multiple comparisons. The results showed significant statistical differences between ZN and ON ($p<0.05$), also between ON and HN ($p<0.5$), but not between ZN and HN. This was observed in the three groups (treated, untreated and intact rats). When comparing the EFP´s amplitudes of the ON conditions between the experimental groups, we observed a significant statistical difference between the treated rats compared with the not treated ($p<0.05$); in contrast not significant differences were observed between the intact and the treated rats. These results suggest that tamoxifen can restore the functionality of the barrel cortex. Also, stochastic resonance phenomena improves the EFP´s response to almost normal values, which lead us to speculate that an optima level of noise applied in the injured brain can be a part of an integral treatment for traumatic brain injury.

Disclosures: L.M. Anguiano-pacheco: None. **J.M. Duenas-jimenez:** None. **N. Huidobro:** None. **P. Linares:** None. **S.H. Duenas-jimenez:** None. **E. Manjarrez:** None. **B. De la torre:** None.

Poster

049. Somatosensory Cortical Circuits

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 49.12/U3

Topic: D.03. Somatosensation: Touch

Support: Max-Planck-Institute for Brain Research

Title: Combined in-vivo functional and structural imaging in mouse barrel cortex L4

Authors: *Y. HUA[1], V. PAWLAK[2], J. N. D. KERR[2], M. HELMSTAEDTER[1];
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Abstract: In spite of substantial efforts, the relevance and computational contribution of neuronal circuits in early sensory cortex of mammalian brains remains elusive. Even for the first stage of cortical processing, the transformation of sensory inputs arriving from the thalamus in layer 4 of sensory cortex is not understood. Hypotheses range from a simple amplification of the thalamic signals to a translation into a proprietory cortical signal code. Here we set out to test these hypotheses by combining in-vivo 2-photon calcium imaging of large populations of excitatory neurons in a “barrel” of a cortical column in mouse primary somatosensory cortex with connectomic analysis of the very same tissue volume. Using a simple correlation method, we detected at least 3 unique neuronal response classes in response to a realistic object-impact stimulus. We then exposed the same barrel to large-volume 3D EM using SBEM and reconstruct the underlying circuitry to ask what fraction of the functional responses can be explained by intracortical circuits - and thus decide whether the observed responses are purely an amplification of thalamic inputs or result from local intracortical computations.

Disclosures: **Y. Hua:** A. Employment/Salary (full or part-time): Max-Planck-Institute for Brain Research. **V. Pawlak:** A. Employment/Salary (full or part-time): research center caesar. **J.N.D. Kerr:** A. Employment/Salary (full or part-time): research center caesar. **M. Helmstaedter:** A. Employment/Salary (full or part-time): Max-Planck-Institute for Brain Research.

Poster

049. Somatosensory Cortical Circuits

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 49.13/U4

Topic: D.03. Somatosensation: Touch

Title: Connectomics of three-layered reptilian and mammalian cortices

Authors: P. BASTIANS[1,2], M. HELMSTAEDTER[2], G. LAURENT[2];
[1]MPI of Neurobio., Martinsried, Germany; [2]MPI for Brain Res., Frankfurt am Main, Germany

Abstract: Next to mammals, reptiles are the only other animal class possessing a cerebral cortex. Reptilian cortex is three-layered, similar to mammalian piriform cortex and hippocampus. While the overall cytoarchitectonic differences between three-layered cortex and mammalian 6-layered "iso"cortex are obvious, the organization of these cortices at the microcircuit scale is not known. Here, we set out to study the local innervation principles of three-layered cortices in turtle and mouse. We acquired local 3D EM datasets using SBEM and are currently analyzing the connectomic fingerprints of these cortices. First results indicate fundamentally different innervation principles especially with respect to inhibitory connections in the 3-layered reptilian dorsal cortex when compared to 6-layered mouse primary somatosensory cortex.

Disclosures: P. Bastians: None. **M. Helmstaedter:** None. **G. Laurent:** None.

Poster

049. Somatosensory Cortical Circuits

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 49.14/U5

Topic: D.03. Somatosensation: Touch

Title: Automated detection of glia cells for the analysis of connectome-specific neuron-glia interactions in cerebral cortex

Authors: *A. MOTTA, M. HELMSTAEDTER;
Connectomics, Max Planck Inst. For Brain Res., Frankfurt, Germany

Abstract: While the exact proportion remains debated, it is known that the mammalian cerebral cortex contains a substantial number of glial cells, possibly exceeding the number of neurons in some species. Glial cells have long been hypothesized to be involved in memory formation and it is speculated that they provide a correlate of local learning signals. Both hypotheses require specific interactions between glial cells and neurons that are related to the neuronal connectome rather than only to the tissue geometry. To test whether such specificity occurs in local circuits of mouse cerebral cortex, we developed a supervised machine learning classifier based on image and shape features for automatically detecting and reconstructing glial processes at high precision. We are currently analyzing the relation of glial processes to the connectomic structure.

Disclosures: **A. Motta:** None. **M. Helmstaedter:** None.

Poster

049. Somatosensory Cortical Circuits

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 49.15/U6

Topic: D.03. Somatosensation: Touch

Title: Automated synapse detection in large scale connectomics data

Authors: ***B. STAFFLER**[1], M. BERNING[1], K. M. BOERGENS[1], P. VAN DER SMAGT[2], M. HELMSTAEDTER[1];
[1]MPI Brain Res., Frankfurt, Germany; [2]TUM, Munich, Germany

Abstract: Nerve tissue contains a high density of chemical synapses, about 1 per μm^3 in the mammalian cerebral cortex. Thus, even for small blocks of nerve tissue, dense connectomic mapping requires the identification of millions to billions of synapses. While the focus of connectomic data analysis has been on wire reconstruction, synapse detection becomes limiting when datasets grow in size and dense mapping is required. Here, we report a method for automated synapse detection from 3D electron microscopy image stacks that scales to very large volumes: SynEM. The approach is based on a pre- segmentation of the image data and focuses on classifying borders between neuronal processes as synaptic or non-synaptic. SynEM yields 96% precision and 95% recall in realistic binary cortical connectomes with no user interaction; it provides full synaptic input maps of single neurons at 95% precision and 95% recall with human effort of about 7 work hours per neuron. We exemplify SynEM for a local connectome of 90 times 38 from mouse somatosensory cortex and the complete synapse input map of a local layer 4 spiny neuron comprising 1334 input chemical synapses. This first locally complete input weight distribution is best fitted by a lognormal distribution, with however a slightly stronger

tail. SynEM provides the tools required for large-scale synapse detection for connectomics in the cerebral cortex and beyond.

Disclosures: B. Staffler: None. **M. Berning:** None. **K.M. Boergens:** None. **P. van der Smagt:** None. **M. Helmstaedter:** None.

Poster

049. Somatosensory Cortical Circuits

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 49.16/U7

Topic: D.03. Somatosensation: Touch

Title: Local connectomic patterns in septal versus barrel circuits

Authors: *P. LASERSTEIN, M. HELMSTAEDTER;
Max Planck Inst. For Brain Res., Frankfurt, Germany

Abstract: The projections from thalamus to cortex in primary somatosensory cortex of mouse distinguish “septal” regions in which projections from the posterior medial nucleus (PoM) dominate, and “barrel” regions in which projections from ventral posterio-medial nucleus dominate. Whether the local cortical circuit principles differ between septa and barrels is poorly understood. Here, we use EM-based circuit reconstruction to investigate the local connectomic patterns in septa and barrels of mouse P28 S1 cortex. We acquired a dataset sized 400 x 400 µm and comprising ~3000 sections (30nm) using serial block face electron microscopy (SBEM) and reconstructed 52 spiny and 12 interneurons located in septa and barrels. We then investigated the synaptic input to these, finding first evidence for separated local circuits.

Disclosures: P. Laserstein: None. **M. Helmstaedter:** None.

Poster

049. Somatosensory Cortical Circuits

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 49.17/U8

Topic: D.03. Somatosensation: Touch

Title: Local connectome statistics in mouse barrel cortex

Authors: *K. M. BOERGENS, M. BERNING, B. STAFFLER, A. MOTTA, S. LOOMBA, M. HELMSTAEDTER;
Max Planck Inst. of Brain Res., Frankfurt am Main, Germany

Abstract: The degree to which local circuits in the mammalian cerebral cortex are specific and the statistical order which these circuits obey are not known. Hypotheses range from random local wiring to high-order innervation precision. Here we set out to measure the local connectome statistics in volumes sized about $(100\ \mu m)^3$ acquired using SBEM. We first focused on two volumes from layer 4 and 2/3 of mouse barrel cortex. We used skeleton reconstruction, focused annotation, local automated volume segmentation and manual and automated synapse detection to obtain the first complete local cortical connectomes of datasets this size. Our analysis unravels highly specific pairwise innervation statistics for inhibitory axons that are selective for the nature of the postsynaptic dendritic targets

Disclosures: K.M. Boergens: None. **M. Berning:** None. **B. Staffler:** None. **A. Motta:** None. **S. Loomba:** None. **M. Helmstaedter:** None.

Poster

049. Somatosensory Cortical Circuits

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 49.18/U9

Topic: D.03. Somatosensation: Touch

Title: Developmental analysis of cortical connectomes

Authors: *A. G. GOUR, M. HELMSTAEDTER;
Dept. of Connectomics, Max Planck Inst. For Brain Res., Frankfurt, Germany

Abstract: With the emergence of highly specific wiring principles in local circuits of adolescent (P28) mouse neocortex (Boergens & Helmstaedter) the question arises whether such wiring specificity is already present at the time of formation of first synapses in postnatal development (around P5-6). To what degree does the establishment of these wiring "rules" require pruning of previously existing connections? Is some fraction of the connectome specificity enhanced or created by sensory experience? As a first step towards answering these questions, we have acquired EM datasets using SBEM from barrels in mouse somatosensory cortex at postnatal days 9 and 14. First results from the P14 cortex indicate the inclusion of new innervation target for

Axon initial segments (AIS) innervating inhibitory axons in L4 when compared to P28. While we are still analyzing these developmental connectomes, preliminary data suggests the specificity of these circuits at P14 to be different with respect to P28.

Disclosures: A.G. Gour: None. **M. Helmstaedter:** None.

Poster

049. Somatosensory Cortical Circuits

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 49.19/U10

Topic: D.03. Somatosensation: Touch

Title: Exploring the role of BDNF in the organization of borders in adult cortical representations of S1

Authors: *L. GONZALEZ, P. W. HICKMOTT;
Psychology, UC Riverside, Riverside, CA

Abstract: The primary somatosensory area, S1, contains a topographical representation of the entire surface of the body. When assessed physiologically, discontinuities are found in the responsiveness of the cortical map, forming distinct borders that serve to constrain the spread of excitation between neighboring representations. Furthermore, the organization of the S1 map is dynamic, and can change in response to a variety of manipulations, notably changes in the patterns of incoming sensory activity to S1. These changes in large-scale organization result from changes in synaptic, intrinsic and anatomical properties of the intracortical circuit. However, little is known about the cues that link changes in activity to coordinated changes in circuit properties.
Research in developmental plasticity has found brain-derived neurotrophic factor, BDNF, to be released in the somatosensory cortex during changes in neural connectivity. BDNF has also been found to display activity dependent expression and release during developmental plasticity. These characteristics have suggested BDNF to be a possible contender for providing a link between the anatomical and physiological bias observed at representational borders. BDNF has been well studied in the developing brain; however, its effects on adult organization have not been heavily researched.
Our previous studies focused on the border separating the forepaw and lower jaw representations in S1. Recent work has found that the local field potential (LFP) of connections crossing a representational border (CB) are smaller than connections that project within a representation (NCB) in our mouse model. When BDNF was bath applied it was found to depress the LFP of CB and NCB populations. Additionally, BDNF had a greater depressing effect on NCB versus

CB synapses.
These results have formed a hypothesis that BDNF is involved in the depression of CB excitatory synapses or in the potentiation of inhibitory synapses. BDNF has previously been shown to be involved in both conditions. To verify the hypothesis, this project will focus on rapid changes in synaptic properties mediated by BDNF after NCB synapses have been depressed using a long –term depression (LTD) paradigm. The results of these studies will shed further light on the role BDNF has on the organization of borders in adult cortical representations of the primary somatosensory area.

Disclosures: L. Gonzalez: None. **P.W. Hickmott:** None.

Poster

049. Somatosensory Cortical Circuits

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 49.20/U11

Topic: D.03. Somatosensation: Touch

Title: The effects of microstimulation on the behavioral report of single neuron stimulation

Authors: *G. DORON, M. BRECHT, M. VON HEIMENDAHL;
Humboldt Univ. of Berlin, Berlin, Germany

Abstract: The activation of individual neurons in the rat barrel cortex using nanostimulation can have an impact on behavioral responses in a detection task (Houweling and Brecht, 2008). Detectability of single neuron spiking depended on the regularity and frequency of the stimulus, as well as the type of the stimulated neuron (Doron et al., 2014). However, the mechanisms that underlie these effects of single neurons on sensation remain unknown. Here we examine the effect of microstimulation on the detectability of single regular spiking (RS) and fast spiking (FS) neurons in the rat somatosensory cortex. We juxtacellularly recorded and stimulated single RS and FS neurons using a combined tungsten electrode and glass pipette device and analyzed the behavioral report to nanostimulation. Interestingly, single neuron stimulation was likely to be detected when preceded by recent (less than 10s) microstimulation. Spectral analysis of the local field potential (LFP) 1 sec before and 1-4 seconds after the microstimulation revealed that perception of the stimulus was related to a significant decrease in delta power (1-4 Hz), as well as an increase in slow frequencies (6-30 Hz) and gamma power (40-100 Hz) within the first 3 seconds following stimulation, compared to no-stimulation trials. Surprisingly, examination of the effects of irregular nanostimulation pattern, which we previously reported to be the best perceived by the subjects, resulted in similar LFP patterns to those of microstimulation within the first 2 seconds following stimulation. These findings suggest that microstimulation as well as

certain single neuron stimulation patterns might influence the local network dynamics and alter their sensitivity to weak perturbations. We are currently assessing the effects of microstimulation on individual RS and FS spiking dynamics. Preliminary data suggest that firing rates are affected in these neuronal classes differently.

Disclosures: G. Doron: None. **M. Brecht:** None. **M. von Heimendahl:** None.

Poster

049. Somatosensory Cortical Circuits

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 49.21/U12

Topic: D.03. Somatosensation: Touch

Title: Somatotopic organization within the rat trunk sensory cortex

Authors: *G. H. BLUMENTHAL, B. NANDAKUMAR, K. A. MOXON;
Sch. of Biomed. Engineering, Sci. & Hlth. Systems,, Drexel Univ. Sch. of Biomed. Engin. Sci. and Hlth. Systems, Philadelphia, PA

Abstract: In both human and animal studies, the intensity of chronic neuropathic pain (CNP) has been directly associated with the degree of reorganization in the primary somatosensory cortex (S1). In patients suffering from phantom or below-level pain after deafferentation injury, cortical representations surrounding the painful, deafferented regions tend to expand, but patterns of cortical reorganization with development of at-level pain are not as well characterized. Mid-thoracic spinal cord contusion in the rat is an appropriate model for studying at-level CNP, because animals can easily be tested for several phenotypes of neuropathic pain and be selected for at-level CNP only. Although mid-thoracic SCI in rats may produce a practical model for at-level pain, the S1 trunk cortex in the naive rat is not defined well enough to study detailed reorganization in that region. This study aims to develop a detailed somatotopic map of the trunk sensory cortex in the rat.
Naive female Sprague Dawley rats were anesthetized with 1.5 g / kg urethane and placed in a stereotaxic frame. A grid of 1 cm^2 squares were drawn on the shaved trunk. A craniotomy with the coordinates 1.5 - 4.5 mm rostral/caudal and 1.5 - 4.5 mm medial/lateral relative to bregma was performed, and a ground screw was inserted into the skull. Dura was removed, and a high-impedance tungsten microelectrode was slowly lowered through the cortex as single unit neurons were identified. Upon identification, the animal's skin was lightly stimulated with a cotton swab as the cell was classified as responsive or non-responsive to the stimulation. If responsive, the receptive field of the cell was determined in relation to the grid drawn on the animal. This process was repeated in several locations within and surrounding the trunk sensory cortex. In a

subset of animals, the single electrode was replaced with a multi-channel shank (NeuroNexus, A2x16), and sensory evoked potentials (SEPs) were recorded through all layers of the cortex simultaneously using electrical stimulation to the trunk.
Our results support a somatotopic organization of the trunk within the trunk sensory cortex, with a caudal to rostral sensory receptive field orientation over the medial to lateral cortical area, and a dorsal to ventral receptive field orientation over the caudal to rostral cortical area. Cortical locations related to specific dermatomes of the thoracic region were also identified. This work provides the basis for studying cortical remapping of specific areas of trunk sensory cortex which receive information from the areas of the body affected by at-level CNP after spinal cord injury.

Disclosures: **G.H. Blumenthal:** None. **B. Nandakumar:** None. **K.A. Moxon:** None.

Poster

049. Somatosensory Cortical Circuits

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 49.22/U13

Topic: D.03. Somatosensation: Touch

Title: The role of mouse barrel cortex in tactile trace eye blink conditioning

Authors: ***J. I. HOFMANN**[1], B. JOACHIMSTHALER[2], C. SCHWARZ[2];
[1]Ctr. For Integrative Neuroscience; Ag Schwarz, Tuebingen, Germany; [2]Univ. Tübingen, Tübingen, Germany

Abstract: Mouse whisker-related primary somatosensory cortex (barrel cortex, BCx) is required to form an association between a behaviorally relevant tactile stimulus and its consequences, only if the first (conditioned stimulus, CS, here a single whisker deflection), and the latter (unconditioned stimulus, US, here a corneal airpuff) are separated by a brief memory period (‘trace’). We trained three mice on a tactile trace eye blink conditioning task (TTEBC) to assess learning related functional plasticity of BCx by recording LFPs and multi unit (MU) spiking from 4-shank laminar silicone probes (8 electrodes per shank, inter-shank distance 200 μm) spanning the depths of the principal barrel column and its neighbors. Current source density analysis (CSD) showed the known short latency sink in L4 and L6 during CS presentation but also a novel source at the same depthsduring the trace period. Both were consistently attenuated during TTEBC acquisition. Onset MU spike response to the CS (at a latency of <30 ms) was increased in some units while steady state CS-response (~50ms-250ms) typically decreased below the pre-learning level. Spiking during the trace period depressed as well during learning. These plastic changes were observed in neighboring shanks at a horizontal distance of up to 400μm. These findings show that BCx is functionally involved in TTEBC acquisition. We next

asked whether the involvement of BCx during the trace period has any causal role. We employed a well-established mutant mouse line that,due to expression of channelrhodopsin2 in inhibitory neurons (Guo et al., 2013), allows to block virtually all spikes in a column using illumination with blue light with great temporal precision. We found that BCx functionality was required during CS presentation. However mice learned normally when blocking BCx during the trace period. After learning, BCx activity during CS and trace was entirely dispensable for task performance. In summary, we demonstrate that the barrel column is tightly involved in acquiring the TTEBC association. Nevertheless, the plasticity of the neuronal response in the trace period is a non-causal reflection of learning, and after learning BCx is not needed for task performance at all. The role of BCx thus seems compatible with an instructor that couples other structures according to the task demands. We are currently investigating whether other tactile cortical representations are involved as well.

Disclosures: J.I. Hofmann: None. **B. Joachimsthaler:** None. **C. Schwarz:** None.

Poster

049. Somatosensory Cortical Circuits

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Studienstiftung des deutschen Volkes

Title: Relationships between sensory-evoked synaptic input and long-range target-related spiking output of cortical layer 5

Authors: *M. OBERLAENDER[1], R. EGGER[2], G. ROJAS-PILONI[3], R. T. NARAYANAN[2], J. M. GUEST[2], C. P. J. DE KOCK[4], D. UDVARY[2];
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Abstract: Even the simplest stimuli evoke highly heterogeneous responses in thousands of neurons in the related primary sensory areas of the mammalian neocortex. This intracortical (IC) representation of the stimulus is integrated by specific output populations in cortical layer 5 (L5), which transmit the results of cortical sensory information processing to several distant brain areas. How local IC activity is transformed into cortical output, and whether the transformations are related to the specific long-range targets is unknown. Here we combined injections of retrograde tracer agents with *in vivo* recordings and computational modeling to determine relationships between local synaptic input and sensory-evoked responses of individual L5 neurons with identified subcortical targets. We found that cortico-subcortical (CS) neurons in L5 of rat primary somatosensory cortex (S1) are subdivided into four disjoint projection types, which are embedded into the cortical circuitry in a target-related manner. Our results demonstrate that several CS output channels, in parallel, transform IC activity into target-related spiking patterns, potentially to extract disjoint features from the same stimulus.

Disclosures: **M. Oberlaender:** None. **R. Egger:** None. **G. Rojas-Piloni:** None. **R.T. Narayanan:** None. **J.M. Guest:** None. **C.P.J. de Kock:** None. **D. Udvary:** None.

Poster

050. Taste: Behavior and Processing

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 50.01/U15

Topic: D.04. Olfaction and Taste

Support: HHMI International Student Research Fellowship

NIH Grant DC007703

Title: Stochastic transitions in cortical ensemble dynamics can predict the timing of taste ingestion egestion orofacial behavior

Authors: *N. MUKHERJEE, J. WACHUTKA, D. B. KATZ;
Brandeis Univ., Waltham, MA

Abstract: Single neurons in the taste/gustatory cortex (GC) display temporally dynamic responses to taste stimuli. Their responses are seen to evolve gradually over time through a stereotypical series of 'epochs' (Katz et al., 2001, 2002), the last of which (~0.8 secs post stimulus) is rich in palatability (the ingest-egest decision variable)-related firing. However, using ensemble analysis techniques (Hidden Markov Models) to look at patterns of population firing, it turns out that the emergence of this epoch of decision-related firing is much more

sudden than revealed by trial-averaged single neuron responses. GC neural ensembles flip suddenly and spontaneously between a set of stereotypical population firing 'states', and the emergence of decision-related firing varies from trial-to-trial (Jones et al., 2007). Here we test the hypothesis that this sudden and variable emergence of palatability/decision related firing holds behavioral significance on single trials – using chronic, multi-electrode recordings of GC neurons (~5-30 neurons) in awake behaving rats, we show that the variability in palatability-related dynamics of GC ensembles, hitherto dismissed as 'noise', correlates strongly with the onset of ingestion-egestion related orofacial behavior on a trial-by-trial basis. We go on to test the functional significance of this brain-behavior correlation – we show that optogenetic inactivation of GC populations during taste processing affects the timing and probability of palatability-specific ingestion-egestion orofacial movements. Finally, we develop a novel optotrode system to probe the stochastic and dynamic states of GC ensemble activity with brief, targeted optogenetic inactivation and explore its behavioral consequences. All in all, our work starts to unravel the role of stochasticity in the processing of taste stimuli in GC ensembles and its behavioral significance in the context of brainstem controlled ingestion-egestion orofacial behavior.

Disclosures: **N. Mukherjee:** None. **J. Wachutka:** None. **D.B. Katz:** None.

Poster

050. Taste: Behavior and Processing

Location: Halls B-H

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Program#/Poster#: 50.02/U16

Topic: D.04. Olfaction and Taste

Support: NIDCD grant RO1 DC006914

Title: Gustatory cortical input onto the nucleus of the solitary tract refines neuronal firing patterns and enhances learning in the awake rat

Authors: ***J. D. SAMMONS**[1], C. E. BASS[2], J. D. VICTOR[3], P. M. DI LORENZO[1]; [1]Psychology, Binghamton Univ., Binghamton, NY; [2]Pharmacol. and Toxicology, Univ. at Buffalo, SUNY, Buffalo, NY; [3]Feil Family Brain and Mind Res. Inst., Weill Cornell Med. Col., New York, NY

Abstract: Taste responsive neurons in the nucleus of the solitary tract (NTS, the first relay in the central gustatory pathway) are almost all broadly tuned across taste qualities. In addition to input from the tongue, the NTS receives centrifugal input from multiple brain regions including the gustatory cortex (GC). In the NTS, spike patterns (rate envelope and spike timing) are known to

convey more information about taste quality than spike count alone. However, whether direct GC-NTS input can shape spike patterns of NTS responses is unclear, as is its behavioral relevance. Here, we used optogenetic tools to manipulate GC-NTS input while recording from the NTS in awake, freely licking rats during taste-driven behaviors. We then used a Go-no-Go (GnG) paradigm to study the behavioral effects of selective enhancement or inhibition of GC-NTS input. We first infused viral constructs containing channelrhodopsin 2 or halorhodopsin genes bilaterally into the GC. After 2-4 weeks, we implanted an optrode consisting of a fiber optic implant attached to a bundle of 8 tungsten microwires into the taste responsive portion of the NTS. Following recovery, rats were moderately water deprived and placed in an experimental chamber where they experienced a "taste-only" or GnG paradigm. In the taste-only paradigm, trials of 5 consecutive licks (12μL/lick) of a tastant (0.1 M NaCl, 0.1 M sucrose, 0.1/0.01 M MSG/IMP, 0.01 M citric acid, 0.001 M quinine, or artificial saliva) were interspersed with 5 licks of artificial saliva presented on a VR5 schedule. After several days of taste-only recording, rats were switched to the GnG paradigm. Each trial in the GnG paradigm started with a single cue stimulus lick of 0.1 M NaCl followed by 5 dry licks and then 3 test stimulus licks (0.1 M MSG/IMP or 0.1 M KCl). If cue and test stimuli matched, continued licking produced a 3-lick 0.5 M sucrose reward; if cue and test stimuli differed, continued licking produced a 3-lick 2 mM quinine punishment. Withholding licking for 1 s enabled them to avoid this punishment. Incorrect responses also included a 5 s timeout. In a random half of each trial type, GC-NTS input was manipulated optically (25 Hz of 473 nm or 532 nm laser light at 8-10 mW) for a maximum 1 s following each test lick. Results show that stimulation or inhibition of GC-NTS input can change the temporal characteristics of taste-evoked spike trains in the NTS. Further, GC-NTS enhancement improved GnG learning only for difficult tasks while inhibition of GC-NTS input impaired GnG performance. These data demonstrate a role of direct GC-NTS input in shaping NTS firing patterns and that these changes are functionally expressed by changes in performance in a GnG paradigm.

Disclosures: **J.D. Sammons:** None. **C.E. Bass:** None. **J.D. Victor:** None. **P.M. Di Lorenzo:** None.

Poster

050. Taste: Behavior and Processing

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Topic: D.04. Olfaction and Taste

Support: NIDCD grant RO1-DC013770

Title: Patterned activation of amygdalo-cortical projections induces synaptic and intrinsic plasticity in rat primary gustatory cortex.

Authors: *M. HALEY, A. FONTANINI, A. MAFFEI;
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Abstract: Projections from the basolateral amygdala (BLA) to the agranular portion of primary gustatory cortex (aGC) show laminar-specific and target-specific properties. Previous studies that used a combination of pharmacology, behavior, and extracellular recordings demonstrated that input from BLA to GC is necessary for learning about the hedonic value of a taste and predictive cues anticipating taste stimuli. These learning processes are hypothesized to induce plastic changes between BLA and GC, but the capacity for plasticity of BLA-GC synapses has not been investigated. Here we used whole-cell patch clamp in acute GC slices combined with optogenetic activation of BLA terminal fields to investigate 1) the patterns of BLA afferent stimulation that induce plasticity at BLA-GC synapses, 2) whether BLA-GC plasticity induction and expression may vary depending on target neuron identity and location. We show that LTD is the dominant form of plasticity induced at BLA inputs onto PYR neurons in supragranular GC (sGC). The magnitude of the LTD differed by induction paradigm (% changes, phasic bursts - 20Hz: -27.7 ± 3.3; 40Hz: -48.3 ± 9.8; Tonic firing for 6s: -43.3 ± 6.2). In infragranular GC (iGC), LTD was induced at BLA-PYR synapses using phasic busts at 20 and 40 Hz (% changes, phasic bursts - 20Hz: -28.0 ± 3.5; 40Hz: -36.5 ± 6.3). However, differently from PYRs in sGC, induction using tonic activation of BLA-GC afferents induced LTP (% change, tonic firing: 98.1 ± 19.0). We also report that in both sGC and iGC, tonic activation of BLA afferents induced intrinsic plasticity of PYR neurons, increasing their excitability. Our results indicate that BLA-GC synapses show laminar differences in their capacity for plasticity, suggesting that input from BLA to the superficial and deep layers of aGC may subserve different functions in taste learning.

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Poster

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Topic: D.04. Olfaction and Taste

Support: NIH/NIDCD R01DC012543

Title: Layer specific sensorimotor activity in the gustatory cortex of licking mice.

Authors: *G. N. DIKECLIGIL, D. M. GRAHAM, I. M. PARK, A. FONTANINI;
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Abstract: Gustatory Cortex (GC) neurons in alert rodents display rich temporal dynamics encoding the chemical and somatomotor features of a gustatory experience. How these dynamics are represented across layers and cell types in GC is unknown. Specifically, little is known on how patterns of somatomotor -related activity differ across elements in GC circuits.
To address this question we performed extracellular recordings targeted to deep (Layers 4-6) and superficial layers (Layer 2/3) of GC in head restrained mice engaged in a cued-licking paradigm. Mice received a single drop (~2 μL) of taste solution in each trial and were trained to continue licking at 7 Hz for 750 ms to ensure stereotyped somatomotor behavior across trials.
Isolated single units with narrow waveforms (peak to through 100-275 μs) were classified as putative inhibitory cells and those with wide waveforms (peak to through 300-500 μs) were classified as putative pyramidal cells. A total of 202 single units were recorded with 39/202 deep layer inhibitory cells (DI), 89/202 deep layer pyramidal cells (DP), 71/202 superficial layer pyramidal cells (SP) and 3/202 superficial inhibitory cells (SI). SI cells were not analyzed further due to small sample size.
Principal component analysis of normalized peri-stimulus time histograms revealed that DI cells display strong pre-stimulus modulations and tonic stimulus-evoked response profiles. DP cells show small pre-stimulus modulation with a mixture of rhythmic and tonic evoked responses. In contrast with deep layer cells, SP cells show very strong coupling to licking rhythm with negligible pre-stimulus modulations. The percentage of neurons that show a significant modulation in the pre-stimulus epoch (300 ms prior to first lick) is greater for DI cells compared to DP and SP cells ($p<0.05$, DI: 79% , DP: 47%, SP: 33%).
To relate pre-stimulus modulations to mouth movements we performed cross-correlations between continuous mouth movement signals (obtained by video analysis) and firing rates. 92% of pre-stimulus modulated neurons showed significant correlations with mouth movements. GC firing could both precede and follow mouth movements, suggesting that pre-stimulus activity could reflect both preparation and execution of licking.
Our data show that response dynamics of GC circuits are layer and cell type specific and this differentiation extends to periods prior to the arrival of taste stimuli. The bias of pre-stimulus activity to putative interneurons in deep layers suggests that inhibition may play an important role in preparing GC neurons for receiving taste and in shaping taste response dynamics.

Disclosures: G.N. Dikecligil: None. **D.M. Graham:** None. **I.M. Park:** None. **A. Fontanini:** None.

Poster

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Topic: D.04. Olfaction and Taste

Support: R01 DC006666-00

R01 DC007703-06

R03 DC014017

Title: Holistic processing of taste mixtures in rat gustatory cortex

Authors: *J. WACHUTKA[1], N. MUKHERJEE[2], S. ZARMSKY[2], D. KATZ[2];
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Abstract: Taste plays a crucial role in the feeding behaviors of animals--acting as the last line of defense against ingestion of potentially harmful stimuli. We avoid bitter and sour foods that may be potentially harmful in favor of sweet, salty, or savory flavored foods—tastes generally associated with nutrients and calories that we require. However, natural foods rarely feature only one basic taste component, and there are some situations—when an animal encounters a sweet yet harmful fruit, for example—in which animals must learn to avoid foods due to physiological affects rather than discriminating by pleasantness of taste alone. This presents the taste system with a difficult problem: it must process a diverse range of sensory stimuli and post-ingestive memory in varying contexts while reliably producing the correct behavioral output. Here, we show that rats are capable of responding to a mixture of two taste cues in the opposite ingestive manner as they do to either taste when presented in isolation following conditioned taste aversion learning: taste mixtures can be processed not as a combination of component parts, but rather as a single unique, holistic entity. We hypothesize that this holistic processing leads animals to generalize taste learning to mixture components on the basis taste quality similarity, rather than processing the mixture as the co-occurrence of two independent features. Future electrophysiological work in rat gustatory cortex will explore single unit responses to binary taste mixtures and single tastes to test the prediction that mixture responses do not simply reflect the simultaneous activation of individual taste response patterns, but in fact reveal a unique pattern reflecting our observed holistic treatment of taste.

Disclosures: **J. Wachutka:** None. **N. Mukherjee:** None. **S. Zarmsky:** None. **D. Katz:** None.

Poster

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Topic: D.04. Olfaction and Taste

Support: JSPS KAKENHI Grant Number 26463192

Title: Modification of the gustatory responses in rat insular cortex by the olfactory stimulation.

Authors: ***N. MIZOGUCHI**[1], M. KOBAYASHI[2], K. MURAMOTO[1];
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Abstract: Flavor is a sense that is important to identify foods in a mouth and is related to a good taste and the satisfaction of the meal. When eating foods, taste information is integrated with odor cues to form a flavor sensation in a certain brain region. Several studies reported previously that the insular cortex (IC) is received the olfactory input in addition to taste information. However, it has been unclear by what kinds of mechanism two chemical senses are integrated in the IC.
To address this issue, we examined the spatiotemporal dynamics of excitatory propagation in cerebral cortex induced by simultaneous electrical stimulation (5 train pulses at 50 Hz) of the chorda tympani nerve (CT) and main olfactory bulb (mOB). Rats were anesthetized by intraperitoneal injection of urethane, and cortical responses to stimulations of gustatory and/or olfactory pathways were observed by an *in vivo* optical imaging technique using a voltage sensitive dye. (RH1691). Our surgical operation permitted a clear visualization of cortical areas around the IC, including the ventral mOB, lateral olfactory tract, anterior and posterior PC. We observed the optical responses in these cortical regions to electrical stimulation of the CT and/or the ventral mOB. The principal responding region with the CT stimulation was located in the rostral part of the dysgranular IC (DI). On the other hand, ventral mOB stimulation evoked excitatory propagation that spread in the piriform cortex (PC) and the agranular region (AI), which locates ventrally adjacent to DI.
Next, we examined the effects of simultaneous stimulation of CT and ventral mOB on the IC response by changing an interval between two stimulations. Ventral mOB stimulation was applied at three timings: simultaneous, 150 ms before, and 150 ms after CT stimulation. Simultaneous stimulation of CT and ventral mOB additively increased the response-amplitude in AI, and shorten the time to peak of excitation in DI without changing its signal amplitude. Ventral mOB stimulation 150 ms before or after CT stimulation evoked the response that peaked just after CT stimulation in DI and after mOB stimulation in AI. These results suggest that DI principally responses to inputs from CT, and in contrast, AI is more sensitive to inputs from mOB than CT. It was showed that the short time lag between odor and taste inputs affected the

responses in the IC.
Our data suggest that the gustatory and the olfactory information is at least partially integrated in AI and DI to form flavor prior to the orbitofrontal cortex.

Disclosures: **N. Mizoguchi:** None. **M. Kobayashi:** None. **K. Muramoto:** None.

Poster

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Topic: D.04. Olfaction and Taste

Support: Consejo Nacional de Ciencia y TecnologIa of Mexico Grant 179484

Fronteras de la Ciencia Grant 63

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Title: Neural correlates of sweet taste intensity in the insular and orbitofrontal cortex

Authors: *E. G. FONSECA DE LA CRUZ[1,2], A. S. MATSUMOTO[4,2], M. VILLAVICENCIO[2], A. I. HERNANDEZ-COSS[3], S. A. SIMON[5], R. GUTIERREZ[2];
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Abstract: Food consumption of any taste quality is modulated in an intensity dependent manner. Rodents taste intensity processing neural correlates has been described from the periphery to the insular cortex (IC). Nonetheless, methodological ways to measure taste sensitivity requires an improvement in order to assess neural correlates in freely moving animals which are actively acquiring sensory information to construct a taste percept. On the other hand, despite Orbitofrontal Cortex (OFC) is also known as secondary gustatory cortex its role in intensity processing has been left aside. To address this issues, we performed single unit recording in IC and OFC of rats trained in a novel sucrose intensity discrimination task that allow us to measure behavioral and neuronal taste identification sensitivity by using psychophysics. During training sessions subjects had to emit differential responses (left or right) according to the taste intensity cue received: low(3%) or high(18%). Once subjects learned the task, rats accessed classification session: rats had to classify different sucrose intensities (3, 4.75, 7.5, 11.75 or 18%) as “low” or “high”. Afterwards, rats were stereotaxically implanted with a 16 channel multielectrode array for recording single unit activity while they were performing in training and classification

sessions. The task was divided in the following epochs: 1) Cluster Start (CS), first dry lick, 2) Cue delivery, 3) Cluster End (CE) last after cue lick, 4) PreRew, 300 ms before reward delivery and 5) Reward delivery. Preliminary data from 538 (training) and 319 (classification) OFC neurons, and 80 (training) and 50 (classification) IC neurons has been obtained. IC and OFC neurons processed orosensory and motor information during different epochs, responding in an inhibitory, excitatory phasic, excitatory tonic or licking-coherent way. In training sessions, we found OFC neurons that discriminates between concentration during Cue, CE and PreRew epochs. OFC neurons responding in an intensity dependent manner were found. These intensity processing neurons classifies intensities in one of two ways: phasic/categoric or monotonic with gradual increases or decreases in their firing rate in an intensity dependent manner. Similar responses are expected to be seen in Insular Cortex. Neural sensitivity to identify sucrose intensities will be calculated for neurons in both areas using psychophysics approximation.

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Poster

050. Taste: Behavior and Processing

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Topic: D.04. Olfaction and Taste

Support: UVa CHARGE/NSF ADVANCE Grant # HRD1209197

Title: Gustatory thalamus in the tree shrew and the rodent are not similar

Authors: *E. MAHER[1], M. PRILLAMAN[1], H. PETRY[2], A. ERISIR[1];
[1]Psychology, Univ. of Virginia, Charlottesville, VA; [2]Psychological and Brain Sci., Univ. of Louisville, Louisville, KY

Abstract: Sensory thalamic nuclei display remarkable similarities in their inputs, intrinsic circuitry, synaptic organization, and neurotransmitters, the gustatory thalamus in particular remains the most sparsely studied sensory nuclei in the mammalian brain. We have recently provided the first characterization of the synaptic circuitry in the gustatory thalamic nucleus (VPMpc) in the rat (Holtz et al., 2015), including its primary input from parabrachial nucleus (PBN) and feedback projections from the insular cortex. We demonstrated that the synaptic circuitry in the VPMpc was unique compared to other mammalian thalamic nuclei: PBN axons synapse exclusively on proximal relay cell dendrites; glomerular organizations of primary inputs and triadic arrangements, two hallmark morphologies in the sensory thalamus, are missing; and dense core vesicles and CGRP label mark inputs from multiple origins. We argued that these unique properties of the rat gustatory thalamus were either specific for chemical senses, or they were a consequence of pathway differences between primates and rodents. In primates, the gustatory thalamus receives its primary input directly from nucleus of the solitary tract (NTS), whereas in rodents, the NTS instead projects to the PBN, which in turn, projects to the thalamus and amygdala. In order to test for these possibilities, we examined the gustatory thalamus in the tree shrew, a close relative to non-human primates. Indeed, while retrograde tracers in the VPMpc of rodents do not result in any label in the NTS, a similar injection in ventral thalamus of the tree shrew confirmed the presence of solitario-thalamic cells in the rostral NTS. Unlike in the rat, the tree shrew gustatory thalamus, the VPMP, has well differentiated cytoarchitectonic borders, delineated by myelin and cytochrome oxidase stains. Unlike in the rodent VPMpc, but similar to other mammals, the tree shrew VPMP is immuno-stained for VGluT2. Also unlike in the VPMpc, where CGRP is a marker for inputs including those from PBN, no CGRP+ terminals were encountered in the tree-shrew VPMP. Electron microscopy revealed that unlike in rodents, the VPMP in tree shrew contains triadic arrangements and glomeruli where VGluT2+ terminals are located. The characteristic morphology of PBN terminals mentioned above were not encountered in the tree shrew. Overall, our data confirm the hypothesis that the synaptic

organization of the rodent gustatory thalamus is substantially different than in the tree shrew, a species with a direct solitario-thalamic pathway, and suggest that rodent taste thalamus circuitry is not representative of the taste thalamus in primates.

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Poster

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Ajinomoto Co., Inc

Title: Pungency and pain and *In vivo* imaging of mouse trigeminal ganglion neurons

Authors: S. LEIJON[1], J. M. BREZA[2], N. CHAUDHARI[1], *S. D. ROPER[1];
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Abstract: Certain plant-derived compounds such as capsaicin, allyl isothiocyanate (AITC), and menthol add pungency to food and beverages and change the perception of oral thermal sensations. Capsaicin is an agonist of TRPV1, a channel that also transduces nociceptive stimuli. AITC stimulates TRPA1 and menthol activates TRPM8, both of which are associated with environmental cold. The trigeminal ganglion receives somatosensory signals from the oral cavity, including pain, pungency, and temperature. Neurons in this ganglion express TRPV1, TRPA1, and TRPM8, with many neurons co-expressing combinations of these channels (e.g. TRPV1/TRPA1). We are exploring the representation of pungency, pain and temperature in the trigeminal ganglion, imaged *in vivo*. Mice were deeply anesthetized and cortical tissue overlying area V3 of the ganglion (identified as containing neurons with receptive fields in the oral cavity) aspirated via a cranial window. Using confocal Ca^{2+} imaging in mice that express the Ca-indicator GCaMP3 in sensory neurons (Kim *et al*, *Neuron* **81**:873, 2014), we could monitor up to 800 trigeminal ganglion cells simultaneously to record orally-evoked neuronal responses. Artificial saliva containing capsaicin (10 to 1000 μM), menthol (1-5 mM), AITC (20 mM), or artificial saliva alone at temperatures ranging from nociceptive cold (2^{o}) to nociceptive hot (45^{o}) were delivered retrogradely via a catheter inserted through the esophagus as 10 second oral lavages. All stimuli elicited responses in neurons having significantly smaller diameters (mean =

19.2 ± 0.4 μm, n=73) than the average diameter of trigeminal neurons (24.5 ± 0.4 μm, n=204; $p<0.0001$), consistent with nociceptors and thermoreceptors. Capsaicin, AITC, and menthol elicited responses in distinct populations of ganglion neurons, despite there being overlapping co-expression of TRPV1 and TRPA1 channels. Interestingly, nociceptive cold (2^{o} C) applied orally stimulated a different subset of neurons than the same stimulus (in Tyrode buffer) applied topically onto the trigeminal ganglion. There was only 6% overlap between these two subsets. Capsaicin (100 μM) applied orally produced a long-lasting (minutes) enhancement of responses to oral lavage with warm stimuli. Menthol (1-3 mM) appeared to have a similar effect for cold stimuli. In addition to exploring the neural basis of everyday somatosensory experiences during food intake, this preparation lends itself as a promising tool for studying orofacial pain, such as thermal allodynia and inflammatory evoked hyperalgesia.

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Poster

050. Taste: Behavior and Processing

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Program#/Poster#: 50.10/V6

Topic: D.04. Olfaction and Taste

Support: University of Nebraska at Omaha Office of Research and Creative Activity

Title: Developmentally dependent microglia increase following chorda tympani transection in rats

Authors: *A. J. RIQUIER, S. I. SOLLARS;
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Abstract: The taste system provides a useful model for the study of neuroplasticity, due in part to the dynamic and relatively rapid changes that occur as a result of normal development and in response to injury. The chorda tympani (CT) is a gustatory nerve that transmits information from taste buds in fungiform papillae to the rostral nucleus of the solitary tract (rNTS) in the brainstem. Transection of the CT (CTX) performed at various ages in the rodent model is a

paradigm used to examine adaption to neural insult of both developing and mature gustatory systems. Since the CT runs ipsilaterally from the taste buds to the rNTS, the side contralateral to a CTX is often used as a control. Adult (≥ 40 days of age) CTX results in temporary loss of taste buds and moderate changes to rNTS terminal fields. Previous work from our lab showed that CTX performed in neonatal rats (≤ 10 days of age) results in profound and permanent loss of taste buds, fungiform papillae, and rNTS terminal fields. While the mechanisms underlying this terminal field loss are unknown, microglia have been shown to increase in number in the rNTS four days following unilateral CTX in adult mice (Bartel, 2012). However, microglia response to CTX performed at pre-adult ages has not been examined. In the present study, four rats received unilateral CTX at 10, 25, or 50 days of age and were sacrificed four days later. Animals were perfused and brains extracted, post-fixed in 4% paraformaldehyde, and sectioned horizontally at 40 μm on a vibratome. Sections were processed for the microglia marker ionized calcium-binding adapter molecule 1 (Iba1). Iba1+ cells in the rNTS were counted using the program Neurolucida (MBF Bioscience). Microglia density was calculated by dividing the number of Iba1+ cells by the area of the rNTS. An ANOVA revealed a significant main effect of both age ($p < .05$) and surgery ($p < .05$). Paired-samples t-tests were used to compare the number of microglia in the rNTS on the transected side to the contralateral intact side. Microglia increased in number in the rNTS when CTX was performed at 50 ($p < .01$), 25 ($p < .01$), or 10 ($p < .05$) days of age. The percentage increase was calculated for each age to allow for comparisons across age conditions. While CTX performed at 25 or 50 days of age elicited a similar microglia response ($p > .05$), 10-day CTX resulted in significantly less response in comparison to 25 ($p < .05$) and 50 ($p < .01$) CTX. These findings show that, although microglia increased in number following CTX in all ages tested, the magnitude of the increase differed between the youngest and the two older ages. These findings may shed light on the developmentally differing degree of terminal field loss observed following CTX.

Disclosures: **A.J. Riquier:** None. **S.I. Sollars:** None.

Poster

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Topic: D.04. Olfaction and Taste

Support: NIDCD 04846

Office of Research and Creative Activity, University of Nebraska at Omaha

Title: Transganglionic degeneration of the chorda tympani nerve terminal field following neonatal denervation

Authors: *L. J. MARTIN[1], K. K. SAMSON[2], A. H. ORAND[1], S. I. SOLLARS[1];
[1]Univ. of Nebraska At Omaha, Omaha, NE; [2]Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Taste afferents project to the nucleus of the solitary tract (NTS) where their terminal fields overlap considerably - creating the opportunity for competition between nerves. We have found that chorda tympani transection (CTX) in rats leads to reorganization of glossopharyngeal and greater superficial petrosal nerve terminal fields when surgery occurs at postnatal day 5 (P5), such that both of these nerves have a larger volume in the dorsal NTS. This effect does not occur following CTX at P10. Since the chorda tympani (CT) does not regenerate if CTX occurs at P10 or earlier, it is unclear why the extent of reorganization differs between these surgical ages. To understand how this altered projection pattern relates to CT degeneration, we analyzed CT terminal field volumes after CTX at P5 or P10. The CT was first accessed in neonatal rats (n=5/surgical group) and either transected (CTX) or left intact (Sham). Following 50-51 days survival, the CT was cut in the tympanic bulla (central to the initial transection) and biotinylated dextran amine was applied to the proximal end of the nerve. Animals were perfused 48 hr later and brain tissue was sectioned at 50 μm in the horizontal plane. Sections were processed using diaminobenzidine (DAB) to visualize the CT terminal field. Images were taken of each section containing CT terminal field, and a pixel analysis (ImageJ) was performed to determine terminal field volumes. Transection of the CT at either age resulted in a significantly lower terminal field volume relative to controls; CTX at P5 and P10 led to CT terminal field volumes that were 12% and 24% of sham volumes, respectively. The loss of terminal field is much greater than that observed after adult CTX (Reddaway et al., 2012). While there was a tendency for terminal field volumes to be smaller after P5 CTX relative to transection at P10, this difference was not statistically significant (p = .08). After CTX at P5 or P10, CT volumes were significantly lower than Sham volumes in the dorsal, intermediate, and ventral NTS. CT volumes following CTX at P5 were not significantly different from those of P10 CTX rats for any zone of the NTS. Volumes in the dorsal zone in particular were very similar after CTX at P5 or P10 - representing 13% and 14% of sham volumes, respectively - suggesting that the CT degenerates to a similar extent following denervation at these ages. Thus, the expansion of the glossopharyngeal and greater superficial petrosal nerves into the dorsal zone following CTX at P5 appears to be caused by factors other than CT retraction alone. These findings suggest that there may be a sensitive period whereby nerves are best equipped to reorganize in response to degeneration of an adjacent nerve.

Disclosures: **L.J. Martin:** None. **K.K. Samson:** None. **A.H. Orand:** None. **S.I. Sollars:** None.

Poster

050. Taste: Behavior and Processing

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 50.12/V8

Topic: D.04. Olfaction and Taste

Support: NIDCD Grant 014428

University of Michigan, M-Cubed funding

Title: Thermal and tactile responses of the rat chorda tympani nerve

Authors: Y. YOKOTA, A. KUMARI, C. M. MISTRETTA, *R. M. BRADLEY;
Univ. Michigan Sch. Dent., Ann Arbor, MI

Abstract: Previous investigations of the properties of primary afferent neurons of the chorda tympani nerve (CT) have emphasized responses to stimulation of the anterior tongue with chemicals. However it has been known for some time that the CT also responds to thermal and tactile stimulation of the tongue dorsum. Recently we have shown that inhibiting Hedgehog signaling using a pharmacological blocker treatment in mice results in loss of taste bud integrity and CT electrophysiological responses to chemical stimulation (Kumari et al. J. Neurophysiol. 2015). However, CT responses to tactile and cold stimuli are retained indicating that these two modalities are mediated by different sensory receptors than taste. We have investigated the receptive field properties of geniculate ganglion/chorda tympani (GG/CT) neurons using extracellular recordings and stimulation of the tongue with chemicals (Yokota and Bradley, J. Neurophysiol. 2016). Currently we are characterizing the cold and tactile responses of GG/CT neurons. Receptive field location and size of the neurons were determined by electrical stimulation of individual fungiform papillae. To date 7 GG/CT neurons have been isolated that responded only to cold stimulation of the tongue. Receptive field size of these neurons was 1.6 ± 0.5 (mean, S.D) papillae, significantly smaller than average field size of neurons responding to chemical stimulation. The thermal responding papillae were located at the tongue tip. We have also isolated GG/CT neurons that innervate fungiform papillae responding exclusively to mechanical stimulation. These neurons also have small receptive fields consisting of 1 or 2 papillae. While receptor mechanisms of tongue chemoreceptors have been extensively investigated little is known about the identity of the thermal and mechanosensitive lingual receptors innervated by the CT. Experiments are in progress to identify possible receptors by evaluating the effect of receptor antagonists on GG/CT responses. In recent experiments we have used TRPA1 receptor antagonists AP18 and HC-030031 injected into the tongue to explore a possible role in the CT response to 4ºC water. Based on all results it is apparent that fungiform papillae are complex sensory organs responding to multiple modalities that can serve a role similar to the finger tips as an oral organ of sensory exploration.

Disclosures: **Y. Yokota:** None. **A. Kumari:** None. **C.M. Mistretta:** None. **R.M. Bradley:** None.

Poster

050. Taste: Behavior and Processing

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 50.13/V9

Topic: D.04. Olfaction and Taste

Support: NIH Grant R01AG004085-26 from the NIA to CM.

Title: Metabolic syndrome is associated with altered functional connectivity of the primary and secondary taste cortices and eating disinhibition

Authors: ***E. E. PONGPIPAT**, A. JACOBSON, C. MURPHY;
San Diego State Univ., San Diego, CA

Abstract: Metabolic Syndrome (MetS) is a cluster of risk factors that increases the risk of heart disease, stroke, diabetes, and late-life cognitive impairment and dementia. The risk factors include hypertension, elevated resting blood sugar/insulin resistance, hypertriglyceridemia, abdominal obesity, and abnormal cholesterol levels. The present study examined the indirect relationship of MetS risk factors and eating disinhibition mediated by the functional connectivity of primary and secondary taste cortices in middle-aged adults. Thirty-four middle-aged adult participants between the ages of 43 and 54 years underwent functional Magnetic Resonance Imaging (fMRI) while tasting and rating the pleasantness of sucrose after a 12-hour fast. The functional connectivity was analyzed and obtained using psychophysiological interaction methods. Additionally, participants completed the Three-Factor Eating Questionnaire (TFEQ) to measure eating behavior such as eating disinhibition. A path-analytic model tested the indirect relationship from the number of MetS risk factors to the eating disinhibition score from the TFEQ via the functional connectivity of primary and secondary taste cortices (i.e. right frontal operculum and left caudolateral orbitofrontal cortex, respectively) as a mediating variable. The target model fit well statistically, ($\chi^2(1, N=28) = 1.465$, $p = .226$) and descriptively (CFI = .956, RMSEA = .129, SRMR = .061). With respect to the relationship specified within the model, individuals were significantly more likely to have an increased positive functional connectivity of primary and secondary taste cortices as the number of MetS risk factors increased ($r = .417$, $p < .015$). The eating disinhibition score was also significantly more likely to increase as the functional connectivity of primary and secondary taste cortices increased ($r = .461$, $p = .006$). For middle-aged adults, these findings suggest that the relationship of eating disinhibition in individuals with MetS may be mediated by an altered functional connectivity of primary and

secondary taste cortices. Future studies may consider examining whether behavior modifications such as healthy eating, exercise, or medication and surgery can ameliorate MetS and its downstream effects of altered functional connectivity of primary and secondary taste cortices and eating disinhibition. We thank Dr. Erin Green, Dr. Lori Haase and Elissa McIntosh for participation in MRI and neuropsychological data acquisition and analyses, Drs. Santiago Horgan, William Perry and Charlie D. Morgan for assistance with subject recruitment, and Dr. Tom Liu and the UCSD Center for fMRI.

Disclosures: E.E. Pongpipat: None. **A. Jacobson:** None. **C. Murphy:** None.

Poster

050. Taste: Behavior and Processing

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 50.14/V10

Topic: D.04. Olfaction and Taste

Support: CRC 1052 Obesity Mechanisms (A01)

Title: Differences in neural gustatory processing between lean and obese individuals revealed by evoked responses.

Authors: *S. HARDIKAR[1], R. WALLROTH[2], A. VILLRINGER[1], K. OHLA[2];
[1]MPI for Human Cognitive and Brain Sci., Leipzig, Germany; [2]German Inst. of Human Nutr., Nuthetal, Germany

Abstract: The sense of taste serves to help us in identifying nutritional food sources and avoiding harmful ones. The sensory appeal of food, including taste, motivates food intake even after satiation, leading to over-nutrition. Yet, little is known about the relationship between taste perception and obesity. Especially the spatio-temporal processing of gustatory stimuli has never been explored in relation to Body Mass Index (BMI). In this study, we presented two suprathreshold intensities of sweet (10 g/100 mL and 5 g/100mL sucrose) and salty (2.5 g/100mL and 1.25 g/100mL NaCl) tastants to 30 lean (BMI < 25) and 24 obese (BMI > 30) individuals using a gustometer suited to elicit gustatory evoked responses without concomitant somatosensory activation, while recording head-surface electroencephalography (EEG) from 62 channels.
All participants rated intensity as higher and pleasantness as lower for high compared to low taste intensities. Global Field Power (GFP) was higher in lean than in obese, and for salty than for sweet taste in both groups, which is in line with previous reports of an increased signal-to-noise ratio for salty compared to sweet taste responses; no effect of intensity modulation was

found. Electric field distributions for all conditions differed significantly between lean and obese (130-158 ms, and from 430 ms onward) as well as showing a group*taste quality interaction (from 196 ms onward), indicating differential neural networks in both groups.
Between-subjects multivariate pattern analysis was used to examine group-specific properties in the neural processing of taste by training shrinkage Linear Discriminant Analysis (LDA) classifiers to discriminate between lean and obese participants for each of the tastes and intensities separately. Model performance was evaluated with data from participants not part of the training set. Significant decoding performance for group membership was observed for all tastes starting at 370 ms and mainly driven by salty taste for which significant group decoding was found at both high and low concentrations beginning as early as 190 ms.
The results suggest that lean and obese individuals process sweet and salty tastes differently, although the behavioural consequences of these differences remain to be explored.

Disclosures: **S. Hardikar:** None. **R. Wallroth:** None. **A. Villringer:** None. **K. Ohla:** None.

Poster

050. Taste: Behavior and Processing

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 50.15/V11

Topic: D.04. Olfaction and Taste

Support: AG004085-26

Title: Cortical correlates of metabolic syndrome risk factors and hunger in middle aged and older adults

Authors: ***R. B. VERTREES**[1], E. E. PONGPIPAT[1], E. MCINTOSH[1], C. MURPHY[1,2,3]; [1]San Diego State Univ., San Diego, CA; [2]SDSU/UCSD Joint Doctoral Program in Clin. Psychology, [3]UCSD, San Diego, CA

Abstract: Metabolic syndrome (MetS), which affects nearly 35% of U.S. adults and 50% of older adults (60+), is defined as the assemblage of risk factors for development of cardiovascular disease and type 2 diabetes. The risk factors are composed of 5 elements (i.e. high blood pressure, high triglyceride level, low HDL cholesterol level, raised fasting glucose, and abdominal obesity) as defined by the Joint Scientific Statement by K. Alberti et al., *Circulation (*2009). Possessing 3 of the 5 risk factors qualifies an individual for a diagnosis of MetS. The purpose of the current study was to examine the effect of MetS risk factors on hunger (as measured by the Three-Factor Eating Questionnaire) and how this relationship is affected by structural brain changes within taste cortices in middle-aged and older adults. The primary taste

cortex is located within the insula and is responsible for intensity and identification of taste. The secondary taste cortex, which includes the orbital frontal cortex (OFC), evaluates the reward and pleasantness of taste. Both taste cortices contribute to an individual's food selection, and are, therefore, crucial to issues of unbalanced energy intake/ expenditure such as exhibited in individuals with MetS. Analyzing 33 middle-age and 32 older adult participants using structural MRI, the study found that, for both middle aged and older adults, the greater the number of MetS risk factors, the higher their score on the hunger scale of the TFEQ. For middle aged adults, individuals with greater OFC thickness exhibit a weaker relationship between number of MetS risk factors and hunger ratings ($p=.038$, $\eta^2=.141$). For older adults, individuals with greater insula thickness exhibit a weaker relationship between number of MetS risk factors and hunger ratings ($p=.021$, $\eta^2=.177$). These findings suggest an important interaction between cortical thickness and risk factors of MetS on feelings of hunger, and suggest the importance of further study of taste cortical structure and MetS correlates.
Acknowledgements: Supported by NIH R0I AG004085-26 from NIA to CM. We would like to thank Dr. Lori Haase, Dr. Erin Green, and Mr. Aaron Jacobson for data acquisition, Drs. Santiago Horgan, William Perry and Charlie D. Morgan for assistance in subject recruitment, as well as Dr. Tom Liu and the UCSD Center for fMRI.

Disclosures: R.B. Vertrees: None. **E.E. Pongpipat:** None. **E. McIntosh:** None. **C. Murphy:** None.

Poster

050. Taste: Behavior and Processing

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 50.16/V12

Topic: D.04. Olfaction and Taste

Support: Consejo Nacional de Ciencia y TecnologIa of Mexico Grant 179484

Fronteras de la Ciencia Grant 63

Productos Medix Grant 1275

Title: Sucrose binge eating selectively alters taste palatability but not sweet taste identification

Authors: *A. S. MATSUMOTO[1,2], E. G. FONSECA[3,5], S. A. SIMON[6], R. GUTIERREZ[4]; [1]Pharmacol., Ctr. De Investigación Y De Estudios Avanzados De, Distrito Federal, Mexico; [2]Fac. of Psychology, UNAM, Mexico, Mexico City, Mexico; [4]Pharmacol., [3]Lab. of Neurobio. of

Appetite, Dept. of Pharmacology, CINVESTAV-IPN, Mexico City, Mexico; [5]Inst. of Cell. Physiology, UNAM, Mexico, Mexico City, Mexico; [6]Duke Univ. Sch. of Med., Durham, NC

Abstract: Binge eating (BE) is characterized by the consumption of highly caloric food in a brief period of time (≤ 2h). This pathological feeding pattern might be due to an altered taste perception. However, it is not clear whether sweet intensity sensitivity (SIS) and/or palatability responses are affected by BE development. Although this 2 process normally correlates, they can be separated. To address this issue we measured SIS using a psychophysical sucrose intensity discrimination task before and after sucrose BE induction. During training sessions 24 rats had to emit differential responses (left or right) according to the taste intensity cue received: low (3 %) or high (18 %). Once subjects learned the task, classification sessions where rats had to classify different sucrose intensities (0, 3, 4.75, 7.5, 11.75 or 18 %) as "low" or "high" were introduced. Response probability to high intensity compartment was obtained and a sigmoid function was fitted to obtain psychophysical SIS. Subsequently, rats were divided into 4 groups (n=6/group) with different access to sweet liquids during 21 d: 1) Intermittent sucrose (2 h, INTsuc), 2) *Ad libitum* sucrose (24 h, ALsuc), 3) Intermittent sucralose (2 h, INTscl), and 4) No sweet access (Ctrl). We found that SIS was not modified by any feeding protocol. Subsequently using a Brief Access Test we evaluated palatability responses evoked by different sucrose intensities. INTsuc displayed higher licking rates for 0 and 3 % intensities, while ALsuc had higher licking rate for all concentrations in comparison to INTscl and Ctrl groups, indicating an increased palatability responses. Licking microstructure was analyzed with a 20 min freely licking test with access to different solutions across days: 1)1-3 d water, 2) 4-8 d sucrose 18%, 3) 9d extinction, 4) 10-13 d reinstatement/sucrose 18%. All groups ingested less sucrose than Ctrl, nevertheless ALsuc had lick bouts almost twice longer than other groups during the first 10 min, while INTsuc during the last 10 min indicating increased palatability responses. INTscl showed a reduced palatability as suggested by shorter bouts than Ctrl during this sessions. During reinstatement we found an increase in palatability during the first 15 min for ALsuc and the last 10 min for INTsuc and INTscl. As well, ALsuc showed higher palatability measured by longer sucrose bouts in comparison with the other groups. Together these data shows that intermittent and *ad libitum* sucrose access did not change the SIS. Nonetheless, it selectively induced a long-term potentiation of sweet palatability responses, leading to pathological overconsumption patterns which is a hallmark in BE disorder.

Disclosures: A.S. Matsumoto: 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; Consejo Nacional de Ciencia y TecnologIa of Mexico Grant 179484, Fronteras de la Ciencia Grant 63, Productos Medix Grant 1275. **E.G. Fonseca:** 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; Consejo Nacional de Ciencia y TecnologIa of Mexico Grant 179484, Fronteras de la Ciencia Grant 63, Productos Medix Grant 1275. **S.A. Simon:** 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; Consejo Nacional de Ciencia y TecnologIa of Mexico Grant 179484, Fronteras de la Ciencia Grant 63, Medix Grant 1275. **R. Gutierrez:** 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; Consejo Nacional de Ciencia y TecnologIa of Mexico Grant 179484, Fronteras de la Ciencia Grant 63, Medix Grant 1275.

Poster

050. Taste: Behavior and Processing

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 50.17/V13

Topic: D.04. Olfaction and Taste

Title: Normosmic subjective ageusia

Authors: *M. K. ALHAIDAR[1], A. R. HIRSCH[2];
[1]neurology, George Washington Univ. Hosp., Washington, DC; [2]Smell and Taste Treatment and Res. Fndn., Chciago, IL

Abstract: Introduction: Subjective ageusia in the presence of objectively normal gustation, orthonasal olfaction, and retronasal olfaction, has not heretofore been described.
Methods: case report: A 59 year old right handed female, with history of geographic tongue, presented with a 3 year course of sudden onset of burning of her tongue and palate, followed the next day by absent ability to taste. She affirms normal olfaction.
Results: Abnormalities in physical examination: white coating in the anterior and center of the tongue. Café au lait spot, right gluteus, 4x6 cm. Telangiectasia, right forearm and arm. Neurological examination: Cranial nerves: Cranial nerve II: anisocoria OD: 4mm OS: 2mm. Fundoscopy: pale discs OU. Motor exam: drift testing: Mild right pronator drift. Reflexes: 1+ both upper extremities. 3+ bilateral quadriceps femoris. All Chemosensory testing normal with Brief Smell Identification Test: 12. Retronasal Smell Index: 5. Propylthiouracil Disk Test: 10. MRI of brain with and without infusion: normal.
Conclusion: Food induced BMS pain may accelerate swallowing, minimizing transit time in the mouth, reducing gustatory and retronasal stimulation. When the patient used the word taste, she may have been describing other components of food perception. In those who complain of taste loss with normal chemosensory testing, more detailed chemosensory assessment is warranted.

Disclosures: M.K. Alhaidar: None. **A.R. Hirsch:** None.

Poster

050. Taste: Behavior and Processing

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 50.18/DP03 (Dynamic Poster)

Topic: D.04. Olfaction and Taste

Support: Internal funds from Opertech Bio,Inc.

Title: Application of an automated rapid throughput taste measurement system to signal detection theory (SDT) using human subjects.

Authors: *R. K. PALMER, M. K. STEWART;
Opertech Bio, Philadelphia, PA

Abstract: Introduced as an alternative to traditional psychophysics, SDT is a conceptual framework that takes into account the bias inherent in any sensory system as a determinant of stimulus discrimination. Although successfully applied to measurements of responses in a broad variety of sensory modalities, application of SDT to taste measurement has been limited by the low throughput capacity and subjective nature of traditional taste panel methods. We have developed an automated rapid throughput system for the objective measurement of human taste, called TaStation^{TM} . The technology and accompanying methodology is based on presentation of randomly selected tastant solutions from a 96-well plate via an automated pipette mounted on a robotic gantry. Sample volumes are small (200 ul) and are self-administered by the subject upon the appearance of a prompt on a touch-sensitive computer display. Subjects are trained to associate a taste stimulus (representative of the basic tastes of sweet, salty, bitter, sour and water) with specific loci in a visual field on the display. The associations are achieved by rewarding correct touch-responses with virtual poker chips having assigned point value, whereas incorrect responses are penalized by subtraction of points from the tally. Subjects typically complete all 96 trials of taste stimuli in less than 45 minutes. We applied this methodology to an SDT design in which a subject's ability to discriminate between the tastes of 270 and 300 mM sucrose was quantified. In each of 6 consecutive test sessions, a single subject was randomly presented 16 trials each of water, 10 mM citric acid (sour), 0.5 mM quinine (bitter), 100 mM NaCl (salty), 270 mM sucrose and 300 mM sucrose (both sweet). In sessions 1 and 2, no reward or penalty occasioned a response on any trial, and a hit rate (H) of 0.71, false-positive rate (F) of 0.78, d' (a measure of discriminability) of -0.219, and c (decision criterion) of -0.663 were calculated, indicating a "liberal" bias on the part of the subject expressing a tendency to accept a high false positive rate on 300 mM sucrose trials to enhance capture of hits on the 270 mM sucrose trials. Sequentially adding a positive consequence (40-cent poker chip for correct responses) to sessions 3 and 4, and both positive and negative consequences (subtraction of 40 cents) in sessions 5 and 6, resulted in shifts of all parameters, with H=0.6562, F=0.5625, d'=0.245, and c=-0.280. Thus

by manipulating the consequences of responses to taste stimuli in the discrimination task, the bias of the subject was shifted to less liberal and discriminability improved.

Disclosures: **R.K. Palmer:** A. Employment/Salary (full or part-time): Opertech Bio, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ownership in the company and listed as inventor on the patents covering the invention. **M.K. Stewart:** A. Employment/Salary (full or part-time): Opertech Bio.

Poster

050. Taste: Behavior and Processing

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 50.19/V14

Topic: D.04. Olfaction and Taste

Title: Rapid generation of concentration-response functions for taste in human subjects using an automated 96-well platform and interactive algorithms for taste measurement.

Authors: *M. M. STEWART, D. J. LONG, R. K. PALMER;
Opertech Bio, Philadelphia, PA

Abstract: Taste is a chemosensory phenomenon mediated by receptors expressed in highly specialized cells of the tongue. As such, taste responses in principle should be amenable to the methods of pharmacology for quantitative analysis of receptor-mediated processes. Critical to any pharmacologic analysis is the establishment of robust concentration-response functions. Applying the principles of pharmacology to the study of human taste has been impeded by prevalent low-throughput methodologies that rely upon large numbers of subjects subjectively evaluating a few samples at a time. We have developed a rapid throughput technology and methodology for human taste measurement, referred to as TaStation^{TM}, with the capacity for establishing robust concentration-response functions for taste in individual subjects within single 45-minute test sessions. An automated pipette mounted on a robotic gantry withdraws 200 ul of taste stimulus solution from a randomly selected well in a 96-well plate. The pipette then is presented to a subject seated before a touch-sensitive display. Prompts appear on the display that instruct the subject step-by step. When prompted, the subject removes the pipette from the gantry and self-administers the 200 ul of taste stimulus. After tasting, the subject then is prompted to respond by touching the display. Underlying a visual field on the display is a Cartesian grid in which specific sets of coordinates have been designated to be associated with basic taste standards (sweet, sour, bitter, salty, and water). Subjects learn the locations of the target coordinates through trial-and-error and are rewarded for correct responses by the appearance of

virtual poker chips carrying point values. Incorrect responses are penalized by reductions in the point tally. Once trained under this procedure, subjects are presented with novel stimuli (i.e., test articles that are not standards) and on these trials responses made anywhere on the display are rewarded. Using this approach we established robust concentrations-response functions for individual subjects within single test sessions. Nonlinear regression of data from a single test of Subject 1 (representative) yielded taste EC50s of 0.08, 4, 32, and 65 mM for quinine (bitter), citiric acid (sour), sucrose (sweet), and NaCl (salty), respectively. In a test of bitter taste, EC50s for denatonium and caffeine were 132 nM and 19 mM, respectively, were measured for Subject 1. The robust concentration-response functions generated through this technology enable a pharmacologic analysis, and thereby a more rigorous quantification of human taste than previously has been available.

Disclosures: **M.M. Stewart:** A. Employment/Salary (full or part-time): Opertech Bio. **D.J. Long:** None. **R.K. Palmer:** None.

Poster

050. Taste: Behavior and Processing

Location: Halls B-H

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Program#/Poster#: 50.20/V15

Topic: D.04. Olfaction and Taste

Support: Supported by NIH grant #AG004085-26 from the National Institute on Aging to CM.

We thank Dr. Tom Liu and the UCSD Center for fMRI.

Title: Neural processing of sweet taste in Hispanic young adults: An fMRI study.

Authors: *J. SZAJER[1], A. JACOBSON[2], E. GREEN[3,4], L. HAASE[5], C. MURPHY[1];
[1]Joint Doctoral Program in Clin. Psychology, SDSU/UC San Diego, San Diego, CA; [2]San Diego State Univ., San Diego, CA; [3]Dept. of Veteran's Affairs, VA Palo Alto Hlth. Care Syst., Palo Alto, CA; [4]Dept. of Psychiatry and Behavioral Sci., Stanford Univ. Sch. of Med., Palo Alto, CA; [5]Dept. of Psychiatry, Univ. of California San Diego, San Diego, CA

Abstract: Research on what is known as the “Hispanic Paradox” has revealed that individuals of Hispanic ethnicity have a higher prevalence of and risk for diabetes than Caucasians. Increasing evidence points to biological bases for these differences, as well as lifespan environmental and lifestyle factors (e.g. diet and eating behavior). Notably, brain response to the physiological states of hunger and satiety has been shown to differ as a function of factors related to risk for diabetes, and differences in brain activation have been associated with childhood diabetes in

Hispanic populations. However, there has been insufficient research on the neural correlates of ethnic and cultural differences in the risk for diabetes in adulthood. Research suggests the typical Hispanic diet includes high consumption of high energy food, such as fructose. Taste is a primary determinant of risk factors like food selection. Differences in the neural response to taste stimuli have been associated with risk factors for diabetes such as obesity and poor nutrition. As such, understanding the neural response to sweet taste stimuli in healthy Hispanic adults is an important first step in characterizing the potential neural mechanisms for this behavior. We used fMRI to examine differences in brain activation during the hedonic evaluation of a sweet taste stimulus (sucrose) as a function of ethnicity (Hispanic vs. Non-Hispanic young adults) and hunger state. Taste stimuli were administered orally during two sessions using a GE 3T-750 scanner: in a fasted state (hunger) and after a caloric preload (satiety). Stimuli were rated for pleasantness on a generalized labeled magnitude scale during each scan session. Data were analyzed at the group level controlling for BMI and age, using the AFNI 3d Mixed Effects Meta Analysis (3dMEMA) program. During hunger, Hispanics had significantly less activation than non-Hispanics in regions critical for taste and reward valuation (e.g. frontal operculum, and frontal and orbitofrontal cortices). During satiety, Hispanics had significantly greater activation in voxel clusters including memory (e.g. hippocampus, entorhinal cortex) and emotion (e.g. amygdala). Differences in processing of sweet tastes have important clinical and public health implications, especially considering increased risk of diabetes in Hispanic populations. Future research aimed at better understanding relationships between health risk and brain function in Hispanic adults is warranted to better conceptualize and develop interventions for these populations.

Disclosures: J. Szajer: None. **A. Jacobson:** None. **E. Green:** None. **L. Haase:** None. **C. Murphy:** None.

Poster

050. Taste: Behavior and Processing

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 50.21/V16

Topic: D.04. Olfaction and Taste

Title: Paradoxical gustatory intensification from traumatic anosmia

Authors: T. LOPES[1], *G. HANSRA[2,1], A. R. HIRSCH[3];
[1]Caribbean Med. Univ., Willemstad-Curacao, Netherlands Antilles; [2]Chicago, IL; [3]Smell & Taste Treatment and Res. Fndn., Chicago, IL

Abstract: Objective: Up to 90% of flavor is due to retronasal smell. Despite this, traumatic anosmia without a reduction in flavor has been reported. Contrastingly, actual flavor enhancement due to orthonasal anosmia has not been reported.
Method: Case Study: A 19-year-old female flipped over a golf cart while driving and sustained a skull fracture with loss of consciousness and intracranial hemorrhage, with anterograde amnesia for 20 minutes and retrograde amnesia for 5 minutes. Shortly thereafter, she noted inability to smell other than for occasional olfactory windows with a whiff of odor. There is no dysosmia, phantosmia, parosmia, or cacosmia. She did admit to flavorful eructation. Despite such loses, not only did she deny any taste loss but actually noted that her sense of taste was exaggerated. Before the trauma her sense of taste was 100% (normal), whereas afterwards, her sense of taste grew to 120% (hypergeusia), such that garlic paste was now too intense and hot sauce was too strong. Her taste acumen remained impeccable such that she was easily able to differentiate foods with mild flavor differences including; types of chocolate, blueberry vs. strawberry yogurt, flavors of almond milk, flavors of rice, and even 312 beer vs. Bud Lite beer. The taste of foods were so magnified that it was "an explosion in [her] mouth-unexpected." This amplified flavor perception has persisted for over 4 months since the trauma. Her preferences have changed in that before she despised alliaceous vegetables and now is partial towards the flavor and seeks them out. Moreover, before she preferred food bland but now prefers them highly seasoned.
Results: Abnormalities on physical examination: Neurological examination: Mental status examination: Digit span: 5 forwards and 3 backwards. Recent recall: 3 of 4 objects in 3 minutes without improvement with reinforcement. Chemosensory: Olfactory: (anosmic). Q-SIT Quick Smell Identification Test: 1 of 3 (anosmic). Olfactometer Identification Test: left nostril 7.00, right nostril 3.00 (anosmic). Brief Smell Identification Test: 7 (abnormal). Snap and Sniff Phenylethanolamine Threshold: left > -2.0, right > -2.0. Alcohol Sniff Test: 0. Retronasal smell: Jelly Bean Difference Test: 2 (abnormal). Gustatory: 6-n-propylthiouracil Disc Test: 10 (normal).
Discussion: Possible mechanisms include; olfactory loss induced disinhibition of gustatory sensation, narcissistic loss with overcompensation and unconscious denial, multisensory input induced illusion, and oral sensory epiphany in the absence of warning smell. The new preference for alliaceous and spicy food suggests compensatory trigeminal and gustatory stimulation.

Disclosures: T. Lopes: None. **G. Hansra:** None. **A.R. Hirsch:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Owner.

Poster

050. Taste: Behavior and Processing

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 50.22/V17

Topic: D.04. Olfaction and Taste

Support: USF NRG Award

Title: Role of insulin-1 in conditional taste aversion learning paradigm

Authors: *S. PANGULURI;
Col. of Pharm., Univ. of South Florida, Tampa, FL

Abstract: Conditioned taste aversion (CTA) is an adaptive behavior that benefits survival of animals including human, and is a powerful model to study the neural mechanisms of learning. Accumulating evidence suggests that this learning-dependent modulation of taste preference relies on direct axonal projections from the PBN to the ventral forebrain. We also know that long-term memory formation is a necessary component of CTA learning, which requires protein synthesis and altered gene expression. Of the forebrain areas the role of amygdala (CeA and BLA) in CTA has been studied most for its role in long-term memory formation. There is evidence demonstrating that CTA learning is correlated with altered expression of specific genes like Fos and Jun in forebrain and brainstem nuclei, and, at least for Fos expression in the amygdala, a functional role is implicated. Undoubtedly, the regulation of additional unidentified genes plays a role in the establishment and maintenance of taste preference/aversion memories. Other than the expression pattern of these immediate-early genes, little is known about the regulation of target gene expression induced by CTA. As a first step to address this gap in knowledge, we utilized rat whole genome chips to identify genes in the amygdala whose activity either increased or decreased following CTA learning. Using rigorous filtering of the data set followed by validation and confirmation with qRT-PCR and Western blotting or ELISA, we identified 4 of 10 select genes including adrenergic receptor 2b, insulin-1, oxytocin, and major histocompatibility complex class I-C play a role in CTA learning specifically in amygdala. Finally, behavioral analyses demonstrated that blockade of insulin signaling centrally using a high affinity insulin receptor antagonist disrupted CTA learning. To further understand the exact role of insulin-1 in CTA behavior, we utilized bilateral injections of lentivirus carrying insulin-1 specific shRNA in amygdala (both BLA and CeA) of rats. Control groups were injected with scrambled shRNA lentivirus. One week after lentiviral injection, these rats were subjected to CTA behavior test and the data was analyzed. Based on our data, specific injection of insulin-1 shRNA in amygdala does not disrupts the CTA acquisition in rats, but effects long-term memory formation. Molecular insight of insulin in amygada for CTA learning is therefore warranted to understand its complete mechanism of action.

Disclosures: S. Panguluri: None.

Poster

050. Taste: Behavior and Processing

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 50.23/V18

Topic: D.04. Olfaction and Taste

Support: NIDCD DC006666

Title: Innocuous experience enhances aversion learning and affects aversion-related neural activation

Authors: *V. FLORES, D. LEVITAN, T. PARMET, D. B. KATZ;
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Abstract: In conditioned taste aversion (CTA), an animal learns to avoid a particular taste that has been paired with malaise. Familiarity with future conditioned taste stimuli (CS) is known to influence the strength of aversion learning; the effect of familiarization with innocuous tastes—tastes other than the CS—has received little investigation, however. Our previous work demonstrated that pre-exposure to salty and sour tastes strengthened a later learned aversion towards novel sucrose, and showed that the phenomenon scaled with both the number of tastes in the pre-exposure array and with the number of pre-exposure sessions. The present studies begin an inquiry into the neural underpinnings of this phenomenon, using c-FOS, optogenetics, and (in future work) electrophysiology. The use of c-FOS enabled us to survey many possible relevant brain sites, but inquiry focused on gustatory cortex (GC), which is known to be involved in the integration of experience with taste behavior. Rats that had been pre-exposed to innocuous tastes demonstrated higher levels of c-FOS in GC after exposure to novel sucrose, compared to animals that had been pre-exposed to water alone. Furthermore, optogenetic inhibition of GC during pre-exposure sessions reduced the magnitude of a later CTA to novel sucrose, rendering taste pre-exposed rats identical to rats exposed to water alone. These results suggest that GC plays a controlling role in determining the impact of innocuous experience on learning-related behavior.

Disclosures: V. Flores: None. **D. Levitan:** None. **T. Parmet:** None. **D.B. Katz:** None.

Poster

050. Taste: Behavior and Processing

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 50.24/W1

Topic: D.04. Olfaction and Taste

Support: NIH/NIDCD R01DC014420

Title: Transcription factors to define subsets of taste neurons in the geniculate ganglia of adult mice

Authors: *G. DVORYANCHIKOV[1], D. HERNANDEZ[1], N. CHAUDHARI[1,2];
[1]Physiol. and Biophysics, [2]Program of Neurosci., Univ. of Miami Miller Sch. of Med., Miami, FL

Abstract: Sensory neurons included in dorsal root (DRG) and cranial ganglia are highly diverse, functionally and morphologically and concordantly, express various molecular markers. The determinants of this molecular diversity during development and in the adult are transcription factors (TFs) that orchestrate the gene expression program to generate functionally specialized neurons. While the transcriptome of somatosensory ganglia (dorsal root and trigeminal) have been assessed, the ganglia that include taste afferents are less explored. To understand the basis of the diversity of taste afferent neurons, we examined transcription factors expressed in individual neurons of the geniculate ganglion (VIIth nerve) in adult mouse. RNAseq data were obtained from mouse geniculate ganglia and compared to those from trigeminal ganglia. To identify candidate TFs that may determine neuronal diversity, we screened for those expressed at levels ≥ 2-fold higher in geniculate relative to trigeminal ganglia. From this list of TFs we selected 15 to analyze further by RT-PCR, first in 4 pools of 8-10 neurons each. Seven of these TFs were further analyzed by single-cell RT-PCR on 20 taste afferent neurons (labeled with TRITC-dextran via the chorda tympani taste nerve). Of the 7 analyzed, 2 TFs (Skor2 and Zbtb7c) were not detectable in labeled taste neurons. The remaining 5 TFs (Phox2b, Eya1, Eya2, Pbx3, and Tcf4) were found to be expressed in variable numbers of taste neurons. Specifically, Phox2b, previously reported as taste-associated, was detected in 17 of 20 taste neurons. 3 of 20 neurons displayed expression of all 5 of these potential taste-related TFs. Eya2 expression was detected in 8 of 20 (40%) taste neurons. To examine their pattern of expression, we label neurons from the chorda tympani and greater superficial petrosal nerves (which carry afferent fibers to tongue and palate respectively) and use the ganglia for immunofluorescent histochemistry for TFs. We are currently testing TFs identified as above to assess which are expressed in non-overlapping sets of taste neurons and thus may represent distinct functional groupings.

Disclosures: G. Dvoryanchikov: None. **D. Hernandez:** None. **N. Chaudhari:** None.

Poster

050. Taste: Behavior and Processing

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 50.25/W2

Topic: D.04. Olfaction and Taste

Title: Effect of color on taste thresholds

Authors: *S. NAGAHAMA;
Human care, Teikyo Heisei Univ., Tokyo, Japan

Abstract: Several reports have demonstrated that color can influence a person's ability to distinguish threshold levels of the four basic tastes (sweet, salty, sour and bitter). Maga (1974) reported that red colored water solution did not affect the sensitivity of sweet and sour, but decreased bitter taste sensitivity. Color did not affect salty taste sensitivity significantly. Kostyla and Clydesdale (1978) found that red increased sweetness perception and other color (blue and yellow) had only minor effects on sweetness. Clydesdale (1993) concluded that color had little effect on the perception of saltiness. The author hypothesized that the effect of color on taste threshold is affected by food culture which varies by country and/or generation. This study reported the influence of color solution on recognition thresholds of four basic tastes by untrained Japanese college-age (ranging from 20 to more than 22 years of age) students. Participants were 15 female and 15 male healthy volunteers not complaining of taste disorders. Simultaneously, participants were all non-smokers. In this study, the whole mouth gustatory method was prepared. The series of taste solution presented the four basic tastes, i.e., sweet, salty, sour and bitter, which were prepared using sucrose, common salt, acetate and quinine, respectively. Each taste test was done individually and distilled water was available at all times. Results of the threshold tests showed that red color had the effect of lowering the threshold of sweet and salty taste, while sour and bitter taste sensitivities were not affected. Blue color decreased the thresholds of saltiness and bitter taste. In the case of sweet and sour taste, both of the thresholds were increased by blue color. These results showed that the influence of color on four basic tastes was different from the previous reports.

Disclosures: S. Nagahama: None.

Poster

050. Taste: Behavior and Processing

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 50.26/W3

Topic: D.04. Olfaction and Taste

Title: When matter matters: taste of solids versus liquids

Authors: *S. KANWAR[1], M. R. CHAND[2], A. R. HIRSCH[2];
[1]Neurol., Smell and Taste Treatment and Res. Fndn., Miramar, FL; [2]Smell and Taste Treatment and Res. Fndn., Chicago, IL

Abstract: Objective: Whether in liquid or solid form, the substance remains the same. One would anticipate that variation between the states of matter would not affect taste since they have equivalent concentrations of specified ingredients. It's been noted that in normogeusic individuals, solid sugar tastes less sweet than the same sugar in liquid form. In those with chemosensory disorders, loss of taste of solids with preservation of liquids has not heretofore been described. Methods/Results: **Case 1:** A 60 year old female with eight years of Burning Mouth Syndrome presented with a four month history of being able to taste sweet in liquid form but not in solid or semi-solid. Chemosensory testing: Brief Smell Identification Test (BSIT): 10 (hyposmia). Retronasal Smell Index: 7 (normal). Propylthiouracil disk taste test: 6 (normogeusia). Intensity of 1 gram dextrose, maltodextrin, sucralose (Splenda) applied onto tongue in solid form: 3/10, liquid form: 7/10. **Case 2:** A 60 year old female presented with a one year impaired ability to taste, dysgeusia, cacogeusia and salty phantogeusia. She is unable to taste solid food, but her sense of taste to liquids is normal. Physical Examination: General: Scalloped tongue. Chemosensory testing: Olfaction: BSIT: 11(hyposmia). Quick Smell Identification Test: 2 (hyposmia). Retronasal smell index: 2 (abnormal). Gustation: Propylthiouracil disk taste test: 9 (normogeusia). Discussion: Theories postulated for the effect of taste between solids and liquids are myriad. Spatial summation of taste with liquid dispersion throughout the oral cavity; taste enhancement with colder temperatures, with liquid being cooler than solid; and counter-stimulus of the solid granules inducing pressure sensation through stimulation of the Pacinian corpuscles thus inhibiting taste. Sucralose is often mixed with dextrose and maldextrin as bulking agents from corn, which composes majority of the molecule as a filling agent of splenda. When crystallized, it may cover the sucralose component causing decreased sweet sensation, but when in water, the sugar component is released which is more potent. The T1Rs in taste suggest that sweet and umami taste receptors share a common subunit. With this, there may be a dominance of glutamate (umami) over glucose (sweet) taste in solids, which causes umami taste more than sweet. In liquids, it is reversed and there's a predominance for sweet more than umami. Formal assessment of liquid versus solid taste in those with chemosensory dysfunction is warranted.

Disclosures: **S. Kanwar:** None. **M.R. Chand:** None. **A.R. Hirsch:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Smell and Taste Treatment and Research Foundation.

Poster

050. Taste: Behavior and Processing

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 50.27/W4

Topic: D.04. Olfaction and Taste

Support: NIH NIDCD R01 DC0114428

Title: Role of innervation in HH signaling in the adult mouse fungiform taste papilla

Authors: A. KUMARI[1], B. L. ALLEN[2], R. M. BRADLEY[1], A. A. DLUGOSZ[3], *C. MISTRETTA[1];
[1]Univ. Michigan Sch. Dent., Ann Arbor, MI; [2]2Department of Cell and Developmental Biology, Med. Sch., [3]Dept. of Dermatology, Med. Sch., Univ. of Michigan, Ann Arbor, MI

Abstract: Inhibition of the Hedgehog (HH) signaling pathway leads to disruption of fungiform papilla (FP) taste organs and loss of taste buds (TB) but innervation to taste organs is retained. However, it is known that cutting the innervation to TB and FP organs also leads to loss of taste buds. To study potential interactions between innervation and HH signaling, we unilaterally severed the combined chorda tympani/lingual nerve (CT/LN) in mice carrying a HH-responsive *Gli1lacZ* allele, and stained for β-Galactosidase activity to identify cells with active HH signaling. We removed a few mm of the nerve where it runs deep in the neck to permanently interrupt the chorda tympani nerve (CT, Cranial Nerve VII) innervation to taste buds and the lingual nerve (LN, Cranial Nerve V) innervation to the FP organs and anterior tongue epithelium. In the contralateral Control side of the tongue the CT/LN nerve was exposed but not cut. In initial studies, after 21 days we measured effects on FP and TB integrity and the location of HH-responding cells, and used immunohistochemistry to identify CT fibers (P2X3 antibody), CT/LN fibers (NF), HH ligand (SHH) and taste bud cells (K8). In FP on the uncut Control side of the tongue, TBs were observed in about 85% of FP, SHH was within TB cells, and HH-responding cells were distributed throughout basal epithelial cells in lateral papilla walls, in the perigemmal epithelial cells around the taste bud, and in stromal cells of the FP. In the tongue half with nerve cut, TBs were undetectable in about 70% of FP; cell remnants of TBs were observed in about 10% of FP; and, typical TB were seen in about 20% of FP. Loss of taste bud cells after nerve cut was associated with loss of TB-restricted SHH and elimination of HH-responding, *lacZ+,* perigemmal cells and basal cells in epithelium of the FP. However, in a majority of FP, HH-

responding cells remained in the FP stroma. Whereas P2X3+ fibers were eliminated in FP with TB loss, some NF+ fibers remained in about 70% of the papillae without TBs and these were in close proximity to the stromal HH-responding cells. In summary, TBs, SHH ligand and HH signaling activity are largely eliminated in the FP epithelium after CT/LN nerve cut. P2X3+ fibers also are eliminated and NF+ fibers are decreased in the FP. Interestingly, the presence of NF+ fibers in the FP stromal core correlates with the presence of HH-responding cells in the stroma that associate with these nerve fibers. We propose that NF+ fibers transport HH ligand into the FP core that can maintain HH signaling in the stroma even in the absence of TB-derived SHH. Supported by NIH NIDCD R01 DC0114428.

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Poster

050. Taste: Behavior and Processing

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 50.28/W5

Topic: D.04. Olfaction and Taste

Title: Normosmic normogeusic subjective ageusia, why is food tasteless if I can smell and taste?

Authors: *P. KHEIRKHAH[1], A. HIRSCH[2];
[1]Neurosurg., UIC, Chicago, IL; [2]Smell & Taste Treatment and Res. Fndn., Chicago, IL

Abstract: Introduction: Complaints of ageusia with normal taste, normal orthonasal smell, and normal retronasal smell has not heretofore been described. Two such cases are pre- sented.
Method: Case study:
Case 1: A 59 years old female with a lifelong history of geo- graphic tongue. One year prior to presentation, developed a flu and burning in her mouth. The next day she noted total loss of taste without recovery since then. She admits to occasional dysgeusia, but denies flavorful eructation or any smell dysfunction.
Results: Chemosensory testing: Dirhinous Olfactory Testing: Brief Smell Identifi- cation Testing: 12 (normosmia). Retronasal Smell Testing: Retronasal Smell In- dex: 5 (normal). Gustatory Testing: Propylthiouracil Disk Taste Test: 10 (normo- geusia).
Case 2: A 53 year old right handed male, was nasute until falling and striking his head with loss of consciousness, two years prior to presentation. Initially he had no ability to smell at all, but it gradually returned to what he perceives to be 85% of normal. He does admit to flavorful eructation. Concurrent with smell loss there was impaired taste and continues detecting only highly spicy foods. Results: Chemosensory testing: Dirhinous olfactory testing: Brief Smell

Identifica- tion Test: 11 (normosmia). Pocket Smell Test: 3 (normosmia). Alcohol Sniff Test: 17 (normosmia). Olfactometer Smell Identification test: left nostril 18 (normosmia), right nostril 15 (hyposmia). Retronasal Smell Testing: Retronasal Smell Index: 9 (normal). Gustatory Testing: Propylthiouracil Disk Taste Test: 9 (normogeusia). Taste threshold : normogeusia to sodium chloride, sucrose, hy- drochloric acid, urea and phenylthiocarbamide.
Discussion The origin for these patient's complaints is unclear. The possibility of this being a primary psychiatric problem, or chemosensory malingering should be entertained. On the other hand, the primary problem may be in the testing strate- gy. The tests might not have been sensitive enough to detect a mild sensory deficit- presenting with high intensity testing stimuli may register normal, even when a true deficit is present (false negative). Alternatively, they may have had an exceptionally good taste or retronasal smell prior to loss, and after the loss it dropped a little bit, but still within the normal range, but low for their baseline abil- ity. This combined with a personality which may have induced them to be ultra- sensitive to small losses of function, may have been sufficient to cause them to perceive that all their taste is gone. Given the above, detailed chemosensory and psychiatric tests in those who complain of smell or taste loss, in the absence of chemosensory deficits, is warranted.

Disclosures: P. Kheirkhah: None. **A. Hirsch:** None.

Poster

050. Taste: Behavior and Processing

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 50.29/W6

Topic: D.04. Olfaction and Taste

Title: Temperature dependent dysgeusia: a reverse Goldilocks syndrome

Authors: *A. DUNN[1], A. HIRSCH[2];
[1]Lake Forest Col., Lake Forest, IL; [2]Smell and Taste Treatment and Res. Fndn., Chicago, IL

Abstract: Introduction: Dysgeusia inhibited by extreme hot and cold temperature has not been described. Such a case is presented. **Methods:** Case study: A 60 year old right handed woman was nasute until 7 months prior to presentation when she slipped, striking the back of her head with loss of consciousness. A few days later she noted trouble tasting. She can taste pickles and sweet food but nothing else. She feels her ability to taste is 20% of normal. She also complains of salty dysgeusia, which is temperature dependent, for instance water at room temperature tastes salty but very hot or ice water tastes normal. Similarly, solid semisweet Tollhouse chocolate chips taste salty, but when heated into liquid form, they taste like chocolate. Some foods only obtain flavor when heated, for instance chocolate brownies and tomato soup at room temperature

are without taste, but when heated, the brownies have a normal flavor. **Results:** Chemosensory testing: olfaction: Brief Smell Identification Test: 11 (normosmia). Alcohol Sniff Test: 19 (normosmia). Pocket Smell Test: 2 (hyposmia). Quick Smell Identification Test: 2 (hyposmia). Retronasal smell testing: Retronasal Smell Index: 9 (normal). Gustatory testing: propylthiouracil disc taste test: 9 (normogeusia). Taste threshold: normogeusia to urea, phenylthiocarbamide, sucrose, and hydrochloric acid. Mild hypogeusia to sodium chloride. Taste quadrant testing: impaired citric acid on the palate. Whole mouth weakness to sodium chloride and sucrose. Fungiform papillae count right: 18, left: 22 (normal). CAT scan of the brain: mild to moderate small vessel ischemic changes. **Discussion:** High and low temperatures may stimulate small C-fiber pain discharges, and the pain may act to inhibit the distorted taste. Possibly temperature extremes cause cognitive distraction, leading the patient to focus on the temperature instead of the distorted taste, therefore reducing the perception of taste. Maybe the salty dysgeusia is a minor manifestation of burning mouth syndrome, both representing pathology in the gustatory nerves, and thus, as temperature extremes help burning mouth syndrome, it would help other gustatory nerve dysfunction, i.e. dysgeusia. Since those with dysgeusia are often unable to eat due to the unpleasant taste, and with subsequent loss of weight, one possible approach for this condition would be providing foods at temperature extremes, reducing dysgeusia, hence inducing greater palatability of foods.

Disclosures: A. Dunn: None. **A. Hirsch:** A. Employment/Salary (full or part-time): Smell and Taste Treatment and Research Foundation.

Poster

050. Taste: Behavior and Processing

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 50.30/W7

Topic: D.04. Olfaction and Taste

Title: Objective chemosensory testing in subjective salty hypergeusia; report of three cases

Authors: *K. KAVEH[1], K. NATASHA[2], A. R. HIRSCH[2];
[1]Smell and Taste Treatment and Res. Foundation,, Chicago, IL; [2]Smell and Taste Treatment and Res. Foundation, Chicago, IL, chicago, IL

Abstract: Introduction Three patients with subjective salty hypergeusia who underwent chemosensory testing are reported. **Methods: Case Studies:** Case 1: Four years prior to presentation, a 49 year old left-handed (familial) woman, suffered a subdural hematoma and intracranial hematoma. One month later, she lost all smell and taste except for salt too intense, at 150% more than normal. **Results**: Chemosensory testing: olfaction: Anosmia on all olfactory

tests including: Quick Smell Identification Test (QSIT), Alcohol Sniff Test (AST), Sniff Magnitude, Olfactometer Butanol Threshold and Identification, Brief Smell Identification Test (BSIT), Suprathreshold Amylacetate Odor Intensity and Amylacetate Hedonics, Sniff-N-Sticks and Retronasal Smell Index (RSI). Gustation: normogeusic to Propylthiouracil Disk and Threshold to sodium chloride, sucrose, hydrochloric acid, urea, and phenylthiocarbamide. Case 2: A 60 year old right-handed woman struck her head with loss of consciousness. A few days later, noted loss of taste, with ability to only taste pickles and sweet, dysgeusia wherein water and most foods taste salty, a constant salty phantogeusia, and salt hypergeusia hypergeusia, where salt tastes 200% more intense than it should. **Results**: Chemosensory testing: olfaction: normosmic to BSIT and AST. Hyposmic to QSIT and Pocket Smell Test. RSI: 2 (abnormal). Gustatory testing: normogeusic to propylthiouracil disk and Threshold to sucrose, hydrochloric acid, urea, and phenylthiocarbamide. Mild hypogeusia to sodium chloride. Case 3: A 60 year old right handed woman presented with eight years of intermittent burning mouth syndrome. Four months prior to presentation, she noted a constant salty taste followed two weeks later by total absence of taste, wherein everything tasted sour, gradually changing to metallic. Salty foods were 120% more salty than normal. **Results**: Chemosensory testing: olfaction: normosmia to AST, Pocket Smell, BSIT and RSI. Gustation normogeusia to Propylthiouracil Disk and Threshold to sodium chloride, hydrochloric acid, urea, and phenylthiocarbamide. Ageusia to sucrose. Other: MRI of brain with and without infusion: right cerebellar hemisphere, small infarction. **Discussion** The common findings in this group were abnormal AST and normal Propylthiouracil Disc Taste Test. Despite this normal taste test, there may be an impaired ability to taste, since this test only assesses the ability to taste bitter. The impaired olfactory ability based on AST may indicate a primary defect in olfaction; smell may act to inhibit intrinsic salt taste discharge. With smell impairment, salt perception would be disinhibited, thus increasing salt perception.

Disclosures: **K. Kaveh:** None. **K. Natasha:** None. **A.R. Hirsch:** None.

Poster

051. Auditory Processing: Temporal and Frequency

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 51.01/W8

Topic: D.05. Audition

Support: NIDCD Grant DC011843

Hearing Research, Inc.

Coleman Memorial Fund

Title: Characterizing receptive fields in awake primate auditory cortex using principled correction of the spike-triggered average

Authors: *J. BIGELOW, R. E. BEITEL, B. J. MALONE;
UCSF, San Francisco, CA

Abstract: Characterizing auditory cortical receptive fields has been the subject of numerous studies, the majority of which have been conducted in anesthetized cats and ferrets. Comparatively little is known about auditory cortical receptive field structure in nonhuman primates. Considering the extensive homologies between human and nonhuman primate auditory cortex, characterizing auditory receptive fields in primates could serve as an important model of auditory cortical processing, with particular relevance for developing neural prostheses. Because anesthesia is known to affect response properties of auditory cortical neurons, studies using awake preparations are especially needed. The current study characterized spectrotemporal receptive fields (STRFs) in primary auditory cortex of two alert squirrel monkeys (*Saimiri sciureus*). Extracellular single- and multi-unit recordings were obtained using 16-channel linear multielectrode arrays (150 μm spacing), with neurophysiological traces sampled at 30 kHz. The spectrotemporal preferences of each unit were probed with a 30-m dynamic moving ripple (DMR) stimulus presented through a free field speaker from a central distance of ~40 cm. A novel 30-s DMR segment was subsequently presented (50 repetitions) to assess the prediction accuracy of the STRF estimates. STRFs were estimated by computing the average DMR spectrogram preceding each spike (spike-triggered average [STA]), at a resolution of 193 frequency bins and 200 time lags (1 ms). Although the STA can theoretically reflect an unbiased estimate of the STRF, we found that a two-step correction procedure nearly doubled prediction accuracy across units, presumably reflecting more accurate STRF estimates. First, a conventional amplitude threshold was used identify regions of the STRF that exceeded chance values obtained from simulated STRFs computed for each unit using circularly shifted spike times to preserve the spike rate and inter-spike interval distribution. Second, a cluster size threshold was used to limit clusters that survived the amplitude threshold to those that exceeded chance cluster size values obtained from simulated STRFs subjected to the same amplitude threshold. The amplitude and cluster size thresholds were optimized for each unit by cross-validation to maximize the correlation between predicted and observed responses to the repeated DMR segment. On average, this procedure resulted in a liberal amplitude threshold but a conservative cluster size threshold. The resulting corrected STRF estimates are used to provide a detailed characterization of spectrotemporal response properties of awake primate auditory cortex.

Disclosures: J. Bigelow: None. **R.E. Beitel:** None. **B.J. Malone:** None.

Poster

051. Auditory Processing: Temporal and Frequency

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

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Topic: D.05. Audition

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Hearing Research Inc.

John C. and Edward Coleman Memorial Fund

Title: Noise-robust encoding of frequency modulation in awake primate cortex

Authors: *B. J. MALONE[1], M. HEISER[2], R. BEITEL[1], J. BIGELOW[1], C. SCHREINER[1]; [1]Otolaryngology & Head & Neck Surgery, UCSF Sch. of Med., San Francisco, CA; [2]Dept. of Psychiatry, Child and Adolescent Div., UCLA Semel Inst. for Neurosci. and Behavior, Los Angeles, CA

Abstract: Natural listening environments are often characterized by multiple 'noise' sources that compete with 'signals' of interest. The presence of noise is thought to degrade central representations of competing signals by suppressing responsiveness via shifts in threshold, or obscuring the representation by eliciting responses unrelated to the signal. We tested this assumption by recording multi-unit cortical responses to logarithmic frequency-modulated (FM) sweeps against backgrounds of white noise at signal-to-noise ratios (SNRs) spanning over 20 dB. Awake squirrel monkeys were trained to sit quietly in an acoustically transparent primate chair. Recordings were obtained using 16 channel linear probes while sounds were presented on a free field speaker. FM sweeps were presented at 6 different velocities designed to encompass the range of FM rates typical of vocalizations in this species (e.g., twitter calls). Ascending and descending sweeps were presented for a total of 12 distinct trajectories. One FM sweep was also presented at several sound levels. We evaluated the effects of the SNR condition by comparing the performance of nearest-neighbor Euclidian distance classifiers tasked with decoding and sweep trajectory (i.e., velocity and direction) and sweep level as the noise level was varied. FM sweeps were also presented in isolation for the purposes of comparison. Roughly a third of cortical sites decoded sweep trajectory best for FM sweeps presented in isolation. Another third decoded sweep trajectory as well in presence of moderately unfavorable SNRs (0 to -10 dB) as they did against a silent background. A final third of cortical sites exhibited enhanced decoding performance in the presence of noise. The cortical representation of the FM sweeps consisted largely of sharp peaks in the peristimulus time histogram aligned with the intersection of the

sweep with the site's best frequency. Generally speaking, increasing noise levels and increasingly negative SNRs either reduced or eliminated these peaks, but did not increase baseline firing rates: firing rates during intervals excluding the sweeps but including the noise were essentially constant across SNR conditions. As a result, it was possible to decode information about the SNR condition for identical sweeps only if the analysis interval included the sweeps themselves. Effectively, the SNR context modulated the transmission of information about the sweeps but adaptive mechanisms compensated for global changes in the noise level. These results suggest that signal transmission in the central auditory system is organized to ensure robust information transfer in a wide range of listening contexts.

Disclosures: B.J. Malone: None. **M. Heiser:** None. **R. Beitel:** None. **J. Bigelow:** None. **C. Schreiner:** None.

Poster

051. Auditory Processing: Temporal and Frequency

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

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Topic: D.05. Audition

Support: NIH grant 5R01DC011379-05

Title: Locomotion modifies sound perception in mouse primary auditory cortex

Authors: *A. G. GUSEV, M. WEHR;
Psychology, Inst. of Neuroscience, Univ. of Oregon, Eugene, OR

Abstract: We performed cell-attached recordings from 460 neurons throughout the depth of primary auditory cortex in awake, head-restrained mice. We compared baseline firing rates and and evoked responses in two behavioral states of the mouse: quiet sitting and locomotion on a ball. In the majority of neurons, locomotion decreased baseline firing rates, but a substantial population of neurons showed increased baseline firing rates. We measured sound-evoked responses to either white noise or CF tones, delivered in free field. About 30 percent of neurons were initially suppressed by sound, which could be due to binaural effects of the free-field sounds arriving at both ears. Excitatory responses in the remainder of neurons were either monophasic (a sustained increase in firing rate) or biphasic (an initial increase in firing rate, followed by suppression that typically lasted three times longer than the excitatory phase). Locomotion decreased both baseline and evoked firing in the majority of neurons in cortical layers L2 through L5, although a substantial population of neurons showed increased baseline and evoked activity. Suppression during locomotion transformed responses in two ways:

monophasic excitatory responses were converted into biphasic responses, and biphasic responses were converted into monophasic suppression. Comparison of white noise and CF stimulation revealed distinct effects of locomotion on surround suppression, non-monotonic intensity tuning, and off responses.

Disclosures: **A.G. Gusev:** None. **M. Wehr:** None.

Poster

051. Auditory Processing: Temporal and Frequency

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 51.04/W11

Topic: D.05. Audition

Support: Tateisi Science and Technology Foundation (Japan)

Suzuken Memorial Foundation (Japan)

Grant-in-Aid for Scientific Research (B) (No. 15H02772) (Japan)

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Title: Neural response differences in the rat primary auditory cortex under anesthesia with ketamine versus the mixture of medetomidine, midazolam and butorphanol

Authors: *H. OSANAI, T. TATENO;
Hokkaido Univ., Sapporo, Hokkaido, Japan

Abstract: Although anesthesia is known to affect central auditory processing, it is unclear the extent to which the choice of anesthetic agents exerts different effects on neural responses to sound stimulation. A mixture of three anesthetics (medetomidine, midazolam and butorphanol) was recently developed as an alternative to ketamine, which has potential addictive effects. However, little is known about the effect of this combination of anesthetics on neural responses. In this study, we compared spontaneous activities, tuning properties, the temporal response properties, and the oscillatory activities of primary auditory cortical (A1) neurons under these two anesthetic conditions, using multi-channel electrophysiology. In addition, we identified the A1 using flavoprotein endogenous imaging before electrophysiology, which takes less than half an hour and thus enables efficient electrophysiological recording. Frequency tuning properties did not show a significant difference. In contrast, neural activities under the combination anesthesia showed decreases in the spontaneous and tone-evoked firing rates in a layer-dependent manner. Moreover, the temporal response patterns were also different between the

anesthetics in a layer-dependent manner. On the other hand, the amplitude of sound-driven oscillations was decreased in high gamma activities (over 120 Hz) across cortical layers under the combination anesthesia, whereas we found no differences in activities including lower frequency components (< 120 Hz), which may reflect differences in the anesthetic mechanisms. Our results provide a fundamental view of how response properties in the primary auditory cortex are affected by the choice of anesthesia.

Disclosures: H. Osanai: None. **T. Tateno:** None.

Poster

051. Auditory Processing: Temporal and Frequency

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Topic: D.05. Audition

Support: NIDCD R01DC011580

Title: Response profiles of inferior colliculus neurons in young and old rats

Authors: *E. X. HAN[1,2], A. PARTHASARATHY[3], E. L. BARTLETT[1,2];
[1]Weldon Sch. of Biomed. Engin., [2]Dept. of Biol. Sci., Purdue Univ., West Lafayette, IN; [3]Mass. Eye and Ear, Harvard Med. Sch., Boston, MA

Abstract: The processing of auditory stimuli is affected at different levels of the central auditory pathway by aging. While there are in vivo single-unit studies involving auditory processing across aging in the inferior colliculus (IC), many of them failed to represent or predict the age-related impacts on IC neurons beyond a small number of stimuli dimensions, either due to using simple stimuli measuring one or two aspect, or treating IC neurons as a fundamentally homogenous group with varying spectrotemporal preferences. In this study, we aim to address this issue by analyzing responses towards various spectrotemporal stimuli from individual neurons to establish their “neuronal profiles”. We recorded in vivo single-unit responses from the IC of young and aged F334 rats in response to simple stimuli, such as tones and wide-band or band-passed noise, and more complex sounds, such as random spectrum stimuli. Our study indicates that aging may take different electrophysiological effects on individual IC units. Such effects might be less evident when only a few stimulus dimensions are considered.

Disclosures: E.X. Han: None. **A. Parthasarathy:** None. **E.L. Bartlett:** None.

Poster

051. Auditory Processing: Temporal and Frequency

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Program#/Poster#: 51.06/X1

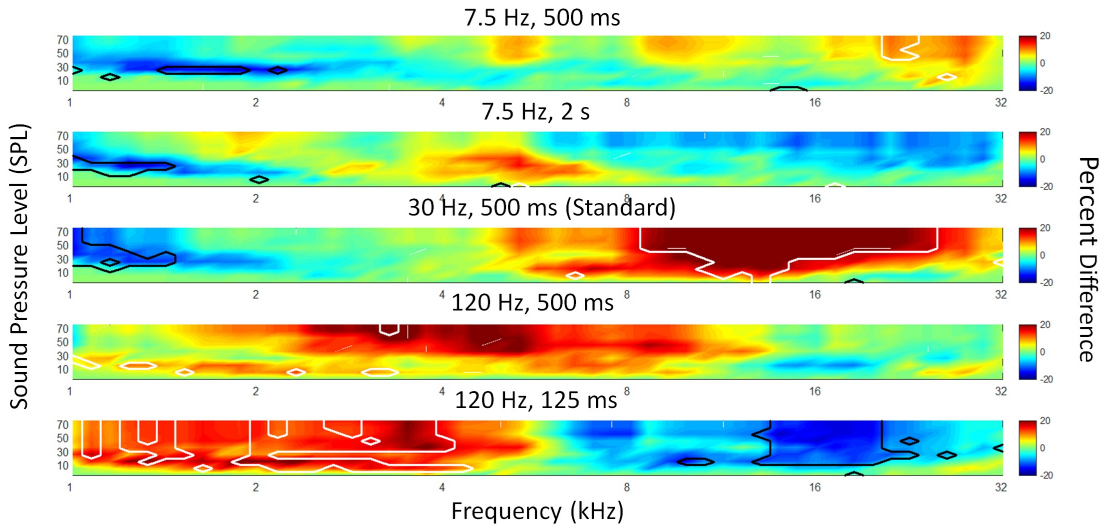
Topic: D.05. Audition

Support: DARP Seedling Grant

Title: Varying frequency in vagus nerve stimulation to optimize plasticity in the rat auditory cortex.

Authors: *E. BUELL[1], M. BORLAND[2], K. LOERWALD[2], M. KILGARD[2];
[1]Univ. of Texas At Dallas, Richardson, TX; [2]Univ. of Texas at Dallas, Richardson, TX

Abstract: Vagus nerve stimulation (VNS) is an FDA-approved treatment option for patients suffering from depression or epilepsy. Repeatedly pairing stimulation of the vagus nerve with a sensory stimulus increases the number of responding cortical neurons. Such changes have been observed in the primary auditory cortex (A1) when pairing a pure tone with VNS, as well as in the primary motor cortex when pairing a movement with VNS (Engineer et al., 2011; Porter et al., 2012). Patients with tinnitus have reported long-lasting relief from VNS treatment paired with tones outside their tinnitus frequency. However, no patient has yet reported having no perception of tinnitus as a result of this treatment. Inverted-U functions have been observed after altering the intensity of stimulation given per treatment session. Specifically, significant cortical changes were observed at 0.4-0.8 mA, but not at 1.2 or 1.6 mA (Borland et al., 2015). This function may exist in other parameters of VNS, as well.
Although it is clear that VNS drives plasticity, optimal parameters under which this treatment is delivered remain undefined. The purpose of this project is to investigate how varying levels of frequency (Hz) of VNS can change the degree of map plasticity, and optimize clinical treatment outcomes. Preliminary data suggest low frequency (7.5 Hz) decreases neuronal selectivity. Preliminary data suggests high frequency stimulation (120 Hz, 15 pulses) reduces neuronal responses to 8-16 kHz tones in the auditory cortex, whereas 30 Hz (standard) has been shown to increase responses in that range (Figure 1). These results indicate levels of stimulation frequency could have a role in efficacy of treatment in the clinical setting.
Figure 1.
Support: The Defense Advanced Research Projects Agency (DARPA) provided funding for this research.



Disclosures: **E. Buell:** None. **M. Borland:** None. **K. Loerwald:** None. **M. Kilgard:** None.

Poster

051. Auditory Processing: Temporal and Frequency

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 51.07/X2

Topic: D.05. Audition

Title: Brain rhythms reveal how words integrate into phrases

Authors: ***A. TAVANO**[1], S. BLOHM[2], C. KNOOP[2], V. WAGNER[2], M. SCHARINGER[2], N. DING[3], O. GHITZA[4], D. POEPPEL[1,5], W. MENNINGHAUS[2];
[1]Dept. of Neurosci., [2]Dept. of Language and Literature, Max Planck Inst. For Empirical Aesthetics, Frankfurt, Germany; [3]Col. of Biomed. Engin. and Instrumental Sci., Zhejiang Univ., Hangzhou, Zhejiang Province, China; [4]Dept. of Biomed. Engin., Boston Univ., Boston, MA; [5]Dept. of Psychology, New York Univ., New York City, NY

Abstract: Low-frequency brain rhythms can track the main constituents in a linguistic hierarchy: phrases and sentences (Ding et al., 2016). Notably, cortical rhythms were cross-linguistically tested using English and Mandarin Chinese; they were found to reflect structure-building operations. Extending this paradigm to German, we here focus more explicitly on the integration of words into phrases. We hypothesized that neural rhythms tracking hierarchical levels of

phrases and sentences can vary in frequency depending on the size of the respective constituents. Participants listened to monosyllabic words (250 ms duration) continuously delivered to both ears at a constant (isochronous) rate of 4 Hz. The stimulus sequences constituted grammatically correct and meaningful four-word sentences with a two-word noun phrase followed by a two-word verb phrase. Phrase and sentence boundaries were not marked by acoustic cues. Stimuli were organized in blocks of 40 sentences, while electroencephalographic activity was recorded using 32 electrodes. The analysis used a Fast-Fourier transform run for the EEG data from each block. Results replicate the main findings of Ding et al. (2016), highlighting the extraction of phrase- and sentence-level parsing rhythms, at 2 Hz and 1 Hz, respectively, despite the absence of any acoustic cues at the corresponding rates. We then tested sentences with one word in the noun phrase and three words in the verb phrase. In this case, the 2 Hz rhythm was absent but substituted by a 1.33 Hz rhythm, which corresponds to the integration rate across the verb-phrase (three words, 750 ms) and which has no harmonic (multiple integer) relationship to the stimulus rate. As a control, we flipped the size of constituents, with three words in the noun phrase and one word in the verb phrase. Again, the 1.33 Hz rhythm surfaced, suggesting that the human brain tracks the size of phrases within a sentence using temporal integration processes reflected in low-frequency brain activity. These data constitute a next important step towards understanding the role of brain rhythms in on-line tracking of natural spoken language.

Disclosures: **A. Tavano:** None. **S. Blohm:** None. **C. Knoop:** None. **V. Wagner:** None. **M. Scharinger:** None. **N. Ding:** None. **O. Ghitza:** None. **D. Poeppel:** None. **W. Menninghaus:** None.

Poster

051. Auditory Processing: Temporal and Frequency

Location: Halls B-H

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Program#/Poster#: 51.08/X3

Topic: D.05. Audition

Support: Department of Defense

Title: Distortion of tonotopic maps in auditory cortex of rats following blast exposure

Authors: ***S. MASRI**[1,2], H. LUO[2], E. PACE[2], J. ZHANG[2], S. BAO[1];
[1]Univ. of Arizona, Tucson, AZ; [2]Otolaryngology, Wayne State Univ., Detroit, MI

Abstract: Blast exposure is often associated with traumatic brain injury (TBI) and central auditory processing disorders. Here we use an established rat model of blast exposure to investigate effects on tonotopic organization in primary auditory cortex (A1). We subjected adult

male Sprague-Dawley rats to a single 10-msec blast shockwave at 14 psi or 194 dB sound pressure level (SPL). Three weeks after exposure, behavioral tests showed that 5 of 7 rats had developed tinnitus. We then mapped A1 in control and blast-exposed rats using established electrophysiological techniques. Control animals showed tonotopically organized cortical frequency maps with all frequencies approximately equally represented. However, animals with blast-induced tinnitus often had expanded cortical representations of a narrow and apparently random frequency band. Consistent with ABR tests, hearing thresholds as measured at cortex were 7 dB higher for animals with blast-induced tinnitus compared to the control animals. Furthermore, cortical tuning properties were significantly broader in blast-exposed rats while cortical response latencies were significantly shorter. These data indicate that blast exposure induces a frequency nonspecific distortion of A1 frequency maps, which may contribute to blast-induced central processing disorders.

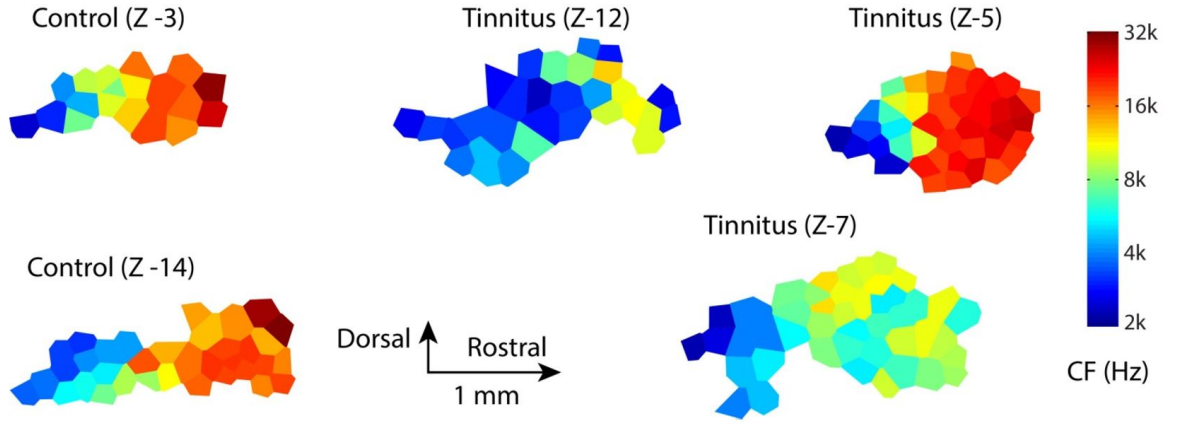

Figure 1. Representative cortical frequency maps showing disrupted cortical map in animals with blast-induced tinnitus. Compared to control maps (Z-3 and Z-14), maps from animals with blast-induced tinnitus often show enlarged representation of a frequency band. Z-12 had enlarged representation of low frequencies (blue colors), Z-7 had enlarged representation of middle frequencies (blue and yellow colors) and Z-5 had enlarged representation of high frequencies (red colors).

Disclosures: S. Masri: None. **H. Luo:** None. **E. Pace:** None. **J. Zhang:** None. **S. Bao:** None.

Poster

051. Auditory Processing: Temporal and Frequency

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ANR-11-0001-02 PSL

ANR-10-LABX-0087

Entendre-SAS

DFG FOR 1732 (TPE)

Title: Responses to sinusoidal frequency modulation in the guinea pig ventral cochlear nucleus

Authors: *N. PARAOUTY[1], A. STASIAK[2], C. LORENZI[1], I. WINTER[2];
[1]Dept. d'études cognitives-DEC IEC, Ecole Normale Superieure Paris, Paris, France; [2]Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Background Many psychophysical studies have investigated the detection of sinusoidal frequency-modulation (SFM) and on the type of sensory information it predominantly relies on: temporal-envelope (ENV) resulting from cochlear filtering, or temporal fine-structure (TFS) cues conveyed by changes in the neural phase-locking pattern over time, or a combination of both. Few neurophysiological studies have addressed this issue and data is still lacking regarding the coding of SFM in the early stages of auditory processing (below inferior colliculus). This work aimed at characterizing the responses of ventral cochlear nucleus (VCN) neurons to SFM tones presented at various modulation depths and sound levels. **Methods** Single-units in normal-hearing anaesthetized pigmented guinea pigs were recorded extracellularly using tungsten-in-glass microelectrodes. Stimuli were 1-second SFM tones played at the unit's best frequency (BF), at modulation rates of 2, 5 and 10 Hz and modulation depths of 2, 4, 8, 16, and 32 % relative to BF. All stimuli were presented at positive and negative polarities and at several sound levels. **Results** VCN responses to SFM varied as a function of sound level, bandwidth and unit type. Shuffled correlogram analyses were carried out in order to assess the relative strengths of ENV and TFS coding for the different unit types in the VCN. For small modulation rates (≤5Hz) and small modulation depths (≤16%), low-CF units showed weak temporal ENV coding but high phase-locking to TFS. In comparison, high-CF units generally followed the stimulus ENV for most conditions. The transition region over which temporal coding changes from being dominated by TFS coding to ENV coding was around 1-2 kHz. **Conclusion** The results provide physiological evidence that SFM is encoded via neural phase-locking to TFS cues for low carrier frequencies and modulation rates, and via neural phase-locking to ENV cues at high carrier frequencies. The results also suggest weaker phase-locking to TFS in guinea pigs compared to other species. Further work will be carried out in order to assess the strengths of these two coding mechanisms in the presence of a hearing deficit.

Disclosures: **N. Paraouty:** None. **A. Stasiak:** None. **C. Lorenzi:** None. **I. Winter:** None.

Poster

051. Auditory Processing: Temporal and Frequency

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Topic: D.05. Audition

Support: R01 DC012947

Title: Thalamocortical circuitry of oscillatory phase reset

Authors: *M. N. O'CONNELL[1], A. BARCZAK[2], T. MCGINNIS[3], D. ROSS[2], P. LAKATOS[2,4];
[1]Nathan Kline Instit, Orangeburg, NY; [2]Nathan Kline Inst., Orangeburg, NY; [3]Nathan KLine Inst., Orangeburg, NY; [4]Dept. of Neurosci. & Physiol., New York Univ. Sch. of Med., New York, NY

Abstract: Our external world is composed of numerous temporally structured rhythmic stimuli, especially in the auditory modality. Several studies have shown that the alignment of internal rhythmic neuronal excitability fluctuations, or neuronal oscillations, to the temporal structure of these stimuli significantly aids in their processing. In fact, an inability to align or entrain neuronal oscillations to the temporal structure of external stimuli in schizophrenia patients has been suggested as a basis for their sensory processing deficits. To be able to align internal to external rhythms, the brain needs a mechanism called phase reset, which is able to modulate the phase of neuronal oscillations. Phase reset is supramodal, and in its purest form - without specific event related responses - occurs in response to non-preferred modality stimuli. It has been speculated that inputs originating in the non-specific, thalamic matrix are responsible for the mechanism of phase reset. In the present study, we directly tested this hypothesis by recording LED flash related responses of neuronal ensembles and single neurons from primary auditory cortex (A1) and the auditory thalamus (MGN) concurrently. Neuroelectric activity was recorded using linear array multielectrodes in awake macaques who were presented with a rhythmic stream of LED flashes. After isolating the activity of single neurons in MGN, we found that less than 20% responded to LED flashes. Individual neurons within this subset reliably responded to the LED across approximately 50% of trials. The onset latencies of these neurons were only slightly delayed compared to responses of LGN neurons, and the majority were located in the dorsal division of MGN. Examination of concurrently recorded A1 laminar CSD response profiles during trials where the visual-responsive MGN neurons fired showed signatures of phase reset and entrainment, accompanied by significant laminar inter-trial coherence (ITC). For the trials when these MGN neurons failed to fire, ITC failed to reach significance in A1 indicating that in these trials ongoing activity was not modulated by visual stimulus related inputs. Lastly, we identified a population of units in cortex that only fired on

trials when “phase reset neurons” of the MGB did, indicating that these are also part of the circuitry that is responsible for phase reset. Our results provide evidence that as previously proposed, phase reset indeed employs a thalamocortical circuitry that involves non-specific, matrix regions of thalamic relay nuclei.

Disclosures: **M.N. O'Connell:** None. **A. Barczak:** None. **T. McGinnis:** None. **D. Ross:** None. **P. Lakatos:** None.

Poster

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Support: NIH/NIDCD Grant R03DC012585

NIH/NIGMS P30GM103340

Title: Modulation of acoustic processing in the auditory cortex by frontal cortex synaptic inputs.

Authors: *J. W. MIDDLETON[1], S. M. BROWN[2];
[1]Cell Biol. and Anat., LSU Hlth. Sci. Ctr., New Orleans, LA; [2]Cell Biol. and Anat., Louisiana State Univ. Hlth. Sci. Ctr., New Orleans, LA

Abstract: Anatomical and functional neural circuitry forms the basis for cortical representations of the sensory world. This circuit architecture needs to be both plastic and adaptable to allow for the cognitive flexibility animals need to survive in complex and dynamic sensory environments. In the auditory system, neural responses to tone frequency exhibit plasticity when acoustic stimuli arrive concurrently with synaptic inputs from modulatory brain centers. This results in a functional reorganization of the auditory tonotopy, i.e. the ordered distribution of frequency specific responses across the surface of the auditory cortex. “Top-down” pathways are responsible for attention, extracting contextual information, cross-modal sensory integration and signaling other internal brain states. The prefrontal cortex (PFC) has a role in many of these higher cognitive functions. We are interested in how a particular PFC sub region, the orbitofrontal cortex (OFC) is involved in mediating plastic changes in the auditory cortex. The OFC plays a critical role in evaluating reward outcomes, signaling aversive stimuli and regulating emotional behavior. It has established anatomical connections with the primary auditory cortex (A1) and can modulate auditory cortical responses. In order to understand how the OFC influences functional circuitry and signal processing in the auditory cortex we

performed *in vivo* multielectrode array recordings simultaneously in the auditory cortex and the OFC. We calculated cross correlations of activity in these two areas to quantify functional coupling. Additionally, we optogenetically activated OFC neurons and measured the resultant LFP responses in auditory cortex. We found that the funcitonal properites of inputs from OFC to auditory cortex were dependent on the rostro-caudal position of the auditory cortex recording. The characterization of this pathway is an important first step in understanding the mechanisms underlying OFC dependent modulation of auditory processing.

Disclosures: J.W. Middleton: None. **S.M. Brown:** None.

Poster

051. Auditory Processing: Temporal and Frequency

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Topic: D.05. Audition

Support: A*STAR NSS

Title: Information processing by synchronized neuronal ensembles in the primary auditory cortex

Authors: *J. SEE, C. ATENCIO, V. SOHAL, C. SCHREINER;
UCSF, San Francisco, CA

Abstract: The primary auditory cortex (AI) contains interconnected populations of neurons that are responsible for auditory information processing. Most studies of information processing in AI involve either single unit spectrotemporal receptive field (STRF) estimation or paired neuronal correlation analyses, and thus assume that AI neurons filter auditory information either as individual entities or, potentially, as pairs. However, mounting evidence suggests that sensory stimuli are processed by interconnected, co-activated populations of neurons. Therefore, determining how AI encodes information will require an integrated approach that combines receptive field and multi-neuronal ensemble analyses. To assess multi-neuronal information processing in AI, we performed multi-electrode extracellular recordings in rat AI while presenting dynamic, broadband stimuli, which allowed us to construct STRFs. We then used dimensionality reduction techniques to identify distinct groups of AI neurons (neuronal ensembles, or NEs) that reliably fired synchronously in AI. Via multiple models, we verified that neurons in the same NE had higher rates of coincident firing than would be by chance or by having similar stimulus preferences. For each NE, we then identified synchronous spiking events, and used the events to assess spectrotemporal information processing. For neurons that

were members of an NE, the spikes associated with the NE conveyed greater information than the spikes that were not associated with the NE. These findings challenge the classical idea that AI neurons produce a homogeneous set of spikes that may be equally weighted to estimate a single STRF. Instead, spikes of AI neurons may represent different pieces of information, and equally weighting all neuronal spikes to form a single STRF ignores this. Therefore, by taking into account the stimulus preferences associated with each NE, we may gain a more complete evaluation of information processing in A1.

Disclosures: **J. See:** None. **C. Atencio:** None. **V. Sohal:** None. **C. Schreiner:** None.

Poster

051. Auditory Processing: Temporal and Frequency

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Topic: D.05. Audition

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NIDCD R93DC013660

Klingenstein Award in Neuroscience

Human Frontiers in Science Young Investigator Award RGY0073/2014

Burroughs-Wellcome Career Award at Scientific Interface

Penn Behavioral/Cognitive Training Grant: NIH NIMH T32MH017168

Title: Adaptation in auditory cortex is actively shaped by somatostatin-positive and not parvalbumin-positive interneurons

Authors: *R. G. NATAN[1,2], W. RAO[1], M. N. GEFFEN[1,2];
[1]Dept. of Otorhinolaryngology, [2]Neurosci. Grad. Group, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Adaptation to repeated stimuli is a ubiquitous property of cortical neurons that is thought to enhance the efficiency of sensory coding. In primary auditory cortex, the vast majority of neurons exhibit stimulus specific adaptation, i.e. firing rate attenuation dependent upon stimulus prevalence, which modulates neuronal frequency tuning properties. Such history-dependent adaptation is thought to support sensory-motor behaviors like stimulus habituation, discrimination and deviance detection, yet little is known about the neuronal mechanisms that control how adaptation is generated. In these experiments, we show how frequency tuning in A1

is differentially shaped by two inhibitory interneuron subtypes prior to and following adaptation. During tone pip trains that induce varying levels of adaptation across frequencies, we measured firing rates of putative excitatory pyramidal neurons (PNs), while using optogenetic manipulation to selectively suppress specific populations of interneurons. Prior to adaptation, i.e. during the first tone of each train, suppressing parvalbumin-positive interneurons (PVs) or somatostatin-positive interneurons (SOMs) each lead to increases or decreases in tone evoked responses in different PNs. Following adaptation, i.e. during the last tone of each train, PVs mediated inhibition increased modestly but continued to bi-directionally modulate tone evoked responses similarly to the first tone. In contrast, SOM mediated inhibition after adaptation increased dramatically and modulation became mostly uni-directional. Thus, SOM mediated inhibition appears to track the dynamics of adaptation more closely than that of PVs. In addition, we tested how inhibition and adaptation shape frequency tuning in PNs. First, we found that adaptation leads to a robust divisive suppression across the tuning curve for PNs, with stronger suppression for preferred frequencies. Matching the aforementioned findings, PV mediated inhibition across frequencies did not change during adaptation. In contrast, SOM mediated inhibition increased more strongly for preferred frequencies than off-peak frequencies, matching the pattern the pattern of suppression observed for adaptation. We further explore circuit mechanisms that may underlie this novel phenomenon. This study reveals a previously unknown functional mechanism of SOMs in generating adaptation.

Disclosures: **R.G. Natan:** None. **W. Rao:** None. **M.N. Geffen:** None.

Poster

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Hearing Research, Inc (San Francisco)

Title: Amplitude modulation coding in awake mice

Authors: *N. HOGLEN[1], E. A. K. PHILLIPS[2], P. LARIMER[3], B. J. MALONE[4], A. R. HASENSTAUB[4];
[2]Neurosci. Grad. Program, Kavli IFN, Ctr. for Integrative Neurosci., [3]Dept. of Neurology, Kavli IFN, Ctr. for Integrative Neurosci., [4]Dept. of Otolaryngology/Head and Neck Surgery, Kavli IFN, Ctr. for Integrative Neurosci., [1]Univ. of California, San Francisco, San Francisco, CA

Abstract: Amplitude modulation is a prominent feature of the vocalizations of many species, including rodents. Sinusoidal Amplitude Modulation (SAM) has been used to define the nature and limits of temporal encoding in central auditory neurons in multiple animal models. The increasing use of mice as a model system for central hearing and the utility of mouse models for circuit dissection using optogenetic tools requires more detailed descriptions of cortical encoding of the temporal dynamics of complex stimuli. To this end, we presented moderate-amplitude, fully-modulated SAM stimuli with modulation frequencies ranging from 2-128 Hz to passive-listening, head-restrained awake mice. Electrophysiological recordings were obtained using 16-channel probes (NeuroNexus) spanning the laminar depth of the cortex. During the experiments the mice were free to move on an air-suspended styrofoam ball. Cortical responses were assessed with respect to both firing rates and response synchronization (Vector Strength) throughout the duration of the stimuli. Simple linear decoders (nearest-neighbor Euclidean-distance classifiers) were also used to classify the modulation frequency associated with individual spike trains to obtain a lower-bound estimate of the mutual information between stimulus envelope features and spiking responses. Multi-unit responses from a sizable fraction of cortical sites exhibited robust response synchronization to stimuli at the low modulation frequencies (< 20 Hz) that dominate animal vocalizations and human speech sounds. Reduced but significant synchronization was occasionally observed at the highest modulation frequency tested (128 Hz) as has been observed in awake nonhuman primate models. These results demonstrate that the temporal precision of cortical responses in awake mice is broadly comparable to that observed in other awake model systems. Further, cortical representation appears sufficiently precise to reward optogenetic experiments aimed at determining how excitatory and inhibitory circuits interact to represent the dynamics of complex signals including mouse vocalizations and human speech.

Disclosures: **N. Hoglen:** None. **E.A.K. Phillips:** None. **P. Larimer:** None. **B.J. Malone:** None. **A.R. Hasenstaub:** None.

Poster

051. Auditory Processing: Temporal and Frequency

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Program#/Poster#: 51.15/X10

Topic: D.05. Audition

Support: NIH RO1 DC002260

NIH RO1 DC014101

Title: Forward masking and synaptic inhibition in the auditory cortex of awake mice

Authors: *E. A. PHILLIPS[1,2], C. E. SCHREINER[1], A. R. HASENSTAUB[1];
[1]Kavli IFN and Coleman Mem. Laboratory, Dept. of Otolaryngology, [2]Neurosci. Grad. Program, Univ. of California, San Francisco, San Francisco, CA

Abstract: The precise temporal relationship between stimulus components is critical for the appropriate interpretation of rapidly fluctuating stimuli, such as speech. In line with this, responses to auditory stimuli are often influenced by recent stimulus history. For example, recent sound exposure can suppress both the perception of, and the neural responses to, a subsequent sound, and the magnitude of this suppression depends on both the spectral and temporal distances between the two sounds. This temporal suppression, called forward masking, is present in the auditory system as early as the auditory nerve, but is enhanced within the cortex. Synaptic inhibition has been hypothesized to mediate this enhancement, but the relative contributions of inhibition versus other potential mechanisms (such as intrinsic adaptation or short-term synaptic depression) remain unclear. Additionally, most prior studies of forward masking in the cortex have been performed under anesthesia, in which synaptic inhibition is greatly altered. This issue is further complicated by the engagement of diverse interneuron populations that may influence temporal processing in unique ways.To test if forward masking is qualitatively similar in the awake animal compared to under anesthesia, we recorded single-unit responses from the auditory cortex of awake mice during the forward masking stimulus (a two-tone sequence). First, we found that masking was qualitatively less robust in the auditory cortex of awake mice compared to prior studies conducted in anesthetized mice or in other anesthetized species. Additionally we found that masking strength weakly correlated with several cellular properties, such as onset latency, average response rate, tuning bandwidth, and spike shape. To test how synaptic inhibition contributes to forward masking within the auditory cortex, we optogenetically inactivated either somatostatin-positive (SST+) or parvalbumin-positive (PV+) interneurons during the forward masking stimulus. Inactivating interneurons produced marked changes in the strength and structure of forward masking. Moreover, by comparing trials with matched firing rates to the first tone, we found that these effects were independent of the response to the first tone, in opposition to intrinsic adaptation models of forward masking. We hypothesize that

forward masking is not only inherited from subcortical structures, but that it is transformed within the cortex, in part, by cortical interneurons.

Disclosures: **E.A. Phillips:** None. **C.E. Schreiner:** None. **A.R. Hasenstaub:** None.

Poster

051. Auditory Processing: Temporal and Frequency

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Program#/Poster#: 51.16/X11

Topic: D.05. Audition

Title: Background EEG activity modulates auditory-evoked response in C57BL/6J mice

Authors: B. H. TRACEY[1], L. SCOTT[2], C. SIOK[2], *D. VOLFSON[2];
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Abstract: Using electrophysiological recording techniques, auditory evoked potentials (AEPs) have been used both clinically and pre-clinically to assess sensory processing in disease and disease model populations. In interpreting AEP data, an important question is to what extent AEP response is modulated by the underlying state of the animal at the time of stimulation. In this study we explored this question using AEPs recorded from the frontal cortex of freely moving adult male C57BL/6J mice, using a paired pulse paradigm. We present changes in the AEPs in response to the novel pre-clinical candidate PDE4 inhibitor, ABI-4, which along with the clinically prescribed antipsychotics risperidone and haloperidol, have been shown to dose-dependently enhance sensory gating. To quantify the underlying state, we utilized frequency domain EEG features from pre-stimulus data from vehicle treated animals. The animal's background activity was sorted into one of several states defined using unsupervised clustering. Similarly clustered traces were averaged at each time point, and wavelet-based denoising was applied to suppress high frequency noise. Signal amplitudes at fiducial points were extracted and a linear mixed model with cluster as a covariate was used to analyze the dataset. The results shown that incorporating background activity significantly improves model fit and that cluster and cluster/treatment interactions are statistically significant factors for many endpoints. The analysis demonstrated that background activity modulates AEP response in mouse model and taking this into account may improve interpretation of AEP studies.

Disclosures: **B.H. Tracey:** None. **L. Scott:** None. **C. Siok:** None. **D. Volfson:** None.

Poster

051. Auditory Processing: Temporal and Frequency

Location: Halls B-H

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Program#/Poster#: 51.17/X12

Topic: D.05. Audition

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Title: Developmental changes in the functional laminar mesoscale organization of primary auditory cortex revealed with multi-scale imaging

Authors: *K. SOLARANA[1], D. WINKOWSKI[2], Z. BOWEN[2], J. LIU[2], N. FRANCIS[2], D. NAGODE[2], P. O. KANOLD[2];
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Abstract: Early experience of the world is fundamental in shaping the structural and functional organization of the brain. Sound inputs are particularly relevant for the proper maturation of the primary auditory cortex (A1), which requires exposure to behaviorally relevant acoustic stimuli during the critical period of auditory development. Previous studies have shown that artificially manipulating environmental sounds during early postnatal life results in wide-scale restructuring of topography in adult rodents, yet little is known how fine-scale A1 organization develops during ear opening and throughout the critical period. Here we investigated how the functional responses of A1 neurons and the mesoscale sound frequency representation in A1 changes and matures in different cortical layers throughout early hearing development. We used *in vivo* two-photon calcium imaging and wide-field imaging in one to four-week old Thy1-GCaMP6s mice and measured fluorescent responses of A1 neuronal populations in thalamorecipient layer 4 and supragranular layers 2/3 in response to amplitude-modulated tones. This age range allowed us to examine A1 development from just before ear opening, throughout the critical period, and into adulthood. We measured response changes on an individual neuronal level (response amplitude, frequency selectivity, sound level threshold) and on a population level (best frequency variability across the imaging window, signal and noise correlations), and found that significant changes occur during the critical period that are reflective of specific modifications in feed-forward and intracortical connectivity as the circuit is refining. A greater understanding of the complexity and plasticity of A1 during development will provide insight into the critical relationship between early sensory experience and the maturation of fine-scale cortical organization.

Disclosures: **K. Solarana:** None. **D. Winkowski:** None. **Z. Bowen:** None. **J. Liu:** None. **N. Francis:** None. **D. Nagode:** None. **P.O. Kanold:** None.

Poster

051. Auditory Processing: Temporal and Frequency

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Klingenstein Award in Neuroscience

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Burroughs Wellcome Career Award at Scientific Interface

NARSAD Young Investigator Award

Title: Two types of cortical interneurons differentially modulate behavioral frequency discrimination acuity

Authors: ***J. BLACKWELL**[1], M. AIZENBERG[1], L. MWILAMBWE-TSHILOBO[1], S. JONES[1], R. G. NATAN[1], M. N. GEFFEN[2];
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Abstract: The ability to discriminate between tones of different frequencies is fundamentally important for everyday hearing. Primary auditory cortex regulates behaviors that rely on frequency discrimination (Aizenberg and Geffen, 2013), but the underlying neural mechanisms are poorly understood. Frequency tuning of cortical excitatory neurons are thought to be shaped by the interplay of excitatory and inhibitory inputs. In the cortex, the two most common classes of inhibitory interneurons are parvalbumin-positive (PV) interneurons and somatostatin-positive (SOM) interneurons. PVs target the soma and initial axon segment, while SOMs target distal dendrites. Therefore, these two interneuron classes may differentially affect responses of excitatory neurons. We recently found that photo-activation of PVs enhanced tone-evoked responses of excitatory neurons, which was correlated with an improvement in behaviorally measured frequency discrimination acuity (Aizenberg et al., PLoS Biology, 2015). We now find

that photo-activation of SOMs diminished tone-evoked responses in the excitatory neurons, by suppressing tone-evoked responses more strongly than spontaneous activity. Interestingly, photo-activation of SOMs also increased the frequency selectivity of excitatory neurons, but had mixed effects on behaviorally measured frequency discrimination acuity. These findings are consistent with the interpretation that PVs and SOMs carry out differential roles in shaping frequency selectivity in the auditory cortex.

Disclosures: **J. Blackwell:** None. **M. Aizenberg:** None. **L. Mwilambwe-Tshilobo:** None. **S. Jones:** None. **R.G. Natan:** None. **M.N. Geffen:** None.

Poster

051. Auditory Processing: Temporal and Frequency

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Topic: D.05. Audition

Support: NIH Grant R01 DC003180

Title: A high-frequency tonotopic reversal in marmoset parabelt auditory cortex

Authors: ***D. GAMBLE**, X. WANG;
Johns Hopkins Univ., Baltimore, MD

Abstract: The current working model of primate auditory cortex comprises the hierarchical arrangement of a series of functionally distinct information processing stages: a primary 'core' region, a secondary 'belt' region, and a tertiary 'parabelt' region. Combined anatomical, physiological, and imaging experiments in monkeys have subdivided core and belt into multiple tonotopic subfields (Kaas & Hackett 2000). Human fMRI investigations have attempted to find homologues of the primate core fields A1 and R by looking for a low-frequency tonotopic reversal near Heschl's gyrus (HG), the histologically-identified site of human primary auditory cortex. While many studies have found such a reversal, a long-standing contradiction exists between results supporting an auditory cortex oriented parallel to HG, and others perpendicular to HG (Baumann, Petkov & Griffiths 2013; Moerel, de Martino & Formisano 2014). Using a single neuron mapping technique in awake and behaving marmosets, we have identified a previously unrecognized high frequency tonotopic reversal in putative parabelt auditory cortex, immediately lateral to the previously known low frequency reversal in lateral belt. Such a spatial arrangement of high and low frequency regions in adjacent fields could conceivably be mistaken for a single field with a tonotopic axis perpendicular to the true cortical configuration. Thus this high frequency region may explain the mutually contradictory patterns of results observed in

human imaging studies. Neurons in this high frequency region exhibit long response latencies, strong selectivity for acoustic parameters, such as bandwidth, intensity, temporal modulation, and spatial location, as well as response modulation by behavioral engagement.

Disclosures: D. Gamble: None. **X. Wang:** None.

Poster

051. Auditory Processing: Temporal and Frequency

Location: Halls B-H

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Program#/Poster#: 51.20/X15

Topic: D.05. Audition

Title: A high-throughput platform for combined optogenetic stimulation and wireless EEG recordings for use with auditory assays in awake-behaving rodents

Authors: *D. J. GRAZIANO[1], A. M. PATINO[2], M. M. SIDOR[2];
[2]Neurosci., [1]Novartis, Cambridge, MA

Abstract: Optogenetics is a powerful tool that that enables the control of neural firing with high spatial and temporal precision. Studies in awake-behaving rodents have provided valuable insight into the neural circuits that underlie normal and disease-related physiological states. There is a growing need for a consolidated platform to study specific circuit perturbations using optogenetics as well as sensory driven perturbations using evoked related potentials, while simultaneously recording the electrophysiological response to these inputs.
Currently available tools, however, are relatively low-throughput in design. Furthermore, the tethered nature of electrophysiological setups makes integrating this technology with optogenetics cumbersome for awake-behaving studies. Here we describe a flexible and versatile platform using a wireless telemetry system for EEG recordings (Data Sciences International) and a custom-made Arduino based auditory delivery system in combination with optogenetic stimulation that permits real-time simultaneous modulation of neural activity and EEG recording in awake-behaving mice. A single optogenetic-EEG recording platform is scalable up to 14 mice. Various TTL triggered optogenetic laser/LED set-ups can be integrated with the EEG recording system, however, we describe an enclosed-laser/LED system comprised of separate but simultaneously controlled lasers, with the ability to individually modulate light intensity output at each fiber end, i.e. at the light-animal interface. Light pulse trains are recorded via an analog-to-digital signal convertor that reliably tracks light pulse trains up to 200Hz in order to time-lock light delivery with EEG activity. Similarly, each auditory assay is time-locked to the EEG signal so that all responses can be temporally correlated. In addition to its high-throughput advantage,

the wireless EEG system is useful for long-term or chronic monitoring of brain activity in awake-behaving mice following optogenetic stimulation.

Disclosures: **D.J. Graziano:** None. **A.M. Patino:** None. **M.M. Sidor:** None.

Poster

051. Auditory Processing: Temporal and Frequency

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Support: ERC ADAM E-138 senior grant no. 295603

Advanced Grant (AdG), LS7, ERC-2013-ADG

Title: Functional organisation of the thalamo-cortical auditory system in awake ferrets using fast ultrasound imaging

Authors: ***C. BIMBARD**[1], C. DEMENÉ[2], C. GIRARD[1], S. RADTKE-SCHULLER[1], S. SHAMMA[1], M. TANTER[2], Y. BOUBENEC[1];
[1]Lab. Des Systèmes Perceptifs CNRS UMR 8248, Paris, France; [2]Inst. Langevin, Waves physics for medecine, Inserm U979, Paris, France

Abstract: Large-scale functional imaging techniques are part of a fast growing field of neuroscience aiming at understanding whole brain activity. Functional Ultrasound Imaging (fUS) is a new method monitoring changes in blood flow with a high spatial (~100µm) and temporal (down to the cardiac time scale) resolution, with a typical imaged section is typically 1cm wide and 2cm deep.It is thus an unequalled modality in the landscape of functional imaging, MRI included. We used this technique to study the functional organization of auditory circuit from the inferior colliculus (IC) to the cortex in the awake ferret. Craniotomies were performed above the auditory cortex, yielding a ~15x10mm window over the brain, and subsequentyly sealed with an ultrasound-transparent TPX cover, embedded in dental cement. First, we characterized the tonotopic organization of several areas of the auditory cortex in three dimensions, including deep sulci which are cortical regions typically inaccessible to standard intrinsic and voltage sensitive imaging techniques. Using a linear classifier algorithm, we found that blood flow activity in the capillaries gave access to a precise representation of the information encoded in the auditory cortex. This information was mostly present in medium and deep layers, mirroring previous studies that showed more refined tonotopic organization in granular and infragranular layers. Taking advantage of the penetration depth of fUS, we then describe for the first time the 3D

functional tonotopic organization of the ferret MGB and of the IC, confirming previous anatomical studies. In summary, we show that fUS is a powerful tool to study the fine organization and connections of brain structures with a great temporal precision and high spatial resolution, all in a short amount of time and with no damage to the brain.

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Poster

051. Auditory Processing: Temporal and Frequency

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Title: Auditory analogues of simple and complex cells: linearity of responses to periodic stimuli

Authors: *P. KUSMIEREK, J. P. RAUSCHECKER;
Neurosci., Georgetown Univ., Washington, DC

Abstract: Recently, S and C neurons were described in macaque auditory cortex based on segregation of “on” and “off” responses in the frequency domain, i.e. in cochlear space (Tian et al 2013). These cell types were proposed to correspond to simple and complex cells of the visual cortex, which are distinguished based on segregation of “on” and “off” responses in retinal space. This suggests a similarity of cortical computational mechanisms across sensory modalities, whereby cortical receptive fields (RFs) are generated from aligned thalamic inputs in their respective sensory epithelium space.
Another way to distinguish simple and complex cells in the visual cortex is by testing the linearity of their responses to sinewave drifting gratings (Skottun et al 1991): the response of simple cells varies linearly with the spatial frequency of the grating (indicated by a high response at the stimulus frequency normalized to DC, F1/F0), while complex cells respond non-linearly (low F1/F0). If auditory cortical neurons show a similar distinction, it would provide further support for the generalized RF model above.

We recorded 189 cells from areas A1 and CM of rhesus auditory cortex. Neurons classified as S based on the difference between "on" and "off" frequency-rate curves, compared to those classified as C, showed smaller distances between "on" vs. "off" best frequency, narrower tuning range and higher frequency selectivity, consistent with Tian et al (2013). In contrast to that study, we found longer response latencies in S cells vs. C cells. However, this was due to a subset of long-latency S cells; in an analysis restricted to latencies <40 ms no difference was found between S and C neurons.
To test linearity of S and C cell responses, we recorded responses of 91 cells to auditory "moving ripple" stimuli, which are equivalent to drifting sinewave gratings. We show that the F1/F0 ratio in auditory S cells is significantly higher than in C cells. This provides new evidence for correspondence between visual simple/complex and auditory S/C neurons. The reported effects were robust against changes in the method of S/C classification, and against restricting the analysis to A1 cells or to cells with confirmed driven responses in both "on" and "off" segments. Tian et al (2013) proposed that S cells might serve as detectors of boundaries between two successive sounds with different spectra, a mechanism that could be important for speech and music processing. However, we did not detect increased firing rate at such boundaries in S cells (n=24). On the other hand, C cells (n=66) decreased their firing rate at boundaries between two different sounds, compared to boundaries between repeated identical stimuli.

Disclosures: P. Kusmierek: None. **J.P. Rauschecker:** None.

Poster

052. Subcortical Visual Pathways: LGN

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

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Topic: D.06. Vision

Title: Subcortical and cortical color representation and attentional modulation

Authors: *S. HONG[1,2], Q. YU[3], W. SHIM[3,4,5];
[1]Psychology, [2]Ctr. for Complex Systems and Brain Sci., Florida Atlantic Univ., Boca Raton, FL; [3]Psychological and Brain Sci., Dartmouth Col., Hanover, NH; [4]Ctr. for Neurosci. Imaging Res. Inst. for Basic Sci., [5]Dept. of Biomed. Engin., Sungkyunkwan Univ., Suwon, Korea, Republic of

Abstract: Subcortical color processing is mediated by two distinct neural pathways, parvocelluar (L-M) and koniocellular (S-(L+M)). Studies on the non-human primate visual system suggest that the strict distinction between these two chromatic channels results in a limited color representation at the LGN level. Using functional Magnetic Resonance Imaging (fMRI) in conjunction with a forward encoding model (Brouwer & Heeger, 2009), we investigate first,

whether and how cardinal and inter-cardinal colors are represented in the human LGN as well as visual cortices by reconstructing population-level color tuning responses, and second, whether these color representations can be modulated by selective attention. On each block, observers viewed equi-luminant concentric ring patterns, composed of either one of four cardinal colors varying on only one channel or four inter-cardinal colors varying on both channels, and equal-energy-spectrum white, which drifted alternately in expanding and contracting directions. Observers performed either a central RSVP task (attention to fixation condition) or a color discrimination task (attention to color condition), which were alternated between blocks within each experimental run. Observers were instructed to report the target (letter 'J' or 'K') among other letters in the central RSVP task or to detect near-threshold level (measured for individual observers and for each color separately) changes in a color probe presented at a random location in the color discrimination task. We found that attention can modulate the population-level color tuning responses to both cardinal and inter-cardinal colors in the LGN. Our results demonstrate that both cardinal and inter-cardinal colors can be represented at the LGN level and that attentional feedback alters population-level responses to chromatic information in the LGN.

Disclosures: S. Hong: None. **Q. Yu:** None. **W. Shim:** None.

Poster

052. Subcortical Visual Pathways: LGN

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Topic: D.06. Vision

Support: University of Oslo

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Title: A firing-rate based simulation tool for studying roles of cortical feedback in lateral geniculate nucleus (LGN)

Authors: *M. HOBBI MOBARHAN[1], G. HALNES[4], P. M. CAÑADA[5], T. HAFTING[2], M. FYHN[1], G. T. EINEVOLL[3,4];
[1]Dept. of Biosci., [2]Inst. of Basic Med. Sci., [3]Dept. of Physics, Univ. of Oslo, Oslo, Norway; [4]Norwegian Univ. Life Sci., Aas, Norway; [5]Univ. of Granada, Granada, Spain

Abstract: The corticothalamic feedback from layer 6 in primary visual cortex (V1) to neurons in the lateral geniculate nucleus (LGN) allows cortex to dynamically shape its own input during sensory processing. Despite several experimental and modeling studies, the functional role of the

cortical feedback is still not well understood. Thus a systematic exploration is needed to understand the effects of the feedback on spatiotemporal responses in the LGN under various conditions. These conditions include different visual inputs and brain states. Recent advances in experimental techniques such as optogenetic and pharmacogenetic methods provide data to better constrain models of the thalamocortical circuit. While biophysically detailed LGN circuit models will be required to properly probe effects of neuronal details (Heiberg et al, 2016, PLoS Comp Biol), simulation of large networks with such models is computer intensive. This often prevents extensive testing of how the model output depends on the (typically uncertain) model parameters.
In order to elucidate notable features of visually evoked cortical-feedback effects, we have developed an efficient, firing-rate based simulator of spatiotemporal responses in the early visual system. The simulation tool is based on a previous model (Einevoll & Plesser, 2012, Cogn Neurodyn) which assumes a phase-reversed push-pull arrangement of ON and OFF cortical feedback, as has been seen experimentally. In this model the LGN cells exhibit linear firing properties despite non-linear firing characteristics of the corticothalamic cells. The advantage of the simulator lies in its computational and conceptual ease, allowing for fast and comprehensive exploration of various scenarios for the organization of the cortical feedback.
We find as a first application of the simulation tool, that even with circularly symmetric feedback it is possible to account for experimental findings on feedback effects on the spatial receptive fields of LGN cells (Sillito et al. 2002, Phil Trans R Soc Lond B). Thus even if the LGN circuit receives feedback from cortical cells of all orientation selectivities, the net resulting cortical feedback may still have circular symmetry. In addition to this, we will also present results for how the cortical feedback affects the temporal response properties of LGN cells.

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Poster

052. Subcortical Visual Pathways: LGN

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Edward R. and Anne G. Lefler Center Predoctoral Fellowship

Title: A reassessment of retinogeniculate convergence using optogenetic stimulation

Authors: E. Y. LITVINA[1,2], *C. CHEN[1];
[1]F.M. Kirby Neurobio. Ctr., Children's Hosp, Harvard Med. Sch., Boston, MA; [2]Program in Neurosci., Harvard Med. Sch., Boston, MA

Abstract: The retinogeniculate synapse is classically described as a simple relay between the retina and the primary visual cortex. Electrophysiological measurements have previously estimated that 1-3 retinal ganglion cell (RGC) synapse onto a mouse geniculate relay neuron (Chen & Regehr, 2000; Jaubert-Miazza et al., 2015). However, technical limitations have set these estimates as the lower bound of possible retinogeniculate convergence. Recent advances in connectomics have yielded much larger estimates of the number of connections between RGC axons and postsynaptic relay neurons (Morgan et al, 2016). Just as slice electrophysiology inherently underestimates input number, anatomical reconstruction lacking a functional readout can overestimate the degree of functional convergence, as not all anatomical contacts may be functional. To address the discrepancy between functional and anatomical descriptions of the retinogeniculate circuit, we took advantage of optogenetics in combination with an improved slice preparation protocol that helps to preserve the health of relay neurons in slice. We determined the upper and lower bounds for average retinogeniculate convergence by comparing the number of inputs estimated with conventional electrical versus optical stimulation of RGC afferents. To express ChR2 in a broad RGC population, we crossed Chx10-Cre mice with Ai32 (ChR2(H134R)-EYFP mice). As with electrical stimulation, we placed an optic fiber over the optic tract several hundred microns from dLGN to stimulate RGC axons with a small spot of blue light. Occlusion experiments showed the same single fiber input can be recruited using either blue light or electrical stimulation. We could reliably isolate single retinogeniculate fibers in Chx10-cre x Ai32 animals using this stimulus, yielding a distribution of single fiber amplitudes similar to that obtained with electrical stimulation of the optic tract. The amplitude of maximal currents we obtained by stimulating retinogeniculate synaptic terminals with full-field blue light illumination were almost twice the electrically-driven maximals, suggesting that optic tract stimulation in slice recruits approximately half of the total retinal input current onto relay neurons. Therefore, our new assessment of the number of RGCs that make contacts with a relay neuron falls between previous functional and anatomical estimates. However, using a simple Monte Carlo simulation, we find that the majority of these contacts make weak synapses while only a few of the inputs are strong.

Disclosures: E.Y. Litvina: None. **C. Chen:** None.

Poster

052. Subcortical Visual Pathways: LGN

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Topic: D.06. Vision

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Whitehall Foundation Research Grant

Title: Corticogeniculate feedback enhances temporal precision of LGN neurons in a stream specific manner

Authors: *J. M. HASSE, F. BRIGGS;
Physiol. & Neurobio., Dartmouth Col. Geisel Sch. of Med., Lebanon, NH

Abstract: An important function of the visual system is to filter relevant visual information from the noisy visual environment. The corticogeniculate pathway, which connects primary visual cortex (V1) with the visual thalamus (lateral geniculate nucleus or LGN) in the feedback direction, may play a role in this filtering process. We are investigating the functional role of the corticogeniculate pathway by optogenetically manipulating the activity of corticogeniculate neurons in anesthetized ferrets. First, we inject a modified Rabies virus expressing m-Cherry and channelrhodopsin2 (ChR2) into the LGN such that corticogeniculate neurons are selectively infected. Following surgical virus injection, we perform an in vivo experiment in which we place electrode arrays into retinotopically-aligned regions of the LGN and V1. Our V1 electrode is paired with a fiberoptic cable such that we can record responses of ChR2-expressing corticogeniculate neurons to both visual stimulation and optogenetic stimulation (via blue LED). We record the activity of V1 and LGN neurons in response to drifting sinusoidal gratings and m-sequence stimuli under conditions in which corticogeniculate neurons expressing ChR2 are light-activated. Our data suggest there are multiple morphological subtypes of corticogeniculate neurons that modulate feedforward LGN neurons in a stream specific manner, and that corticogeniculate feedback enhances the temporal precision of LGN neurons. These results support the notion that corticogeniculate feedback increases the salience of information carried by discrete feedforward processing streams. Funding: Whitehall Foundation Research Grant, NIH R01 EY25219

Disclosures: J.M. Hasse: None. **F. Briggs:** None.

Poster

052. Subcortical Visual Pathways: LGN

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Topic: D.06. Vision

Support: EY014024

Title: Functional mapping of retinogeniculate inputs on mouse thalamocortical relay neurons

Authors: *J. A. BEATTY, C. L. COX;
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Abstract: Anatomical evidence indicates that excitatory retinogeniculate terminals preferentially synapse on proximal dendrites of thalamocortical relay neurons. Retinogeniculate terminals can be part of a triadic arrangement consisting of the retinogeniculate axon terminal, the presynaptic dendrite of a local inhibitory interneuron, and the dendrite of the thalamocortical neuron. In this arrangement, excitatory drive from retinogeniculate terminals may activate inhibitory dendrodentritic synapses (F2 terminals) from thalamic interneuron dendrites onto dendrites of thalamocortical relay neurons. These F2 terminals are also thought to preferentially reside proximal on thalamocortical dendrites based on anatomical data, but to date, there has been little functional evidence to corroborate both of these anatomical findings. Here, we use subcellular channelrhodopsin-2 (ChR2)-assisted circuit mapping of the retinal inputs onto thalamocortical relay neurons of the dorsal lateral geniculate nucleus (dLGN) in mice. Briefly, intraocular injections of an adeno-associated viral vector containing ChR2 were made in 8-10 day old mice. Animals were allowed to recover for at least two weeks, to allow transfection of the virus, before brain slice recording were performed. Whole-cell intracellular recordings were made in dLGN thalamocortical relay neurons using a cesium based internal solution with Alexa-594 to aid in visualizing the dendritic morphology. Depolarization of retinogeniculate terminals by ChR2, and subsequent release of glutamate, was elicited with a small diameter (~2.5 μm) laser beam (473 nm) scanned in a grid (11 X 11, 20 μm spacing) centered over the soma. At hyperpolarized holding potentials (-60 mV) mapping resulted in excitatory postsynaptic currents (EPSCs) at proximal locations (<100 μm from soma) that persisted in the presence of tetrodotoxin (TTX) and 4-aminopyridine (4-AP). At holding potentials of 0 mV, EPSCs were replaced by the presence of inhibitory postsynaptic currents (IPSCs) that were shown to be GABAergic, persisted in the presence of TTX and 4-AP, and were more proximally distributed than the EPSCs (<60 μm from soma). These results functionally mapped retinogeniculate inputs on proximal dendrites of thalamocortical relay neurons. In addition, we were able to elicit inhibitory currents, presumably through F2 terminals, via subcellular ChR2-assisted circuit mapping of

retinogeniculate terminals and these inputs display a more proximal distribution on dendrites of the same thalamocortical relay neurons.

Disclosures: **J.A. Beatty:** None. **C.L. Cox:** None.

Poster

052. Subcortical Visual Pathways: LGN

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Program#/Poster#: 52.06/Y5

Topic: D.06. Vision

Title: Thalamic suppression as revealed in a Hidden Markov Model

Authors: *A. R. CASTI;
Dept. of Mathematics, Fairleigh Dickinson Univ., Teaneck, NJ

Abstract: Visual neurons in the mammalian lateral geniculate nucleus (LGN) reside in an elaborate circuit that includes suppressive input from numerous sources, including local thalamic interneurons and feedback collaterals from the reticular nucleus and primary visual cortex. Although some of the most prominent features of LGN relay cell responses, such as its receptive field, seem to be dominated by feed-forward excitation from retinal ganglion cells (RGC), the importance of thalamic suppression in controlling LGN activity has becoming increasingly clear. There is now strong evidence that a dynamic interplay between excitation and inhibition in the LGN sharpens response precision, primes relay cells for burst spiking, and reduces response redundancy without information loss. While relay cell models that incorporate only retinal input can be quite successful for spatially localized visual stimuli, they usually break down as the stimulus size increases and more of the retinogeniculate and thalamocortical architecture that produces inhibition is engaged.
Detecting the presence of LGN suppression *in vivo* is extremely difficult. For this reason inhibition is often inferred from extracellular spike records using a versatile model like the GLM. In this study, we use an Input-Output Hidden Markov Model (IOHMM) combined with single-cell, “quasi-intracellular” recordings of cat LGN to detect the presence of thalamic suppression and its influence on relay cell activity driven by temporally noisy flashing spots of various sizes. In these recordings, the electrode was placed sufficiently close to an LGN cell body to capture the primary retinal input (S potentials), and often also picked up “interloper” events of unknown origin, which were either fast biphasic or slower monophasic events in the extracellular voltage trace. Of particular interest was whether these interloping events were inhibitory in nature, and whether they could be exploited to improve the predictive power of LGN neuron models. Within the framework of the IOHMM, with S potentials as input and LGN spikes as output, the

hidden variables were interpreted as states of undetected excitation or inhibition. We correlated the hidden state transitions with the recorded "interloper" activity, and found that, for the larger stimulus sizes, interlopers of the fast biphasic type were indeed inhibitory, in that they were associated with hidden states producing a reduced conditional spiking probability in the optimized IOHMM. The results for the monophasic type events were mixed, suggesting in some cases inhibition and in some cases excitation, with more variable degrees of influence on LGN spiking.

Disclosures: A.R. Casti: None.

Poster

052. Subcortical Visual Pathways: LGN

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Support: NIH EY09593

Title: Response profiles of interneurons in the ferret vs. cat lateral geniculate nucleus

Authors: *V. SURESH[1], U. M. CIFTCIOGLU[1], K. R. DING[1], F. T. SOMMER[2], J. A. HIRSCH[1];
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Abstract: Local inhibitory interneurons in the lateral geniculate nucleus of the thalamus (LGN) influence all retinal signals that relay cells convey to cortex. In cat, receptive fields of most interneurons and relay cells are made of concentrically arranged subregions that have the opposite preference for luminance contrast, On or Off. Furthermore, there is a push-pull pattern of excitation and inhibition within each subregion: e.g. in On subregions bright excites and dark inhibits. A simple circuit might explain this pattern of response; the push is supplied by retinal ganglion cells with the same center sign as the postsynaptic target whereas the pull is fed forward from nearby ganglion cells of the opposite sign, via local interneurons. This scheme is difficult to test however, because in cat, as in most species, On and Off cells are intermingled in the LGN. The main layers of the ferret LGN, however, divide into On and Off leaflets, providing a potential means to gain insight into mechanisms of push-pull.
Previously, using whole-cell recording in vivo, we showed that basic synaptic physiological features of interneurons and relay cells are conserved in ferret and cat. In both species, membrane currents recorded from interneurons were dominated by trains of unitary IPSCs and

those recorded from relay cells by individual EPSCs (Wang et al., Nat. Neurosci., 2011). Also, relay cells in both cat and ferret displayed push-pull responses. Unlike cat, however, ferret interneurons lacked push-pull. For these cells, stimuli of the preferred sign did not evoke a sustained push current (as in cat) but instead elicited a response whose envelope comprised a transient hyperpolarization followed by a slowly developing depolarization that drove spikes. Similarly, stimuli of the non-preferred sign did not evoke a maintained pull but rather induced waveforms whose shape varied across the population. To quantify the variations in response pattern among interneurons in the ferret and across species, we developed a method in which the neural signal is represented as the weighted sum of the convolution of the stimulus (a square function) and a set of filters that include a Gaussian function and its first and second derivatives. The weights estimated for responses from ferret interneurons spanned a wide range of values that partially overlapped the distribution measured for cat. It was nonetheless possible to reconstruct the envelope of the pull in ferret relay cells from a composite of On and Off responses of interneurons. Thus, even as push-pull is preserved in relay cells of ferret and cat, the wiring diagram that generates this pattern of response in the two species may not be wholly identical.

Disclosures: **V. Suresh:** None. **U.M. Ciftcioglu:** None. **K.R. Ding:** None. **F.T. Sommer:** None. **J.A. Hirsch:** None.

Poster

052. Subcortical Visual Pathways: LGN

Location: Halls B-H

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Title: Contrast dependent synchrony in the retinogeniculate circuit

Authors: ***P. C. ALEXANDER**[1,2], H. J. ALITTO[1,2], T. G. FISHER[2], D. L. RATHBUN[3], W. M. USREY[1,2];
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Abstract: Across the visual hierarchy, neurons at successively higher stages of processing tend to exhibit an increased sensitivity to stimulus contrast. This phenomenon has been observed in the transition between the retina, lateral geniculate nucleus (LGN), primary visual cortex (V1),

and extrastriate areas. Although we lack a mechanistic understanding for how this process occurs, it seems likely that patterns of multi-neuronal activity play a role. Here we test the hypothesis that contrast-dependent changes in synchronous firing between LGN neurons contributes to the increased contrast sensitivity of V1 neurons. To do so, we made extracellular recordings from pairs of neurons in the LGN of anesthetized cats that showed a prominent peak in their cross-correlation function at zero time lag, suggesting shared retinal input. The tendency of cell pairs to spike with tight synchrony (±0.5 ms) in response to drifting sine wave gratings was assessed across multiple contrast levels while controlling for changes in overall firing rate. Our results show that across cell pairs the proportion of synchronous spikes increases with decreasing contrast, suggesting that contrast-dependent correlations between LGN cells may contribute to elevated contrast sensitivity in V1, as synchronous spikes are more effective in evoking cortical responses. Using a network of leaky integrate-and-fire model neurons we explore the ability of different circuit configurations to account for our findings in the LGN and further examine how the observed correlations might impact V1. Model simulations demonstrate that depression at the retinogeniculate synapse is capable of modulating the probability of synchronous firing among LGN relay cells receiving shared retinal input in a contrast-dependent manner. Ongoing simulations are examining possible influences from other cellular and circuit mechanisms including facilitating inhibitory input from geniculate interneurons.

Disclosures: **P.C. Alexander:** None. **H.J. Alitto:** None. **T.G. Fisher:** None. **D.L. Rathbun:** None. **W.M. Usrey:** None.

Poster

052. Subcortical Visual Pathways: LGN

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Rappaport Foundation

NIMA Foundation

Title: New methods to identify extra-classical receptive fields in macaque lateral geniculate nucleus.

Authors: N. J. KILLIAN[1,2], *J. S. PEZARIS[1,2];
[1]Massachusetts Gen. Hosp., Boston, MA; [2]Harvard Med. Sch., Boston, MA

Abstract: Receptive field mapping techniques for measuring the detailed responses of LGN neurons are built around transfer function estimation techniques using broadband stimuli. Typically such stimuli are constructed to be spatially and temporally white, such as a *m*-sequence, and often presented in either luminance-only or cone-isolating modes. These techniques fundamentally address linear response characteristics alone, and have most often been used in anesthetized, paralyzed animals. We address many of these shortcomings by using an awake behaving macaque preparation and wideband stimuli (RGB noise) with both white and non-white spatiotemporal (1/f-like) characteristics. While the response fields match in fundamental character between the two stimulus conditions, the lower high-spatial frequency energy content of the non-white stimuli produce generally lower signal-to-noise measurements, but allow fitting of non-linear parameters that rely on, for example, local contrast levels. Using additional non-linear post-processing noise-reduction techniques such as same-sign connectivity and annular summation, evidence for extra-classical responses can be identified in many cells.

Disclosures: **N.J. Killian:** None. **J.S. Pezaris:** None.

Poster

052. Subcortical Visual Pathways: LGN

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Topic: D.06. Vision

Support: Howard Hughes Medical Institute

National Science Foundation Graduate Research Fellowship Program

Title: Brain state-dependent modulation of thalamic visual processing by cortico-thalamic feedback

Authors: *K. REINHOLD[1], M. SCANZIANI[2];
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Abstract: The behavioral state of an animal impacts the way that sensory stimuli are processed by the brain. The response of thalamic neurons to temporally modulated visual stimuli, for example, depends on the alertness of the animal. In the alert state the response of thalamic neurons in the dorsal lateral geniculate nucleus (dLGN, the principal thalamic target of retinal axons) is strongly modulated by the timing of the visual stimulus, while during non-alert states this temporal modulation is profoundly reduced. The underlying mechanisms are still unclear.

Here we show that cortico-thalamic feedback through cortical layer 6 neurons plays a critical role in the state-dependent modulation of dLGN responses. As alertness to visual stimulation declines, cortico-thalamic feedback suppresses the amplitude of the response of the dLGN to time-varying visual stimulation. Thus, this top-down mechanism interacts with bottom-up sensory processing to change representations in sensory thalamus as a function of behavioral state.

Disclosures: K. Reinhold: None. **M. Scanziani:** None.

Poster

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Title: Contrast and temporal dynamics of extraclassical suppression in the lateral geniculate nucleus of the alert macaque monkey

Authors: *D. ARCHER, W. M. USREY;
Univ. of California, Davis, Davis, CA

Abstract: Nonlinear extraclassical suppression is a fundamental receptive-field (RF) property for neurons in the early visual system, including the retina, LGN, and primary visual cortex. The influence of extraclassical suppression is evident when stimuli overlapping and extending beyond the classical RF (CRF) noticeably suppress the CRF driven response. Given the extraclassical surround has been proposed to serve as a means for gain control as well as provide context for stimuli appearing in the CRF, understanding the temporal dynamics of extraclassical suppression is critical for gaining insight into the underlying circuits involved. Although, stimulus contrast has been shown to affect the spatial extent and response latency of CRF responses, our understanding of how contrast affects the temporal profile of extraclassical suppression in the LGN is less established. To determine whether stimulus contrast affects the time course of extraclassical suppression in the LGN, we made single-unit recordings from LGN neurons in the alert, fixating macaque monkey and measured responses to stationary sinusoidal gratings varying in size and contrast centered over the receptive field of the recorded cell. We then compared the temporal profiles of responses evoked from optimal size stimuli to profiles

evoked from large size stimuli over a range of contrasts. Across our sample of cells and consistent with the literature, response onset latencies increased as stimulus contrast decreased. Interestingly, suppression latencies also increased as contrast decreased, but to a much greater extent than for response latency. Suppression latencies also increased as stimulus size decreased, further suggesting that suppression latency depends on suppression strength. Taken together, these results indicate that the additional circuits recruited for extraclassical suppression require greater synaptic drive to engage than those underlying CRF responses.

Disclosures: D. Archer: None. **W.M. Usrey:** None.

Poster

053. Visual Cortex: Carnivores

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Topic: D.06. Vision

Support: KAKENHI 22135006

KAKENHI 15H05921

KAKENHI 24700325

Title: What features are matched binocularly for stereopsis?

Authors: *I. OHZAWA[1,2], D. KATO[1], M. BABA[3], K. S. SASAKI[1,2];
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Abstract: The key problem of stereoscopic vision is traditionally defined as accurately finding the positional shifts of corresponding object features between left and right images. Here, we demonstrate that the problem must be considered in a four-dimensional parameter space; with respect not only to shifts in space (X, Y), but also spatial frequency (SF) and orientation (OR). The proposed model sums outputs of binocular energy units linearly over the multi-dimensional V1 parameter space (X, Y, SF, OR). Experimentally, extracellular single-unit recordings were made in area 17 of 17 anesthetized and paralyzed adult cats. A binocular SF stimulus consisted of pairs of dichoptic sinewave gratings with various combinations of left-right SFs (SF_L, SF_R) and phases (ph_L, ph_R). Similarly, a binocular OR stimulus consisted of pairs of dichoptic sinewave gratings with various combinations of left-right ORs (OR_L, OR_R) and phases (ph_L, ph_R). Binocular version of reverse correlation analyses were performed in these domains. Both the theoretical analyses of the model and the physiological experiments show that many

binocular neurons achieve sharp binocular tuning properties by pooling the output of multiple neurons with relatively broad tuning. Pooling in the space domain sharpens disparity-selective responses in the SF domain so that the responses to combinations of unmatched left-right SFs are attenuated. Conversely, pooling in the SF domain sharpens disparity selectivity in the space domain, reducing the possibility of false matches. Analogous effects are observed for the OR domain in that the spatial pooling sharpens the binocular tuning in the OR domain. Such neurons become selective to relative orientation disparity. Therefore, pooling in the 4-dimensional space sharpens or tightens the binocular matching requirement constructively along all dimensions, and allows the visual system to refine binocular information into a form more desirable for stereopsis.

Disclosures: **I. Ohzawa:** None. **D. Kato:** None. **M. Baba:** None. **K.S. Sasaki:** None.

Poster

053. Visual Cortex: Carnivores

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Topic: D.06. Vision

Support: MEXT Grant KAKENHI 22135006

MEXT Grant KAKENHI 15H05921

MEXT Grant KAKENHI 24700325

Title: Fast contrast adaptation in V1 neurons - neural correlates of fading illusion -

Authors: *K. S. SASAKI[1,2], K. KURIHARA[1], I. OHZAWA[1,2];
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Abstract: Vision is a dynamic sense; we move eyes intermittently to see visual objects of interest at the center of gaze. This means that contrast of local visual scene stimulating a portion of retinae can change abruptly just after saccadic eye movements. Thus, neurons in early visual pathway might adapt to these sudden changes in contrast so fast that they can function properly. To the contrary, if retinal images are still for seconds during fixation, dim percepts in periphery fade away gradually and disappear eventually (Troxler fading). Traditionally, this fading is explained by a hypothesis that physical stimulus is cancelled by a negative image generated in the visual pathway, because the negative image is perceived momentarily when the physical stimulus is removed suddenly. Measuring the contrast responses of single neurons, we asked the

following two questions in the striate cortex of anesthetized and paralyzed adult cats. (1) Do these neurons show fast adaptation to change in contrast in the receptive fields? (2) Do these neurons respond to a blank screen in the faded or adapted state as if they actually 'see' the negative image.
We recorded single-unit activity of neurons in area 17 to measure the contrast response function during flashed grating stimuli of various contrasts were presented (7 contrasts in straddle, ±25%, range and pedestal, within 0-50%, range; negative values for phase reversal) in a rapid succession. The orientation and spatial frequency of gratings were fixed at the optimal value of each cell. Recorded spikes were analyzed using a reverse correlation technique.
We found that neurons in area 17 showed a shift of contrast response function during pedestal conditions. This adaptation process appeared to be completed within 500 milliseconds for the majority of our cells. The amount of shift was generally consistent with the arithmetic mean of contrasts in each experimental condition. Furthermore, shifts of contrast response function caused neurons to respond to a blank screen (i.e., 0% contrast stimulus) as if neurons had been stimulated by their preferred stimulus. This result supports the hypothesis that a negative image is generated during visual fading, and it behaves as if it were a real stimulus.

Disclosures: **K.S. Sasaki:** None. **K. Kurihara:** None. **I. Ohzawa:** None.

Poster

053. Visual Cortex: Carnivores

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 53.03/Y13

Topic: D.06. Vision

Title: Serotype-specific tropism of adeno-associated viruses in ferret primary visual cortex

Authors: A. DANIELS[1], T. BOUCHER[2], *K. J. NIELSEN[3,1];
[1]Zanvyl Krieger Mind/Brain Inst., Baltimore, MD; [2]Krieger Sch. of Arts and Sci., [3]Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: Ferrets constitute an important neuroscience research model for both the auditory and visual system. Increasingly, viral vectors are used in ferrets to transduce neurons with novel transgenes for optogenetic manipulation or calcium imaging. Adeno-associated virus (AAV) has become a popular choice for this purpose, owing to its low pathogenicity and stable long-term transgene expression. A number of different AAV serotypes are commonly available. Studies in other species, in particular rodents, demonstrate differences between serotypes in the cell types infected (tropism) and the transport to other brain regions. The choice of serotype is therefore an important factor in designing experiments. Currently, neither tropism nor transport have been

compared across serotypes in the ferret. Here, we sought to quantify tropism and transport for four different AAV serotypes in ferret primary visual cortex (V1).
Our experiments investigated serotypes AAV1, AAV5, AAV9, and AAV-DJ. All viral constructs contained GCaMP6 under control of the human synapsin promoter (hSyn). Viruses were injected in V1 in either one or both hemispheres, and a terminal experiment was performed 14-21 days later depending on the peak expression time for each serotype. The tissue was then fixed in 4% PFA and sagittally sectioned. Immunohistochemistry was performed using a primary antibody against GFP to detect GCaMP6 expression. In addition, sequential sections were stained against parvalbumin, calretinin or calbindin. To quantify tropism, we analyzed multiple sections containing the injection region in each animal. For each section, a tiled image of the whole injection region was taken using a 510 LSM confocal (Zeiss). We then determined which percentage of GCaMP6-infected cells were inhibitory neurons. To quantify transport, we determined the number of somata in the lateral geniculate nucleus that were infected with GCaMP6. Our preliminary data suggest differences in tropism between serotypes: While all serotypes similarly infected excitatory neurons in layers II/III, AAV-DJ infected a larger subpopulation of inhibitory neurons in these layers than the other serotypes.
These data contribute to a growing body of literature on species-dependent changes in AAV tropism and transport. A better characterization of these differences is key for selecting the correct serotype for an experiment, in particular as viral tropism - in addition to the chosen promotor - may be a critical factor in achieving cell-type specific expression of transgenes.

Disclosures: **A. Daniels:** None. **T. Boucher:** None. **K.J. Nielsen:** None.

Poster

053. Visual Cortex: Carnivores

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Support: NRSA Grant 1 F32 NS096877-01 to BSW

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Title: Retinal input and callosal neuron axons regulate activity-dependent elimination of Chandelier cells at the V1-V2 border before eye opening

Authors: ***B.-S. WANG**, Z. J. HUANG;
Neurosci., Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: Proper distribution and wiring of cortical inhibitory interneurons are crucial in regulating the balance of local circuitry and routing of cortical information flow. Although progress has been made in understanding the specification and long-range migration of cortical interneurons, the mechanisms that regulate their density and cortical integration remain unclear. The chandelier cells (ChCs) innervate pyramidal neurons (PyN) at axon initial segment – the spike initiation site, thus the proper distribution of ChCs may play a major role in shaping functional PyN ensembles. After their specification in the late embryonic medial ganglionic eminence, ChCs migrate with stereotyped route and schedule to arrive at designated cortical layers by the end of the first postnatal week. Surprisingly, we found that young ChCs then undergo massive apoptosis throughout the developing cortex between P7 and P14. In particular, the density of ChCs at the V1-V2 border region is reduced by half. The V1-V2 border receives input from callosal neuron (CN) axons of the contralateral visual cortex. By blocking CN growth or activity using Kir2.1 or inhibitory DREADD, we found that contralateral CN activity between P7-14 regulates the survival and density of ChCs at the border region. Further, ectopic CN axon projection induced by monocular enucleation at birth resulted in corresponding ectopic ChC elimination. Importantly, blocking retinal activity by monocular TTX injection between P7-14 had no effect on CN axon projection but reduced ChC pruning at ipsilateral V1-V2 border. Together, these results suggest that the density of ChCs at V1-V2 border is regulated by contralateral CN axons and retinal inputs. CNs receive inputs from the temporal retina that represents the central visual field and contribute to the seamless fusion of the left and right visual field. We hypothesize that activity-dependent elimination of ChCs at V1-V2 border may contribute to the development of a fast bi-lateral signaling pathway between the CNs that correlate their discharges when stimulated by the same orientation stimulus. Current effort is directed toward understanding the role of ChCs in the interhemispheric callosal projection pathway that integrates the cortical representation of the central visual field.

Disclosures: **B. Wang:** None. **Z.J. Huang:** None.

Poster

053. Visual Cortex: Carnivores

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Support: CIHR grant MOP-119498 to CB

Vanier Canada Graduate Scholarship CIHR to AG

Title: Separating ON and OFF pathway inputs to cortical simple cells reveals receptive fields with ON-dominated asymmetric push-pull.

Authors: *A. GHARAT[1], C. L. BAKER, Jr[2];
[1]Integrated Program in Neurosci., [2]Ophthalmology, McGill Univ., Montreal, QC, Canada

Abstract: Simple cells in the early visual cortex are conventionally thought of as linear Gabor-like spatial filters with an output nonlinearity. Spatial linearity of simple cells has been demonstrated in cat Area 17 receptive fields with intracellular recordings (Martinez et al., 2005). This linearity across the receptive field is thought to arise from a symmetric "push-pull" arrangement of inputs from ON and OFF pathways. However the generality of this finding to a wider sample of neurons, and to other early cortical areas, remains unclear.
We used 32-channel multielectordes (polytrodes and linear arrays) to record extracellular single-unit responses of cat Area 17 & 18 simple cells to natural image sequences. We estimated a two-stage (LNLN) receptive field model of individual simple cells using regularized gradient descent optimization. In this model, first stage filters correspond to receptive fields of ON- and OFF-center lateral geniculate nucleus afferents, while the second stage (weight map) corresponds to the spatial layout of their summation by the cortical neuron. This model enables us to visualize the spatial arrangement of excitatory and inhibitory inputs from ON and OFF subcortical pathways to individual receptive fields.
The estimated receptive field models of most Area 17 simple cells have symmetric push-pull, as demonstrated previously by intracellular recordings. That is, excitatory ON input at a given spatial location in the receptive field is balanced by inhibitory OFF input and vice-versa. Interestingly, a large fraction of simple cells in Area 18 do not have such symmetric push-pull receptive fields. Instead, these cells have strong excitatory as well as inhibitory input from ON pathway inputs, and very weak complementary input from the OFF pathway. Some receptive fields receive input only from the ON pathway.
This novel system identification method demonstrates the presence of ON-dominated asymmetric push-pull receptive fields in early visual cortex. Further, these results challenge the generality of the widely accepted model of simple cells as linear spatial filters.

Disclosures: A. Gharat: None. **C.L. Baker:** None.

Poster

053. Visual Cortex: Carnivores

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Topic: D.06. Vision

Support: NIH Grant EY024890

Title: Targeted optogenetic activation of the parvalbumin inhibitory neuron circuit in cat primary visual cortex sharpens orientation tuning of putative excitatory neurons

Authors: *D. C. LYON, A. T. FOIK, Y. LIU, L. A. ZHANG;
Anat. and Neurobio., Univ. of California Irvine, Irvine, CA

Abstract: Major strides have been made in recent years on realizing how different cortical inhibitory neuron subtypes shape neuronal selectivity. For example, optogenetic studies targeting parvalbumin(PV)-positive inhibitory neurons in primary visual cortex (V1) of transgenic mice demonstrate gain control at low PV activation and sharpening of orientation tuning at higher activation (Atallah et al. 2012 Neuron 73, 159-70; Lee et al. 2012 Nature 488, 379-83). While functional differences in several inhibitory neuron subtypes have now been described in transgenic mouse lines, there is little understanding of similar cell types in higher visual species due to a lack of transgenic models. Here, we used a helper virus containing a *Fugu* PV promoter to target infection of a modified rabies for selective delivery of channelrhodopsin-2 (ChR2) to PV neurons, initially (starter cells), and to the neurons providing direct inputs (connected cells) to these PV starter cells. In this local circuit expressing ChR2 in cat V1, both PV cells and putative excitatory neurons projecting directly onto PV cells can be activated through optical stimulation, which in theory can lead to reduction in firing rate of local non-ChR2 expressing neurons. This was confirmed in preliminary analysis, where we found a 10, 11 and 17% reduction in firing rate for the population of recorded neurons ($n > 110$; $p < 0.01$) to low, moderate and high laser intensities, respectively, compared to no-laser. Nevertheless, ~30% of recorded cells instead showed an increase in firing rate to increasing laser intensity and may correspond to cells expressing ChR2. Also found for the entire population at high laser intensities, orientation tuning widths measured by the half-width at half-height (HWHH) were sharpened by 14% ($p<0.05$). Note that the visual stimulus and laser were shown simultaneously for 500 ms and a 1500 ms inter-stimulus time interval was used to allow recovery from ChR2 activation. The above results were found when stimulating the classical receptive field (CRF). In contrast, when visual stimuli also included the extraclassical surround, there was no change in firing rate or HWHH to increasing laser intensity. Thus, our data suggest that for CRF sized stimuli which elicit the maximum response of a cell, local PV neurons in cat V1 play a role in sharpening orientation tuning by reducing the firing rate. However, for larger stimuli, which generally already lead to sharper HWHH and reduced cell firing through surround suppression (Liu et al. 2015 J Physiol 593.19, 4485-98), PV neuron circuit activation is not a factor.

Disclosures: **D.C. Lyon:** None. **A.T. Foik:** None. **Y. Liu:** None. **L.A. Zhang:** None.

Poster

053. Visual Cortex: Carnivores

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Topic: D.06. Vision

Support: Max Planck Society

Title: Synaptic architecture of visual space in ferret visual cortex

Authors: *B. SCHOLL, D. E. WILSON, D. FITZPATRICK;
Max Planck Florida Inst., Jupiter, FL

Abstract: Understanding how single neurons integrate synaptic inputs from myriad sources to generate somatic responses remains a challenge. Despite decades of somatic subthreshold voltage measurements, we still lack a fundamental understanding of the functional properties of individual synaptic inputs and their dendritic integration. Here we address this issue by examining how visual space is represented in the synaptic inputs onto dendrites of neurons in ferret visual cortex. We focused on visual space because retinotopic maps are highly conserved across mammals and mapping spatial receptive fields (RFs) can be accomplished with 1-dimensional noise stimuli. To measure the functional properties of synaptic inputs we used two-photon imaging of cortical cells sparsely labeled with a genetically encoded calcium indicator, allowing the visualization of hundreds of dendritic spines on single neurons. Despite the presence of a retinotopic map in V1, there was no evidence for retinotopic organization in the arrangement of synaptic inputs within the dendritic field. Pooling across the entire population of synaptic responses for individual neurons revealed that synaptic inputs arise from a much wider region of space than the soma; nevertheless, the preferred spatial location of the soma could be predicted from the summed synaptic responses. Individual synaptic inputs were often tuned for regions of space outside the somatic RF and there was considerable diversity in synaptic RF properties. Despite the lack of a clear dendritic retinotopic organization, the arrangement of inputs was not random, and we found that some dendritic branches exhibited clusters of spines with a high degree of similarity in the spatial selectivity of their responses. The summed synaptic input from correlated spines on dendritic branches revealed spatial RFs confined to subregions of the larger summed synaptic RF. In contrast, summed synaptic input from uncorrelated spines spanned much larger areas of the summed synaptic RF. The functional clustering of synaptic inputs responsive to localized regions of visual space suggests that dendritic computations may play an important role in spatial interactions that contribute to cortical neuron RF properties.

Disclosures: **B. Scholl:** None. **D.E. Wilson:** None. **D. Fitzpatrick:** None.

Poster

053. Visual Cortex: Carnivores

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Topic: D.06. Vision

Support: NIH Grant EY022122

Title: Subthreshold spatio-temporal receptive fields in developing ferret visual cortex

Authors: A. ROY, B. MESCHEDE-KRASA, W. ALFORD, *S. D. VAN HOOSER;
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Abstract: In visual cortices of cats and ferrets, selective responses to edges with specific orientation (orientation selectivity, OS) develop prior to eye opening, whereas selective responses to oriented edges moving in specific directions (direction selectivity, DS) develop through visual experience following eye opening. Cortical DS is thought to arise from spatially separated detectors projecting on to cortical neurons with varying time delays such that motion inputs only in the preferred direction summate at the cortical neuron to produce robust spiking. This model predicts slanted spatio-temporal receptive field (STRF) for direction-selective V1 neurons, experimentally demonstrated previously. How does this slanted STRF develop following visual experience? According to a Pre-existing bias model, weak but slanted structure of STRFs already exists at eye opening and visual experience merely allows the maturation of these patterned synaptic inputs. Alternatively, according to a flexible plasticity model, visual experience works via an instructive mechanism to develop slanted STRFs from immature thalamo-cortical inputs that are diffusely organized in space and time. To distinguish between these models it is necessary to map the STRFs directly before visual experience. Neurons in developing cortex exhibit relatively low firing rates, and mapping STRFs with extracellular recordings is problematic. Therefore, we carried out in vivo sharp-microelectrode intracellular recordings from V1 of ferrets at young (P30-35) and older (P40-60) ages, and using white noise reverse correlation technique computed the sub- and supra-threshold STRFs of these neurons. While the STRFs from DS cells in older animals showed distinct slanted structure, STRF structures in younger animals were more diverse. Some non-DS neurons in younger animals showed weakly slanted STRFs, but most immature neurons had diffusely structured STRFs. Implications of these immature STRF structures on visual experience-dependent plasticity will be discussed.

Disclosures: A. Roy: None. **B. Meschede-Krasa:** None. **W. Alford:** None. **S.D. Van Hooser:** None.

Poster

053. Visual Cortex: Carnivores

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Program#/Poster#: 53.09/Z1

Topic: D.06. Vision

Title: Horizontal organization of thalamic inputs in visual cortex

Authors: *A. SIAHKAMARI[1], E. ZABEH[1], J. JIN[2], R. LASHGARI[1], J.-M. ALONSO[2];
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Abstract: Thalamocortical afferents are thought to play a major role in the organization of visual cortical maps, however, we still have a poor understanding of how they are horizontally arranged within the cortex. To address this question, we measured horizontal changes in ON and OFF cortical retinotopy within cat visual cortex by using multielectrode arrays (Neuronexus, 32 electrodes horizontally separated by 0.1 mm from each other). We then performed computer simulations to estimate the horizontal pattern of thalamic axons that best reproduced the ON-OFF cortical changes. Thalamic afferents were first given random values of receptive field position/size/polarity, synaptic strength per cortical point and axon-terminal size, while constraining the parameter range with physiological data (e.g. equal number of ON and OFF afferents, matched size of thalamic receptive field center and cortical receptive field subregion, average thalamic receptive field scatter per cortical point: 2.5 receptive field centers). After randomizing the initial values, thalamic axons went through two different stages of pruning. In the first stage (retinotopic pruning), the synaptic weights and axonal branches changed to match the ON-OFF cortical retinotopy. In the second stage (fine pruning), we tested three different approaches: 1) minimize size of axon terminals, 2) minimize number of thalamic inputs, 3) both. At each pruning stage, we performed a weighted sum of the receptive fields from ON and OFF thalamic afferents and compared the sum with the spatial structure of ON and OFF cortical receptive fields (200 afferents combined in 200 separate simulations). Among the three approaches, minimizing axon terminal size provided the best fits to the cortical data (9% of mean unexplained variance vs. 20% and 12%, $p<0.0001$, Wilcoxon tests here and below) and reproduced more closely the unity slope of the correlation between ON/OFF ratios for axon number and synaptic strength found in physiological experiments (0.89 vs. 0.56 and 0.56, $p<0.0001$). The minimization of axon terminal size also made OFF afferents to cover larger horizontal distances than ON afferents (1.17 vs. 1.06 mm at half-amplitude of exponential distribution, $p<0.0001$) while making each of the multiple axon patches more locally restricted for OFF than ON afferents (221 vs. 286 microns at half-amplitude of exponential distribution, $p<0.0001$). These preliminary results demonstrate that changes in ON/OFF cortical retinotopy can be accurately reproduced from linear combinations of thalamocortical inputs and suggest a

possible difference in the local and long-range organization of ON and OFF thalamic afferents in visual cortex.

Disclosures: **A. Siahkamari:** None. **E. Zabeh:** None. **J. Jin:** None. **R. Lashgari:** None. **J. Alonso:** None.

Poster

053. Visual Cortex: Carnivores

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 53.10/Z2

Topic: D.06. Vision

Support: NIH Grant EY011488

Title: Cellular and synaptic mechanisms of direction selectivity in visual cortex

Authors: *D. E. WILSON, B. SCHOLL, D. FITZPATRICK;
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Abstract: Individual neurons in the primary visual cortex respond selectively to the direction of motion of visual stimuli. In simple cells, previous studies have shown that weak biases in synaptic inputs are amplified by a spike threshold nonlinearity to generate robust direction selectivity. These biases in membrane potential direction selectivity derive from differences in the relative timing of synaptic inputs rather than differentially tuned excitatory and inhibitory conductances. The cellular and synaptic mechanisms of direction selectivity in complex cells have remained largely unexplored. *In vivo* two photon imaging of calcium signals in the dendritic spines of single neurons in ferret visual cortex indicates that direction selective neurons receive excitatory synaptic inputs tuned to both the preferred and null directions, and that direction selectivity cannot be predicted by summing spine inputs and applying a threshold nonlinearity. Despite the prevalence of null-tuned excitatory synaptic inputs, *in vivo* whole-cell patch recordings show that neurons with strong spiking direction selectivity tend to show similarly strong direction selectivity in their subthreshold membrane potential. This highly selective membrane potential tuning may arise from null-direction tuned inhibition in these neurons. Consistent with this notion, we find that the membrane potential variance decreases at the null direction in these neurons, and that this reduction in noise at the null direction is strongly correlated with membrane potential direction selectivity. Finally, hyperpolarizing highly selective neurons strongly reduces their membrane potential direction selectivity. These findings

suggest that inhibitory inputs tuned for the null direction enhance direction selectivity in complex cells.

Disclosures: **D.E. Wilson:** None. **B. Scholl:** None. **D. Fitzpatrick:** None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 54.01/Z3

Topic: D.06. Vision

Support: NSF-SMA 1041755

UCSD FISP Grant G2176

Title: Modeling individual variations in contrast perception as lateral connections within V1

Authors: ***E. C. MORGAN**, V. R. DE SA;
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Abstract: The FLODOG model (Robinson et al., 2007), based on the ODOG model (Blakeslee & McCourt, 1999), has previously demonstrated that perception of contrast illusions can potentially arise solely from lateral interactions between neurons in the primary visual cortex, but as an abstract model it does not have the ability to represent individual differences in human perception of contrast. We are creating a new model based on FLODOG with more biologically realistic connections based on a model of correlation-based connectivity by Troyer et al (1998). This new model shows increased ability to represent individual differences in perception of contrast illusions as found in previously collected psychophysical data, as well as providing the ability to correlate these differences with individual variations in neural connectivity in V1. Further refinements to this model are ongoing, and we expect to improve its ability to represent contrast perception. **References:** Blakeslee, B and McCourt, M.E. (1999). A multiscale spatial filtering account of the White effect, simultaneous brightness contrast and grating induction. Vision Research 39, 4361-4377. Robinson, A. E., Hammon, P. S., & de Sa, V. R. (2007). Explaining brightness illusions using spatial filtering and local response normalization. *Vision Research, 47*(12), 1631-1644. Troyer, T. W., Krukowski, A. E., Priebe, N. J., & Miller, K. D. (1998). Contrast-invariant orientation tuning in cat visual cortex: thalamocortical input tuning and correlation-based intracortical connectivity. *The Journal of Neuroscience, 18*(15), 5908-5927.

Disclosures: **E.C. Morgan:** None. **V.R. de Sa:** None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 54.02/Z4

Topic: D.06. Vision

Title: Color processing in mouse primary visual cortex

Authors: *I. RHIM[1,2], G. COELLO-REYES[1,2,3], I. NAUHAUS[1,2,3];
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Abstract: Mice, in common with other mammals, have dichromatic vision. Most mouse cones coexpress opsins that are sensitive to either 'green' (M-opsin) or 'UV' (S-opsin) light., with varying S:M expression ratios along the retina's dorsoventral axis. In addition to the co-expressing cones, there are pure S-cones intermixed throughout the retina. Opponency (i.e. subtraction) between the M- and S-opsins has been observed in ganglion cells, indicating that the mouse may be a useful model for the study of color vision. However, little is known about color vision in the mouse primary visual cortex (V1). Here, we developed a green-UV visual stimulus to study the balance of cone inputs to V1. Two projectors, one emitting green light (540 nm) and one emitting near-UV light (405 nm), were aligned to a rear-projection screen. We used S- and M-opsin sensitivity functions to generate drifting gratings with equated total opsin contrast in multiple directions of the S/M plane. Neural responses were measured with two-photon imaging within identified locations of the visuotopic map. We identified neural responses tuned to unique directions in the S/M plane, which allowed us to estimate the relative input from S and M components. In addition, orientation and spatial frequency of the gratings were varied to examine spatial properties of cells tuned to each directions of color space. Our results show a diverse population of chromatic tuning in V1: neurons are selective for S+M, S-M, S-isolation, and/or M-isolation. Together, we provide evidence for color-tuned responses in mouse V1 and a useful platform for studying chromatic vision in the mammalian brain.

Disclosures: I. Rhim: None. **G. Coello-Reyes:** None. **I. Nauhaus:** None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

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Program#/Poster#: 54.03/Z5

Topic: D.06. Vision

Support: Wellcome-DBT India Alliance Intermediate Fellowship to SR

Tata Trusts Grant

Title: Large visual stimuli induce two distinct gamma oscillations with different tuning properties in the primary visual cortex of macaque monkeys

Authors: *P. RAVISHANKAR[1], M. V. P. S. DINAVAHI[2], V. SHIRHATTI[3], S. RAY[3];
[1]Ctr. For Neurosci., [2]Ctr. for Neurosci., [3]Indian Inst. of Sci., Bangalore, India

Abstract: Gamma rhythm (30-70 Hz) has been associated with high level cognitive functions and known to be elicited in many parts of the brain. Gamma oscillations elicited in the primary visual cortex have been extensively studied but its functionality is poorly understood. Although recent reports have suggested the presence of two gamma oscillations in the hippocampus, only a single gamma rhythm (whose center frequency varies depending on the properties of the visual stimulus) has been observed in the primary visual cortex. We recorded local field potentials (LFP) from chronically implanted microelectrode arrays in the V1 area of two macaque monkeys and found that large visual stimuli that cover both visual hemi-fields induce a slow gamma rhythm between 20-35 Hz in the primary visual cortex of awake monkeys, in addition to the previously reported gamma between 40-70 Hz. More importantly these two rhythms have distinct orientation preferences that are almost orthogonal to each other. The tuning preference for stimulus parameters like contrast, spatial frequency and temporal frequency are also different for both these rhythms. Lack of evidence from previous studies in observing two gamma rhythms can be largely attributed to the use of small stimuli localized around the classical receptive field. Two gamma rhythms with different tuning preferences might together provide a more extensive representation of the external input and better coding or communication mechanisms in the primary visual cortex.

Disclosures: P. Ravishankar: None. **M.V.P.S. Dinavahi:** None. **V. Shirhatti:** None. **S. Ray:** None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 54.04/Z6

Topic: D.06. Vision

Title: Evidence of nonlinear dynamics in cortical processing of the color VEP

Authors: *V. NUNEZ[1], R. SHAPLEY[2], P. SCHUETTE[1], A. AMIR[1], C. BRITTENHAM[1], A. BUTT[1], N. CHAN[1], S. A. HASSAN[1], P. PEHME[1], C.-S. RIDWAN[1], Y. SONG[1], J. GORDON[1]; [1]Psychology Dept., CUNY Hunter Col., New York, NY; [2]Ctr. for Neural Sci., NYU, New York, NY

Abstract: Recent research has provided support for the notion that the primary visual cortex (V1) is involved in the processing of color form perception through the combined activity of single-opponent and double-opponent cells. While many color researchers subscribe to the view of linear cortical function, receptive field measurements have indicated the possibility of cortical nonlinearities. To investigate the nature of cortical processing for color patterns, we examined human cortical responses to isoluminant color appearance-disappearance checkerboard and full-field patterns as a function of color contrast. The patterns were square-wave modulated from gray to color and back to gray (0.5s on and 1.5s off) and the stimuli used chromatic excitation purities ranging from 0.03 to 0.53. We measured the chromatic visual evoked potential (cVEP) with a 64-channel BioSemi system (but obtained the spatial resolution of a 128-channel system by positioning the 64 electrodes on the back half of the head from Cz using the extended 10-20 system). Cortical topography indicated the cVEPs were highly localized at electrodes near Oz, pointing to V1 cortex as their major source. Responses (30 repeats) were signal-averaged and Fourier-transformed. Remarkably, the cVEP waveform of the checkerboard pattern at Oz showed evidence of two different chromatic mechanisms: one was early, fixed around 100 ms for all excitation purities, whilst the other had a latency that decreased dramatically as excitation purity increased. In full field the N100 was stronger (characteristic of area-responsive, single-opponent cell contributions) and the second mechanism was absent (indicating it had been due to edge-dependent double-opponent cells). Gomes et al (2010) measured the VEP of equiluminant red-green sine-wave gratings as a function of pooled cone contrast; their resulting waveforms did not contain a peak equivalent to the N100 that we had observed, thereby supporting the suggestion that the N100 was due to single-opponent cells. Whilst this mechanism was clearly linear, the double-opponent mechanism was strikingly nonlinear; as was confirmed by Fourier analysis which showed phase advances and large changes in the amplitude spectrum with increasing excitation purity. These data establish the existence of color-contrast-dependent non-linear dynamics in cortical processing.

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Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

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Topic: D.06. Vision

Support: National Natural Science Foundation of China (31571078)

Title: Red predominance of gamma band power in macaque v1 and v4

Authors: ***Y. LIU**[1,2], J. YIN[1], Z. CHEN[1,2], I. M. ANDOLINA[1], W. WANG[1];
[1]Lab. of Visual Perceptual Mechanisms, Inst. of Neuroscience,CAS, Shanghai, China; [2]Univ. of Chinese Acad. of Sci., Shanghai, China

Abstract: Our visual world is rich in spectral information, and the evolution of separate L / M cone and resultant L—M opponent channels in catarrhine and some platyrrhine primates is indicative that distinguishing "red" from "green" serves a critical purpose. Psychophysical studies in humans, and electrophysiology in both human and non-human primate visual cortices suggests a red dominance in driving neural responses in comparison to other colors. Here we used 16-channel laminar probes to record local field potentials (LFP) and multi-unit activity (MUA) across the cortical depth for full-field isoluminant color stimuli in both V1 and V4. We computed band-limited power from the LFP 0-200ms during stimulation induced by seven isoluminant colors (red, orange, yellow, green, cyan, blue, purple). As the gamma (γ) band is commonly assumed to be related to attention and stimulus awareness in visual cortex, we hypothesized that the red may differentially drive gamma band power during visual perception. Consistent with our hypothesis, we found that red stimulation induces significantly higher power at both the γ-low (30-50Hz) and γ-high bands (50-100Hz) across all laminar depths in both V1 and V4. Even when recording within V4 color domains whose color preference was other than red, or V4 form domains with little color preference, red still drove a higher γ-band power response. The fact that red drives a significantly enhanced γ-band response invariant to color or form domain preferences is consistent with the perceptual preference to red seen across primate species.

Disclosures: **Y. Liu:** None. **J. Yin:** None. **Z. Chen:** None. **I.M. Andolina:** None. **W. Wang:** None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

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Topic: D.06. Vision

Support: Research grant from the Whitehall Foundation

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NEI Training Grant 2T32 EY007135-21

Title: Interocular gain control in primate LGN

Authors: *K. DOUGHERTY, M. A. COX, A. MAIER;
Dept. of Psychology, Vanderbilt Univ., Nashville, TN

Abstract: Visual appearance differs rather little when we use one or both eyes despite the drastic difference in sensory input to the visual system. This similarity between monocular and binocular viewing suggests that the brain accounts for the difference in input through a process of interocular gain control. The dorsal lateral geniculate nucleus of the thalamus (LGN) has been identified as a candidate structure for such interocular gain control. The anatomical connectivity of the LGN allows for neurons that receive inputs from one eye to be modulated by neurons that receive inputs from the other eye. However, due to a lack of converging evidence, it remains unclear in how far responses of LGN neurons depend on the views of both eyes. Here we directly test the hypothesis that interocular gain control occurs within the main pathways of the LGN. Towards this aim, we recorded single unit responses from multiple LGN layers simultaneously with a linear multi-contact electrode array in macaques. Animals were trained to fixate while visual stimuli were presented to one or both eyes through a mirror stereoscope. We compared visual responses to varying levels of interocular luminance and contrast differences. For the majority of magnocellular and parvocellular LGN neurons in our sample, visual responses were comparable under monocular and binocular stimulation. However, for a small fraction of LGN neurons the contrast response functions systematically shifted between monocular and binocular conditions. This finding suggests that, while it is possible that interocular gain control begins in the LGN, it seems unlikely that the LGN is the main structure supporting this process in primates.

Disclosures: K. Dougherty: None. **M.A. Cox:** None. **A. Maier:** None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

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Topic: D.06. Vision

Support: Wellcome Trust/DBT India Alliance (Intermediate Fellowship to SR)

Tata Trusts Grant

Title: Effect of stimulus discontinuities and chromatic input on gamma rhythm in primate primary visual cortex

Authors: *V. SHIRHATTI[1], S. RAY[2];
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Abstract: Gamma rhythm (~30-80 Hz) has been hypothesized to play an important role in perception and feature binding, which posits that gamma should be generated by a wide range of stimuli. However, while gamma is elicited strongly in local field potential (LFP) signals by stimuli such as bars and sinusoidal gratings, whether natural images also produce salient gamma is contested (Brunet et al (2013), Hermes et al (2014)). To elucidate this we studied the effect on gamma of two important aspects of natural images - stimulus discontinuities and chromatic properties.

Because a natural image contains arbitrary low-level discontinuities in many dimensions (such as luminance or orientation), it is difficult to determine the effect of such discontinuities on Gamma rhythm using natural images alone. We therefore studied the effect of experimentally controlled and parameterized discontinuities (such as a cut or discontinuity in orientation, contrast or phase) in grating stimuli on Gamma rhythm in LFP recorded from the primary visual cortex of two awake rhesus macaques (Macaca Radiata).

We observed that Gamma rhythm was robustly induced by continuous sinusoidal gratings, but was drastically reduced in the presence of even a small discontinuity. This drop was observed for discontinuities of various types, such as luminance, orientation or phase. In a separate set of experiments we found that chromatic input also significantly modulates gamma rhythm in V1 LFP even though the neuronal responses are largely unaffected.

When presented with natural images, as predicted by these results, Gamma rhythm was found to be weak or absent for most images, consistent with the findings of Hermes and colleagues (2014). Overall, our results show that Gamma rhythm is unlikely to play a major role in the processing or binding of natural images. Instead, it could be a resonant phenomenon that depends critically on the excitation-inhibition balance within the local neuronal network and gets disrupted if this balance is compromised due to discontinuities. Additionally, our results report for the first time that chromatic input is also a major determinant of gamma rhythm in LFP in the

primate primary visual cortex.
References: Brunet, N. et al. (2013). Visual cortical gamma-band activity during free viewing of natural images. *Cerebral Cortex*, bht280. Hermes, D. et al. (2014). Stimulus dependence of gamma oscillations in human visual cortex. *Cerebral Cortex*, bhu091.

Disclosures: V. Shirhatti: None. **S. Ray:** None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 54.08/Z10

Topic: D.06. Vision

Title: Neuronal dynamics related to figure-ground modulations in V1 and V4 of the macaque monkey

Authors: *S. VAN STIJN[1], W. H. BARNES[1], W. SINGER[2,3], S. H. LEE[4,3];
[1]Ernst Strüngmann Inst. (ESI) In Cooperation With Max Planck Society, Frankfurt Am Main, Germany; [2]Max Planck Inst. for Brain Res., Frankfurt, Germany; [3]Ernst Strüngmann Inst. (ESI) In Cooperation With Max Planck Society, Frankfurt am Main, Germany; [4]Max Planck Inst. for Empirical Aesthetics, Frankfurt am Main, Germany

Abstract: An ongoing mystery of visual processing is how fragmented local visual information gets bound into a unified global percept. Feedback from higher order areas with larger receptive fields modulates incoming visual information already at the level of primary visual cortex. To further elucidate the mechanisms of figure-ground segregation, the current study investigates the neural dynamics (MUA and LFP) of macaque V1 and V4 in response to two figure-ground stimuli. We use chronically implanted electrode arrays (Graymatter Research) which allow us to sample from a large number of cells simultaneously with adjustable electrodes.
The first stimulus consists of an array of dense coherently rattling random dots. For a certain time period within a trial, a subset of dots confined to a circular area rattles out of sync to the global rattle, but with an equivalent magnitude. This creates the temporary percept of a pop-out disc while keeping local stimulus properties unchanged for the length of the trial.
The second stimulus consists of an array of randomly placed randomly oriented Gabor patches. At one time point in the trial, the Gabor patches are replotted with a subset of the patches forming either an open or a closed apparent figure. At the beginning of each trial, Gabor patches that will become part of the figure, or the background respectively, are placed over the RFs. These Gabor patches are kept unchanged during the trial. Thus, the effect of a global perceptual change can be assessed while the local stimulus features remain constant. To increase attention

to the figure, the monkey must report whether the figure is open or closed.
We observed an increase in spike rates when the RFs were part of the figure but only when there was a strong pop out (rattling disc) or figure directed attention (Gabor figure task). Furthermore, we observed a bifurcation in spike rates for the Gabor task in the first 100ms after the appearance of the figure. Units with RFs on the figure showed a significant increase in spike rate while units with RFs over elements of the background showed a decrease. In addition there was an increase in synchronous firing of the V1 neurons responding to elements of the figure.

Disclosures: **S. Van Stijn:** None. **W.H. Barnes:** None. **W. Singer:** None. **S.H. Lee:** None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

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Topic: D.06. Vision

Support: National Natural Science Foundation of China (31571078)

Title: The extra integration time window for illusory contour representation in macaque V2 and V4

Authors: ***Z. CHEN**[1,2], J. YIN[1], Y. LIU[1,2], Y. LU[1], X. LI[1], I. M. ANDOLINA[1], W. WANG[1]; [1]Inst. of Neuroscience, CAS, Shanghai, China; [2]Univ. of Chinese Acad. of Sci., Shanghai, China

Abstract: One distinct feature of the primate ventral stream is that neural response latencies monotonically increase accompanying the increasing complexity of encoded physical features and the size of neuronal receptive fields along the hierarchy (Rousselet et al., 2004; Kravitz et al., 2013; Yau, Pasupathy et al. 2013). However, it is less clear for neural representations of visual illusions. Previous studies of neural responses mediating the Kanizsa triangle suggest that this type of illusion is represented in higher visual areas and fedback to early visual cortices (De Weerd et al. 1996; Mendola et al. 1999; Ramsden et al. 2001; Lee & Nguyen 2001; Murray et al. 2002; Halgren et al. 2003; Stanley and Rubin 2003, Cox et al., 2013). Abutting-line illusions represent the simplest illusory contour (IC) whereby aligned local line-ends elicit a global perception of an illusory contour. We hypothesized that this type of IC is mediated through a feed-forward integration of local defining cues, and therefore would show a monotonic relationship along the visual hierarchy. To test this hypothesis, we studied neuronal response latencies for abutting-line IC stimuli of the same spatial scale from V1 to V4 using electrophysiological recording in awake macaque. We found that a longer integration time window is required for the representation of ICs when compared with the representation of real

physical lines or gratings. The representation of ICs takes on average 24ms of extra time than for real contours in V4 (79ms versus 55ms for IC and real contours, respectively). The latencies for real and illusory contours in V4 are significantly longer than in V2, where the extra time taken for the representation of ICs is about 14ms. The average visual response latency of V2 neurons for IC and real contours is about 62 and 48ms, respectively, which agree with re-measurements from the spike responses of V2 neurons from von der Heydt & Peterhans 1989. No neurons recorded in V1 signaled the presence of ICs due to the spatial scale of our stimuli. Thus our findings strongly support that the view that the neuronal representation of abutting-line ICs follows a serial feed-forward integration along the ventral stream, needing extra time for representation when compared with real contours.

Disclosures: **Z. Chen:** None. **J. Yin:** None. **Y. Liu:** None. **Y. Lu:** None. **X. Li:** None. **I.M. Andolina:** None. **W. Wang:** None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

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Topic: D.06. Vision

Support: Wellcome Trust (ref 105624) through the centre for chronic diseases and disorders (C2D2)

Title: Inter-ocular contrast normalization in the amblyopic visual cortex

Authors: ***B. RICHARD**, F. LYGO, D. H. BAKER;
Psychology, The Univ. of York, Heslington, United Kingdom

Abstract: Doubling the input to visual cortex by presenting stimuli to two eyes rather than one has little impact on the magnitude of neural responses (Moradi and Heeger, *JoV,* 9, 1-22). This is well explained by a normalization process whereby each eye contributes equally to the suppression of the other. This type of model can also explain psychophysical binocular vision deficits in individuals with amblyopia when the input from the amblyopic eye is attenuated prior to inter-ocular suppression (Baker et al., *Vis Res*, 48, 1625-1640). Importantly, the model defines the amblyopic binocular deficit as monocular attenuation without any abnormal suppression or structural changes to the binocular combination process. Here we measure neural signals, using both a Steady-State (SSVEPs) and a functional Magnetic Resonance Imaging (fMRI) method, to test the model assumptions in regards to attenuation and inter-ocular suppression in individuals with and without amblyopia. In both studies, stimuli were sinusoidal gratings with a spatial

frequency of 3 cycles/° at five different contrast values (0, 1.5, 6, 24 and 96%). Stimuli flickered (contrast modulation) at a rate of 4Hz and were presented monocularly, binocularly, and dichoptically (stimuli of different contrasts presented to each eye) to observers for trials of 12 seconds. Stimulus viewing conditions were identical in both the SSVEP and fMRI studies. Our control participants showed typical contrast response functions in the monocular viewing conditions, which increased monotonically as a function of stimulus contrast. Under binocular and dichoptic viewing conditions, contrast response functions showed evidence of sub-linear summation, a consequence of inter-ocular suppression. In participants with amblyopia, we observed a reduction in the response of the amblyopic eye, and a rightward shift of contrast response functions under dichoptic conditions. This pattern of results is consistent with monocular attenuation of signals in the amblyopic eye without any modification to inter-ocular suppression. This computational account of amblyopic deficits demonstrates how vision can become functionally monocular without requiring structural changes to binocular combination, or abnormal suppression.

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Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

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Topic: D.06. Vision

Support: EY10834

Title: Multiple mechanisms underlying color appearance and color naming

Authors: ***K. EMERY**[1], V. J. VOLBRECHT[3], D. H. PETERZELL[4], M. A. WEBSTER[2]; [1]Dept. of Psychology (0296), [2]Psychology, Univ. of Nevada, Reno, Reno, NV; [3]Psychology, Colorado State Univ., Fort Collins, CO; [4]Psychology, John. F. Kennedy Univ., San Francisco, CA

Abstract: The number and nature of the mechanisms underlying color appearance remain poorly understood. Standard models posit two opponent channels mediating red vs. green or blue vs. yellow sensations. However, a neural basis for this representation has yet to be identified. We took advantage of the large individual differences in color perception to analyze the processes underlying color percepts in color normal observers, using factor analysis to explore the patterns of variability in both hue scaling and hue naming. 30 observers were shown 36 hues of roughly equal saturation spanning steps of 10 deg around the standard cone-opponent space. The stimuli

were shown on a calibrated CRT as 2 deg uniform fields demarcated by black borders from an equiluminant (20 cd/m2) gray background. In the hue scaling task observers judged the component proportions of red, green, blue, or yellow. In the naming task they used unrestricted names or a fixed set of basic color terms to label each hue. A factor analysis of both data sets revealed multiple factors with each largely confined to a narrow range of contiguous hue angles. These were inconsistent with predictions for classical opponent channels varying in their tuning or relative sensitivity, which instead predict broad and bimodal patterns of factor loadings. The observed factors instead reveal that putatively opponent pairs (e.g. red and green) are constrained by independent processes, and moreover that binary hues (e.g. orange) vary independently of their constituent primaries (e.g. red and yellow). Our results suggest that different narrow angles of color space may each be governed by different mechanisms that can vary across observers, and these are manifest even in the standard hue scaling task that requires observers to perceptually decompose each hue into red-green and blue-yellow components. The pattern we observed is consistent with a cortical representation of color mediated by population coding in multiple "higher-order" mechanisms such that different hues reflect qualitatively different perceptual categories rather than metrical variations within a "neural" color space.

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Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 54.12/Z14

Topic: D.06. Vision

Support: JSPS KAKENHI Grant #15H05916

COI Program from JST

Title: Chromatic interaction profile in macaque area V4

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Abstract: Neurons in area V4 of the macaque monkey plays important roles in color vision. Although color selectivity of V4 neurons has been examined, most studies have measured selectivity to single color stimulus. Objects in natural scene typically have various colors on their

surfaces as well as texture, which provides important cue for our object recognition. Some studies have reported response modulation by surrounding color (Schein and Desimone 1990, Kusunoki et al., 2005), however, combinations of color stimuli were limited in these experiments and it is still unclear how V4 neurons respond to multiple colors presented on the receptive field (RF). In this study, we examined response profile to combinations of colors and tested if the chromatic interaction profile can be predicted by responses to single color stimuli. After identifying RF position/size by hand mapping, color selectivity was measured in conventional way by single color stimulus (16 colors). Then, chromatic interaction profile was measured by presenting color combination stimuli with center and annulus over the RF. Colors of center and annulus were randomly selected from 8 hues that evenly divided an iso-saturation circle in CIE-uv chromaticity diagram. For each neuron, we tested 64 color combinations (8x8 hues). In addition, responses to single color patch (either center only or annulus only) were tested (8 hues each, single patch conditions). We found that about 45% of the V4 neurons tested showed strong modulation of the responses due to color combination. Such response modulation to specific color combination was observed even in neurons that did not show significant color selectivity measured by single color stimulus. The chromatic interaction profile cannot be predicted by linear sum of responses in single patch conditions. Furthermore, in some neurons, such responses to specific color combination disappeared when colors were swapped between the center patch and annulus. This indicates that there is spatial configuration on the chromatic interactions. These results suggest that V4 neurons represent chromatic information in a manner more complex than previously thought. References:1. Schein SJ, Desimone R., Spectral properties of V4 neurons in the macaque., J Neurosci. 1990 Oct;10(10):3369-89. 2. Kusunoki M1, Moutoussis K, Zeki S., Effect of background colors on the tuning of color-selective cells in monkey area V4., J Neurophysiol. 2006 May;95(5):3047-59.

Disclosures: **T.M. Sanada:** None. **H. Komatsu:** None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 54.13/AA1

Topic: D.06. Vision

Title: Green face illusion

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Abstract: We have accidentally discovered a visual illusion; faces (and -seemingly- no other objects) appear greenish under the monochromatic yellow light of a sodium lamp. This is an unusual observation because under monochromatic sodium illumination there exist only one hue, yellow. Thus the greenish appearance of faces cannot be attributed to a retinal origin. We systematically measured the magnitude of this object-based color illusion and its selectivity for faces in comparison to other objects. 6 different objects, 9 Munsell chips, 3 human hands and 3 human faces were presented to the subjects (n=9, normal color vision, age 20-30) in two illumination conditions: sodium and white light. Additionally, to dissociate the effect of the monochromatic light on perceived skin tone from its potential interaction with facial (or hand) features, the whole face/hand, as well as a patch of the face/hand skin (the rest masked by a thick black cover) were presented in both conditions. For both illumination conditions, the test objects were presented in a random sequence and the subjects matched the color of a point on each object using a color-matching paradigm. The color matching display was a computer screen placed inside a black box to avoid contamination of the ambient illumination with the screen light, subjects looked at the screen through a small aperture. We then compared the color matching results with spectral measurements of the tested objects for both illumination conditions. Observed hues and matched hues were significantly correlated for the white light condition ($r>0.68$ $p<0.05$, all subjects) indicating the ability of subjects to perform color matching. This correlation went away when objects were viewed under monochromatic light ($r<0.19$, $p>0.067$). As expected, under the sodium light, the perceived hue for different stimuli was highly similar (CIE x=0.45, STD=0.14, CIE y=0.48, STD=0.12) and all stimuli were matched as different shades of yellow. However, perceived color of faces deviated from other objects under monochromatic yellow light and shifted towards greenish shades (CIE x=0.63, STD=0.11, CIE y=0.59, STD=0.09). This shift happened exclusively for faces and did not occur for other stimuli, it also did not happen for patches of face skin. Chromatic selectivity has been frequently documented throughout the ventral stream, thus shape and color information might be systematically entangled. Our results provide an example of interaction of color perception with shape processing, revealing existence of strong hue-priors for certain object classes (faces in this case).

Disclosures: **M. Hassantash:** None. **A. Afraz:** None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 54.14/AA2

Topic: D.06. Vision

Support: Allergan, Inc.

Title: Comparison of contrast sensitivity in human and non-human primates.

Authors: *W. RIDDER[1], A. KARSOLIA[1], K.-M. ZHANG[2], J. BURKE[2];
[1]SCCO, Marshall B. Ketchum Univ., Fullerton, CA; [2]Allergan, Inc, Irvine, CA

Abstract: **Purpose:** Non-human primates (NHPs) are frequently investigated to understand human diseases of the visual system. A common clinical measurement for many visual diseases is visual acuity. Visual acuity can be measured by employing psychophysical (e.g., the contrast sensitivity function) techniques in NHPs. The purpose of this investigation was to determine the relationship between acuities determined with similar psychophysical techniques in human and NHPs. **Methods:** Ten normal humans and 8 normal NHPs (Macaca fascicularis) took part in this project. The NHPs were placed in a primate chair in a sound isolating chamber. The NHPs were trained to hold and then release a lever when they detected a sine wave grating on the monitor. Threshold was operationally defined as two misses in a row for a descending method of limits (10 spatial frequencies from 0.3 to 20.2 cpd). A similar paradigm was used for the humans except that the descending method of limits was combined with a 2 AFC technique and the spatial frequency range was 0.75 - 18.50 cpd. The contrast sensitivity functions were fit with a double exponential function and acuity was determined by extrapolating the fit to a contrast sensitivity of 1. **Results:** The averaged peak contrast sensitivity, peak spatial frequency, and acuity for the humans were 279.6 ± 37.6 (Mean ± SE), 3.50 ± 0.32 cpd, and 27.3 ± 2.5 cpd and for the NHPs were 112.0 ± 14.0, 4.3 ± 0.45 cpd, and 28.7 ± 3.60 cpd. A two-sample t-test indicated that the peak contrast sensitivities were different ($p < 0.001$). The peak spatial frequencies ($p = 0.13$) and the extrapolated visual acuities ($p = 0.75$) were not different. **Conclusions:** The contrast sensitivity functions for the NHPs had lower peak contrast sensitivities than the humans. The peak spatial frequency and visual acuity extrapolations were the same for the humans and the NHPs. The contrast sensitivity differences and similarities between humans and NHPs need to be considered when using NHPs to study human disease.

Disclosures: **W. Ridder:** 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; Allergan, Inc.. **A. Karsolia:** None. **K. Zhang:** A. Employment/Salary (full or part-time): Allergan, Inc. **J. Burke:** A. Employment/Salary (full or part-time): Allergan, Inc..

Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 54.15/AA3

Topic: D.06. Vision

Support: Wellcome-DBT India Alliance Intermediate Fellowship to SR

Tata Trusts Grant to SR

Title: Stimulus dependence of gamma oscillations (20 - 70 Hz) in human EEG

Authors: *M. V. P. S. DINAVAHI[1], V. SHIRHATTI[2], P. RAVISHANKAR[1], S. RAY[1];
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Abstract: Stimulus characteristics of Cartesian gratings such as spatial frequency, size, contrast, orientation and drift speed have been shown to influence power, centre frequency, and temporal evolution of stimulus-sustained gamma oscillations in local field potentials (LFP) in animals, but how these characteristics influence gamma oscillations recorded in human electroencephalogram (EEG) is not well studied. Here we compared the stimulus dependence of gamma oscillations in EEG recorded from humans versus LFP recorded from the primary visual cortex of monkeys. We found that large Cartesian gratings centred on the fovea generated not one, as reported in previous studies, but two gamma oscillations in human EEG. Specifically, we observed a slower gamma at 20 - 40 Hz along with the traditional band at 40 - 70 Hz. Similar results were observed in monkey LFP recordings as well. The low gamma band was observed in two-thirds of the subjects studied, and about half of the subjects showed at least 1 dB increase in power from the baseline in both the low and the traditional gamma band for the same stimulus set. Also, bipolar reference scheme used in this study for analysis of data revealed strong lateralisation of both the gamma bands in most subjects. The low gamma band showed similar tuning characteristics as the traditional gamma oscillations. In general, both the gamma bands showed a sharp spatial frequency tuning around 2 - 4 CPD (cycles per degree). The power in both the bands increased with increase in size and contrast of the grating. It was maximum for stationary gratings and decreased with increase in the speed of drifting gratings. Similar results were obtained in comparable recordings in the LFP recorded from the monkey. In EEG recordings, both gamma bands showed weak or no tuning to orientation, while tuning was much stronger in monkey LFP recordings with low and traditional gamma bands tuned to different orientations.
Two gamma rhythms can potentially provide better coding or communication mechanisms, more flexible and robust signal for brain machine interfacing applications, and a more comprehensive biomarker for diagnosis of mental disorders such as Alzheimer's disease.

Disclosures: **M.V.P.S. Dinavahi:** None. **V. Shirhatti:** None. **P. Ravishankar:** None. **S. Ray:** None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

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Topic: D.06. Vision

Support: EY020679

EY07556

EY13312

Title: Powerful visual illusion of light/dark perception in human faces

Authors: ***R. BACHY**, J. ALONSO, Q. ZAIDI;
GCVR, SUNY Optometry, New York, NY

Abstract: We present a striking new lightness illusion in face perception. When the grey-level image of a face is half-wave rectified into images above and below the 50^{th} percentile of the luminance histogram, the two images have similar features but non-overlapping grey levels except for the mid-grey. Remarkably, the two faces appear to have widely overlapping ranges of lightness, and we discovered to our surprise that the face with the higher luminance values appears much darker. We quantified this observation in two ways. Images of a face digitally modified to 4 different skin shades from fair to dark, were rectified into high and low luminance versions. Observers who were asked to arrange the 8 faces by perceived skin color from lightest to darkest, ranked every face with the higher luminance as darker than its lower luminance pair. Observers also matched the grey level of a small disk in white noise to the perceived lightness of 8 features of face images in original, high and low versions. The range of matched brightness of the rectified images was shifted and expanded from 50% of the original image to over 90% in some cases. To explain the illusion, a model of time-varying brightness induction was modified for the static case. The model first applies a punctate gain, controlled by local adaptation, to each point in the image, which compresses the luminance range and shifts it lower, simulating photoreceptor responses. Then brightness induction signals from all surrounding points are added for each point. The induction from each surrounding point is calculated as the signed difference between the receptor signals at the two points, gain controlled by the absolute difference between the signals, and weighted by an exponentially decreasing function of the spatial distance. The

parameters controlling the luminance and induction gains, and the space constant of the spatial weighting, were adjusted to fit the data. The model explained the illusion by reproducing the perceived brightness in both high and low luminance images. The luminance-based gain-control reduced the effect of absolute luminance. The induced effect of surrounding points overcame the resulting range contraction, and made perceived lightness largely a function of the surrounding brightness, with the effect of gradual luminance gradients being enhanced by the induction gain. Our illusion also affects the perceived lightness of animal and object surfaces in natural images, thus revealing that neural mechanisms generically expand perceived brightness and enhance the effect of luminance gradients over absolute luminance. Our model shows that local adaptation and surround induction can function as these mechanisms.

Disclosures: **R. Bachy:** None. **J. Alonso:** None. **Q. Zaidi:** None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 54.17/AA5

Topic: D.06. Vision

Title: Attention and amodal completion affect metacontrast masking.

Authors: *V. S. RAMACHANDRAN[1], M. VAJANAPHANICH[2], C. CHUNHARAS[2,3]; [1]UCSD, La Jolla, CA; [2]UCSD, San Diego, CA; [3]Med., Chulalongkorn University, KCMH, Bangkok, Thailand

Abstract: If a square patch of grating target, 1.5 degree, is flashed for 75 ms followed by flanking gratings (mask) flashed for another 75 ms; the target becomes invisible (metacontrast). We cycled the target-mask pair in a continuous cycle with inter-pair interval being 400 ms. The target remained continuously invisible. The degree of masking was measured by having the subject discriminate the orientation of the target grating. Remarkably, when the subject reached out with his index finger to “touch” the invisible target, the target became instantly visible. The effect could be produced using a tiny photo of a finger superposed on the target even though one wouldn’t ordinarily expect continually visible features to interfere with the masking. The photo by itself or pointing behind the computer screen was not quite as effective suggesting a contribution from proprioception, attention, and/or re-afference. We also noticed that if several target-mask pairs were displayed simultaneously on the screen the single unmasked one “popped out”. Next experiment, we asked whether amodal-completed rectangle could be masked by non-occluded flanking rectangles. We found the masking did occurred compare to the control condition of fragmented target without occluder. This demonstrated the image segmentation

based on implied occlusion occurred prior to metacontrast masking. Taken collectively, our experiments demonstrate a striking modulation of metacontrast masking by visual attention, segmentation and proprioception.

Disclosures: V.S. Ramachandran: None. **M. Vajanaphanich:** None. **C. Chunharas:** None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

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Program#/Poster#: 54.18/AA6

Topic: D.06. Vision

Support: U.S. Department of Defense Psychological Health and Traumatic Brain Injury Research Program W81XWH-14-1-0320

Title: Monkeys see snakes like humans do: A comparative analysis of internal noise and efficiency in contour integration

Authors: *A. ZHANG[1], P. KHAYAT[1,2], H. AKHAVEIN[1], A. BALDWIN[1], R. HESS[1], R. FARIVAR-MOHSENI[1];
[1]Ophthalmology, McGill Univ. Hlth. Ctr., Montreal, QC, Canada; [2]Sch. of Optometry, Univ. of Montreal, Montreal, QC, Canada

Abstract: The visual system's ability to detect contours, even in the presence of discontinuities, is essential for interpreting everyday scenes. Previous studies have shown that both humans and monkeys are equally proficient on tasks where they are required to detect a contour embedded in a noisy background. However, it is not clear whether the underlying neural process is similar between species. We designed a novel task that allows us to determine the efficiency and level of internal noise governing contour perception. Internal noise reflects the quality of the visual information received by the brain while efficiency is how well the brain uses that information. Through these measures, we compared how contours are internally represented and processed between species. In our task, contours were presented with external noise added by adjusting the orientation of Gabor elements such that there was no longer good continuation between the elements. On each trial, contours had different degrees of curvatures or degrees of noise. Three macaques were trained to saccade to the contour with better continuation in a two-alternate forced choice task. Psychometric performance as a function of curvature was obtained for each noise level, showing that detection threshold increases with increasing external noise. The thresholds for each condition and subject were then fitted with a Linear Amplifier Model that calculates internal noise and performance efficiency as a function of external noise. At

decreasing external noise, thresholds are increasingly determined by internal noise, so the limit of the fitted function allows us to determine levels of internal noise alone. Macaque data was then compared to human subjects on an analogous task. The results show that contour integration efficiency and internal noise is similar in monkeys and humans. Our findings are indicative that not only do monkeys perform equally well on a contour detection task, contours are internally represented and processed in a similar manner. This evidence suggests that humans and monkeys have a common framework for contour perception and therefore may share an underlying neuronal mechanism for visual integration.

Disclosures: **A. Zhang:** None. **P. Khayat:** None. **H. Akhavein:** None. **A. Baldwin:** None. **R. Hess:** None. **R. Farivar-Mohseni:** None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 54.19/AA7

Topic: D.08. Visual Sensory-motor Processing

Title: Which side of the face is more sensitive to enfacement illusion?

Authors: *E. GÜLBETEKIN;
Dept. of Psychology, Akdeniz Univ., Antalya, Turkey

Abstract: “The experience of observing oneself reflected in the mirror involves the integration of motor, proprioceptive, tactile and visual cues, as every touch on one’s face is mirrored by a compatible visual event” (Tajadura-Jiménez, Lorusso and Tsakiris, 2013). A mirror-like effect can be induced by another face during the‘‘enfacement’’ illusion paradigm. Research on enfacement illusion indicated that this experience affects one’s self face perception. The aim of the experiment is to find out which side of the face is more sensitive for enfacement illusion.
Method
Subjects: Twenty eight male undergraduate students (M = 21.28years, SD = ±1.82) participated the study.
Stimuli: A video of a male model was recorded while his face was stroked with a cotton bud (Fig1, Fig2).

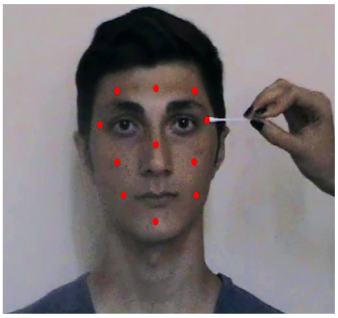

Figure1. 11 points were touched in both the synchronous and asynchronous sessions.

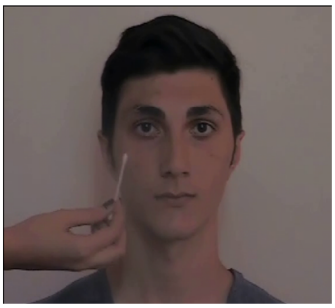

Figure 2. The right side and the left side of the face around cheeck were touched linearly for three times in both the synchronous and asynchronous sessions.

1. While the other person's face was touched I seemed to feel the touch on my own face.
2. It seemed that the touch I felt on my face was caused by the cotton swab touching the other person's face.
3. It seemed that the other person's face was mine.
4. It seemed that the other person's face was part of my body.
5. It seemed that the other person's face belonged to me.
6. I seemed to see my face reflected in a mirror rather than the other person's face.
7. It seemed that the shape of the other person's face began to resemble mine.
8. It seemed that the skin color of the other face began to resemble mine.
9. It seemed that the features of the other person's face began to resemble mine.
10. It seemed that the other person's face would move if I moved.

Strongly Diagree						Strongly Agree
-3	-2	-1	0	1	2	3

Procedure

The participants were asked to watch the video clips from a viewing distance of 150 cm. A synchronous and asynchronous sessions were applied. While watching the video, participants were touched on their face at the same time and the same locations with the video in the synchronous sesions. However, there was a delay between the strokes on the two faces in the asynchronous sessions. After watching the video clips, 10 enfacement questions were asked to the subjects. An additional question was asked to asses on which side of the face a stronger stimulation was sensed while the viewing of the video clips. The order of stimulation type and side were counterbalanced between subjects.

Results

Mean responses for each of the 10 questions were calculated. Wilcoxon signed-rank tests were conducted. The subjects gave higher ratings of agreement to 7 of the 10 illusion questions items after synchronous relative to asynchronous sessions, showing that synchronous stimulation could successfully induce the illusion. Chi-square test indicated that there was a significant difference among face side for enfacement effect X^2 (2, 28) = 17.64, p =.001. The results indicated that the subjects experienced stronger enfacement effect when the right side of the face was stimulated.

Disclosures: E. Gülbetekin: None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 54.20/AA8

Topic: D.06. Vision

Support: Riken Brain Sciences Institute, Visiting Scientist Support

Bournemouth University Research Support

Title: Repulsion of perceived visual motion direction as an emergent property of deciding to unify or segregate sources

Authors: *A. I. MESO[1,2,3], K. HARUHANA[3], G. S. MASSON[2], J. L. GARDNER[4];
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Abstract: To understand dynamic scenes, humans must infer which motion signals should be unified, that is, labelled as coming from the same object, and which should be segregated into signals from different objects. In the face of ambiguities, this underlying computational task of unclassified separation (separating mixed signals with unknown labels) is not trivial and solutions may engender perceptual consequences. We studied the case of motion transparency: simultaneous perception of two directions within the same region in space; and motion repulsion, the phenomenon in which perceived separation between transparent directions is wider than physical separation. In a psychophysical task, six participants made direction comparisons of components of two large random dot stimuli. We varied dot appearance and measured small changes in repulsion size. Judgements were made on controlled simultaneous presentations of two stimuli in the left and right visual field. Cardinal and oblique motion directions were deliberately excluded to avoid known reference attraction biases. Precision of single direction estimates of 0.5-3° were measured across individuals, consistent with a fine-grain sensory representation of individual components. Under transparent presentation, repulsion values of between 4-8 degrees were measured, consistently stronger at 30 than at 60 degrees of separation. Ambiguity in local direction introduced by appearance changes resulted in stronger repulsion. The pattern of shifts in repulsion suggests that the probabilistic component neural representations were consistently pushed away from the average during transparency perception. Incorporating experiment insights, the underlying separation task was modelled using a hierarchical two step Bayesian formulation. In the first step, an initial estimate was made to determine whether a motion signal comes from a unified source or whether there are two segregated sources based on the spread of direction signals. In the second step, this number of source estimates acts as a prior which for two sources inhibits the average direction and shifts the posterior distribution for the

two directions of motion away from each other, thus resulting in motion repulsion. We suggest that determining whether to unify or segregate motion signals could explain motion repulsion effects and that this is just one example of a consequence of such a computation that may appear in other neural processing contexts.

Disclosures: A.I. Meso: None. **K. Haruhana:** None. **G.S. Masson:** None. **J.L. Gardner:** None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

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Program#/Poster#: 54.21/AA9

Topic: D.06. Vision

Support: Academy of Neuroscience for Architecture

Title: Solid field of visibility: first model and test

Authors: *S. GEPSHTEIN[1], G. LYNN[2], A. MCDOWELL[3];
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Abstract: Visual systems are unequally sensitive to different features of the world. One characteristic of the unequal sensitivity is the contrast sensitivity function (CSF) commonly measured with periodic stimuli, such as luminance gratings, viewed over a fixed distance. For a fixed viewing distance, the CSF describes how different amounts of luminance contrast are required to make the stimulus just visible for different spatial frequencies of luminance modulation in the stimulus. When the spatial frequency is fixed but the viewing distance is varied, a similar profile of contrast sensitivity is expected as a function of viewing distance. Using this method, we predict the range of viewing distances over which the stimulus is visible in any direction. A complex optical environment that contains multiple patterns can be described in terms of multiple corresponding solid regions of visibility that may overlap or nest in one another. This structure is usefully summarized as a 'solid field of visibility' in which the value of visibility varies smoothly across location. This description constitutes a sensory counterpart of the light field, which is a radiometric description of the amount of light flowing in every direction through every point in space. We derived a simplified model of the solid field of visibility using an established characterization of human spatiotemporal CSF (Kelly, 1979). We tested the model using large-scale robotics at the UCLA Architectural Robotics Laboratory. A large screen and a projector were mounted on the end arms of two computer-controlled industrial

robots, capable of moving synchronously along linear tracks. An integrated custom-built suite of hardware and software coordinated the positions of robots and presented visual stimuli on the screen. Observers were seated between the tracks and performed the tasks of detection and direction discrimination by means of a wireless computer mouse. First, we confirmed that visibility of static stimuli was confined to the solid spatial regions predicted by the model. Second, the boundaries of visibility were tested for stimuli projected on moving screens. Visibility was found to change across location as predicted by the model, sometimes changing from zero to full visibility over a distance of half a meter, indicating a sharp boundary of the solid field of visibility. Such abrupt spatial transitions of visibility have immediate applications in design of immersive environments: fully virtual and mixed. Knowing the solid field of visibility of an environment allows one to selectively enlarge or diminish the solid regions of visibility for specific parts of the environment and thus control user engagement.

Disclosures: **S. Gepshtein:** None. **G. Lynn:** None. **A. McDowell:** None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

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Topic: D.06. Vision

Support: ERC Starting Grant 311435

Title: Modeling orientation preference in the apical and basal trees of L2/3 V1 neurons

Authors: A. PAPOUTSI[1], J. PARK[2], S. M. SMIRNAKIS[3], *P. POIRAZI[1];
[1]IMBB-FORTH, Heraklion, Crete, Greece; [2]Baylor Col. of Med., Houston, TX; [3]Harvard Med. Sch., Boston, MA

Abstract: Pyramidal neurons receive inputs in two anatomically and functional distinct domains, the apical and the basal tree. Inputs to the basal tree, due to their proximity to the soma, greatly influence neuronal output, whereas the more remote apical tree has less potential to influence somatic activity. How these inputs co-operate to form the functional output of the neurons is currently unknown. In this work we focused on how inputs to the apical and basal trees shape orientation tuning in L2/3 V1 neurons. In particular, we investigated how dendritic integration of orientation tuned inputs to the apical versus basal trees allows for the emergence of stable neuronal orientation preference. Towards this goal a model L2/3 V1 pyramidal neuron was implemented in the NEURON simulation environment. The passive and active properties of the model neuron were extensively validated against experimental data. Synaptic properties, number

and distribution were also constrained according to available data. Using this model neuron we investigated a) the differences in the mean orientation preferences of the two trees and b) the distribution of orientation preferences to individual synapses that allow for the emergence of orientation tuning. In addition, we further identified how apical versus basal dendritic tree ablation would affect neuronal tuning in the different conditions implemented. Model results provide insights regarding the 'tolerance' to different input properties at the apical and basal tree in order to achieve stable orientation preference.

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Poster

054. Mechanisms of Color, Contrast, and Form Perception

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Topic: D.06. Vision

Support: W81XWH-14-1-0320

Title: Cortical mechanisms of stereopsis vision investigated using naturalistic stimuli and magnetoencephalography

Authors: ***Y. CHEN**[1], A. DEHMOOBADSHARIFABADI[1], E. BOCK[2], S. BAILLET[2], R. FARIVAR-MOHSENI[1];
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Abstract: Binocular disparity enhances human visual perception in natural scenes. Previous fMRI studies (Hasson, et al, 2004) have shown that natural viewing of a movie induces inter-subject correlation (ISC) of cortical responses, which has been suggested to increase when stereoscopic depth is added to the movie (Gaebler, et al., 2014). We raise two inter-related questions using an MEG approach in the presence of depth information in the movie stimulus: what is the frequency profile of ISC, and how disparity does addition of disparity affect ISC? 27 subjects were recruited to watch two clips from the movie 'Under the Sea' in both 2-D and 3-D version with central fixation. Eye blinks and cardiac activities were removed by signal-space projection in brainstorm (Tadel F, et al, 2011). Screen noise components were removed using independent component analysis. Brain sources were estimated by minimum norm methods. Hilbert transformation for the brain source response was performed in 6 difference bands: delta (2-4Hz), theta (5-7Hz), alpha (8-12Hz), beta (15-29Hz), low gamma (30-59Hz), and high gamma (60-90Hz). The transformed data were then low-pass filtered to 4Hz to emphasize the low

oscillation pattern for the high frequency amplitude. Comparing 3-D movie viewing condition with 2-D movie viewing condition, we observed greater ISC in the ventral visual areas (fusiform and inferior temporal) in the low gamma band in the left hemisphere. Thus our results support the claim that addition of binocular disparity enhances some aspects of ISC, but that these aspects are highly constrained to late-stage visual areas and to the high frequency range of cortical activity. An additional analysis of the relation between oscillatory activity and depth vs. luminance contrast will be presented.

Disclosures: **Y. Chen:** None. **A. Dehmoobadsharifabadi:** None. **E. Bock:** None. **S. Baillet:** None. **R. Farivar-Mohseni:** None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 54.24/AA12

Topic: D.06. Vision

Support: NIH Grant DP1 NS082121-02

Title: Color signal processing in the larval zebrafish brain

Authors: ***D. A. GUGGIANA-NILO**, F. ENGERT;
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Abstract: Light in the ultraviolet region is invisible to humans, but is ubiquitous in nature as it is one of the components of sunlight. This range of the electromagnetic spectrum is of particular importance to organisms in contact with it, given its higher energy per photon and concomitant potential to generate cellular damage and trigger mutations in DNA. The ability to detect these low wavelengths is present in a variety of organisms, ranging from mammals to insects. One of such organisms is the larval zebrafish (Danio rerio). This small teleost possesses 4 types of cone photoreceptors that can detect wavelengths from the red to the UV and are active as early as 4 days post fertilization (dpf). Additionally, this organism is a newly encumbered model for a variety of fields in biology such as development, pharmacology and neuroscience. This is due to a combination of well-described genetics, rich behavioral and physiological repertoire and optical transparency. In the past we have shown that larvae avoid UV light in an intensity dependent manner and that UV light is able to overcome phototactic behavior in freely swimming animals. In the current work we monitored the neural responses elicited by UV light in a tethered animal preparation. For this we relied on a custom built 2 photon microscope to

capture calcium spike signals from RGC terminals and the Optic Tectum of the animal, and we correlated these responses with the behavioral output of the larva during stimulation.

Disclosures: D.A. Guggiana-Nilo: None. **F. Engert:** None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 54.25/AA13

Topic: D.06. Vision

Support: JSPS KAKENHI 26280047

RIEC H25/A09

Title: Figure-ground discrimination by a population of V4 cells.

Authors: *K. SAKAI[1], M. HASUIKE[2], D. MINOWA[2], Y. YAMANE[3], H. TAMURA[3]; [2]Comp. Sci., [1]Univ. Tsukuba, Tsukuba, Japan; [3]Frontier Biosci., Osaka Univ., Suita, Japan

Abstract: Figure-ground (FG) segregation, a process to separate an object from background in a visual scene, is a crucial step toward object recognition in the visual cortex. Physiological studies on monkeys have reported that V4 cells appear to be sensitive to FG organization [Nat. Neurosci. 5, 1332-1338, 2002; Neuron, 75, 143-156, 2012] although their selectivity to FG in natural images has not been clarified. We investigated whether the intermediate-level visual areas represent information capable of determining FG in natural image patches. We recorded multiple single unit activities from V4 of two analgesized and immobilized macaque monkeys (Macaca fuscata) using 32 channel array electrodes. Eight hundred forty patches that were sampled from natural images with the constrains of diversity [Front. Psych., Article 1685, 2015] were presented for ten times. For a half of the patches, the objects that were therein were filled with black and white. The recordings included the responses from 1725 cells with their classical receptive field (CRF) center located within the patches.
First, we analyzed whether the responses of single cells in monkey V4 depend on FG organization of stimuli with respect to their CRF center. A two-way ANOVA with the factors of FG and contrast at the CRF center showed that 20% (351/1725) of cells showed the significance to FG ($p<0.05$), 30% to contrast, and 12% to the interaction. The mean FG-discrimination rate of the FG-significant cells was 51% that was barely above the chance. These results indicate that a part of cells in monkey V4 show the responses that depend on FG at their CRF center. However, these single cells are hardly capable of discriminating FG correctly for the natural image and

filled patches.
Second, we investigated computationally whether an integration of the responses of multiple cells enables the correct discrimination of FG for the stimulus patches. We used support vector machine (SVM) as an ideal machine for the integration of the responses of multiple cells. If the machine was capable of segregating correct FG from the cellular responses, it indicates that the responses of the cells included the sufficient information for judging FG. Our simulations showed that the responses of fifty V4 cells were capable of achieving 66% correct in the FG discrimination of the natural image and filled patches, suggesting the distributed population representation of FG in the intermediate-level visual area.

Disclosures: **K. Sakai:** None. **M. Hasuike:** None. **D. Minowa:** None. **Y. Yamane:** None. **H. Tamura:** None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

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Topic: D.06. Vision

Support: BMBF grant 01GQ1004A

Munich Graduate School of Systemic Neurosciences (GSN)

Title: Population coding model of color in visual cortex

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Abstract: Color information in the retino-cortical pathways is encoded by cone-opponency. The link of these physiological signals to perception is still unclear. Neither do the cone-opponent signals correspond to perceptually pure hues, nor is the color space defined by these signals perceptually uniform. Various properties of color perception, such as color discrimination and color induction, indicate a non-uniformity of the cone-opponent color space that corresponds to an expansion along the oblique blue-yellow axis. Here we present a model for the cortical encoding of color based on a population code that accounts for this perceptual non-uniformity. The model assumes that stimulus hue is encoded by a population of neurons with bell-shaped color tuning and color preferences distributed in color space. The model further assumes modulatory interactions between neurons with similar preferences, which results in the

prediction of chromatic induction for stimuli in chromatic surrounds. Tuning parameters of the model neurons were adjusted by fitting the predicted induction strengths to empirically determined color shifts for different chromatic surrounds. Fitting was constrained by the assumption of an expansion of color space along a single, adjustable axis. The resulting orientation in color space of the expansion axis fell close to the perceptual blue-yellow line. Compressing the empirical induction data along the expansion axis, i.e., compensating for the predicted non-uniformity of color space, reduced the variability between the induction curves. Small residual differences, which depended on stimulus size, remained for some directions in color space, suggesting an additional direct effect of the center-surround opponency of midget cells. The resulting population code was non-uniform, according to the color space non-uniformity, with tuning widths varying by ±6% around an average width of 44 degrees. The results indicate that a population code for color that incorporates the non-uniformity of cone-opponent color space can account for color induction effects observed in humans.

Disclosures: T. Wachtler: None. **C. Kellner:** None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 54.27/AA15

Topic: D.06. Vision

Title: Chromatic isolumance assessment using pupillary oscillations.

Authors: *P. M. DAYE[1], P. CAVANAGH[2], J. LORENCEAU[3], P. POUGET[4];
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Abstract: Most of the current methods used to assess differences of chromatic equiluminance rely on human perceptual reports or on optokinetic responses to moving colored stimuli (e.g. Cavanagh et al. 1984; Chaudhuri and Albright, 1990). However, all these approaches require some minimum amount of training and cooperation from the participants. Here we report a novel Pupil Frequency Tagging method (PFTM), where oscillatory changes in relative stimuli brightness over time are mirrored by pupil constrictions and dilatations.
PFTM was apply to 10 human participants during 5 sessions of 6 minutes. We computed the spectral density of the pupil response and analyzed signal amplitude at the tagging frequency. As predicted, our results showed that the luminance-induced pupil oscillations are reduced at

equiluminance.
These results suggest that the amplitudes of pupil responses at the tagging frequency closely follow the variation of luminance and therefore provide access to the coding of stimulus luminance without any participant training. PFTM gives us a new opportunity to study chromatic luminance not only in cooperative adult human subjects but also in very young infants, or animals with minimal constraints on training, movement artifacts and length of the recordings sessions.

Disclosures: **P.M. Daye:** None. **P. Cavanagh:** None. **J. Lorenceau:** None. **P. Pouget:** None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

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Topic: D.06. Vision

Support: Blythedale Children's Hospital

The Thomas & Agnes Carvel Foundation

Title: OptokineSys: an automated method of quantifying visual function, even in people who cannot follow commands

Authors: ***J. HILL**[1,2,3], M. SUNER[1,2], J. B. CARMEL[1,2,3], G. T. PRUSKY[1,2,3];
[1]Burke Med. Res. Inst., White Plains, NY; [2]Blythedale Children's Hosp., Valhalla, NY; [3]Weill Cornell Med., New York, NY

Abstract: Brain injury may lead to cerebral visual impairment (CVI), a leading cause of disability in children. Diagnosing CVI is difficult, as brain injury often also leads to cognitive impairments that preclude traditional vision assessment methods. To address this, we developed an automated system to measure vision based on the smooth tracking of moving visual stimuli. Our system, called OptokineSys, moves visual stimuli smoothly across a large computer screen while an eye-tracker measures gaze position. Our algorithm determines in real time whether the eyes smoothly follow stimulus movement. It rewards smooth tracking by playing music of the subject's or caregiver's choice, to motivate continued engagement with the task. The algorithm tolerates reverse saccades, catch-up saccades and changes of direction, but interruptions longer than these will cause the system to pause the music. Smooth tracking in the correct direction and approximately correct speed is evidence that the subject can see the stimulus. An adaptive procedure uses this evidence to alter contrast and spatial frequency and determine a threshold of

visuomotor function. Our use of OptokineSys as a vision assessment tool is based on the principle that, given intact smooth pursuit, these tracking thresholds reflect the subjects' spatial vision.
We used OptokineSys to vary the contrast of a band-limited visual noise stimulus, to measure contrast thresholds as a function of spatial frequency. We also adapted spatial frequency at a fixed contrast level, to obtain spatial frequency thresholds as a single acuity measure. In healthy adult subjects, we found:(1) that the system worked robustly and without needing per-subject calibration; (2) that the resulting contrast sensitivity functions had an inverted-U shape, as expected of a true measure of spatial vision; (3) that contrast sensitivity measures were highly repeatable within and across subjects; (4) that spatial-frequency thresholds were well correlated with eye-chart acuity (LogMAR). In children with brain injury, we found (5) that OptokineSys engages children even when they are unable to follow verbal instructions, and (6) that it provides objective information about visual function, including repeatable spatial-frequency thresholds, even in children who cannot communicate and hence cannot otherwise be measured.
We conclude that our system is a promising tool for assessing spatial vision, even for people who cannot follow commands. Efficient, objective measurement of visual thresholds in people with brain injury should enable future studies to determine incidence, natural history, and treatment of CVI.

Disclosures: **J. Hill:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent applied for: OptokineSys: A Method for Automatic Real-Time Detection of Smooth Eye Movements (application #62/185,983b, 2015). **M. Suner:** None. **J.B. Carmel:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent applied for: OptokineSys: A Method for Automatic Real-Time Detection of Smooth Eye Movements (application #62/185,983b, 2015). **G.T. Prusky:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cerebral Mechanics, Inc., Patent applied for: OptokineSys: A Method for Automatic Real-Time Detection of Smooth Eye Movements (application #62/185,983b, 2015).

Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

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Program#/Poster#: 54.29/AA17

Topic: D.06. Vision

Support: NIH NEI Grant R01EY018839

NSF CRCNS Grant IIS-1309725

NSF GRFP DGE-1256082

Title: Advancing models of shape representation for mid-level vision

Authors: *D. V. POPOVKINA, W. BAIR, A. PASUPATHY;
Biol. Structure, Univ. of Washington, Seattle, WA

Abstract: Visual area V4 is a critical part of the neural pathway underlying object recognition in primates. Our goal is to develop models that explain V4 responses to visual stimuli and thereby elucidate the computations supporting mid-level form processing in the visual cortex.
The current best-performing models of form representation in V4 (e.g. Cadieu *et al*., 2007) emphasize oriented features at the object boundary while disregarding surface attributes such as boundary phase information. Specifically, these contour-template models make the clear prediction that simple, filled 2D shape stimuli should evoke responses very similar to those evoked by only the outlines of the same shapes. However, past experiments in V4 have not explicitly tested the effect of surface fill on shape-selective responses.
We recorded responses from 43 single, well-isolated V4 units in awake, fixating macaques using a large battery of simple shapes (Pasupathy & Connor, 2001; 2002) that were presented either in filled or outline form. Surprisingly, we found that only 8/43 neurons (19%) maintained tuning for filled and outline shapes that was consistent with the predictions of the contour-template model. 19/43 neurons (44%) exhibited strong and selective responses only to filled shapes and 6/43 neurons (14%) only to outline shapes; tuning was poor or absent for the other category of stimuli. The remaining ~23% of neurons responded well to both stimulus types but responses were poorly correlated between types.
To improve the contour-template model to capture these physiological observations, we first verified that differences in spatial frequency content between filled and outline stimuli alone were insufficient to predict response differences. Next, we focused on the early stages of the model hierarchy and tested two ways to retain previously discarded boundary phase information. We allowed early inputs to be phase-sensitive rather than combining phases; alternatively, we added unoriented units to the early phase-pooled inputs. Both approaches explained the data better than the original model for most neurons, with greater improvement for those neurons that responded best to one or the other class of stimuli. Thus, our modifications allowed the model to distinguish filled and outline stimuli without disturbing the form-selectivity architecture.
Together, our results suggest that boundary orientation and surface information are both maintained until at least the mid-level visual representation. This finding supports the idea that in addition to their role in shape processing, V4 neurons can participate in scene segmentation tasks requiring information about object surface.

Disclosures: **D.V. Popovkina:** None. **W. Bair:** None. **A. Pasupathy:** None.

Poster

054. Mechanisms of Color, Contrast, and Form Perception

Location: Halls B-H

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Program#/Poster#: 54.30/AA18

Topic: D.06. Vision

Support: TUBITAK Grant 113K210

Title: Behavioral and neural correlates of the effect of context-dependent lightness on threshold and suprathreshold contrast perception

Authors: *H. BOYACI[1,2], Z. PAMIR[1];
[1]Bilkent Univ., Ankara, Turkey; [2]Giessen Univ., Giessen, Germany

Abstract: Contrast is an important feature for visual performance on many tasks such as object identification, speed or motion detection (Kilpeläinen, Nurminen, & Donner, 2011). Perceived contrast of a pattern depends on the luminance of its background. On the other hand, context often causes a large perceived difference between equiluminant regions (e.g. simultaneous brightness contrast). Thus, characterizing different effects of luminance- and context-dependent lightness on contrast is critical. In this study, we investigate how context-dependent lightness affects contrast judgement using a variant of Adelson's checkerboard illusion stimulus (Adelson, 1995), and psychophysical and fMRI methods. Two series of behavioral experiments were conducted. First, we measured perceived contrast of rectified gratings with incremental and decremental suprathreshold gratings superimposed on equiluminant backgrounds which appeared different in lightness (context-dependent lightness). Participants adjusted the contrast of an external matching grating to match that of the test grating for different levels of frequency, luminance and contrast. Results showed that perceived contrast of gratings superimposed on the perceptually lighter background was higher than those superimposed on the darker background (N=6). However, this pattern was present only for incremental, not for decremental contrast. Second, we measured the contrast detection threshold using an adaptive two alternative forced-choice (2-IFC) procedure. The detection threshold was lower for the gratings superimposed on equiluminant but perceptually brighter target regions (N=6). Thus, we found that not only appearance judgment but also contrast detection threshold is affected by context-dependent lightness. These findings indicate that context-dependent lightness of the target region, not only its luminance, influences the perceived contrast of gratings both at threshold and suprathreshold levels. Next, in an fMRI study we investigated neuronal correlates of this perceptual effect (N=9). Using the same contextual stimulus, we superimposed flickering gratings on equiluminant patches that appeared different in lightness. We found that, parallel to the behavioral results, the BOLD signal in V1 was greater when the grating was superimposed on the perceived-lighter patch. Our findings unveil the significant effect of context-dependent

lightness on contrast perception, thereby provide evidence that contrast perception does not depend only on the contrast of the image formed on the retina, and that higher level context-dependent factors also play a role.

Disclosures: H. Boyaci: None. **Z. Pamir:** None.

Poster

055. Eye Movements I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 55.01/BB1

Topic: E.01. Eye Movements

Title: Scotopic fixation eye movements dependence on eye position: separate visual modes?

Authors: *O. SPIVAK[1], P. THIER[2], S. BARASH[3];
[1]IMPRS For Cognitive and Systems Neurosci., Tuebingen, Germany; [2]Cognitive Neurol., Hertie Inst. for Cinical Brain Res., Tuebingen, Germany; [3]Dept. of Neurobio., Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Last year we presented evidence that, in scotopic conditions, fixation eye movements differ from those in photopic conditions. Scotopic fixations comprise larger but less frequent saccades than photopic fixations. In mesopic fixations, scotopic-like and photopic-like fixation movements are combined, with their relative frequencies contingent on the ambient light intensity. Fixations made with dark background are marked by a shift of gaze direction: the eyes seem to be directed above the target, so that the target's image may fall off the fovea. These results indicate that fixation eye movements reflect different retinal focus regions. In photopic vision, frequent small fixation saccades keep the target's image on the fovea. The larger scotopic fixation saccades might reflect the larger rod-dense region in superior retina, most sensitive in scotopic conditions. The upshift reflects the use of the superior retina instead of the fovea. These observations might solve the paradox, of why there are saccades and visual fixations in the dark, even though the foveal cone receptors are inactive. The tentative solution is that these are indeed the superior retina rods that are activated by the target's image, not the foveal cones. Here we analyze the dependence of the upshift and of the fixation movement parameters on the position fixated. Preliminary analysis shows that the upshift is largest while the eyes fixate positions on the bottom of the screen, and smaller if the fixated target is placed higher. We will present analysis of this dependency on eye position. We have collected data from several monkeys. Most of the data contains visual fixation intervals, each lasting ~2s, with the target appearing in 24 locations, arranged on 3 concentric circles of radii 5, 10, and 15 degrees. Blocks with dark background are run either directly after blocks with bright background (no dark adaptation), or

with 45 min in the dark in between (dark adaptation). There are also blocks with intermediate background intensities, allowing studying mesopic vision. The spectrum of experimental conditions is broad enough to allow us to fully characterize the effect of eye position on the fixation movement parameters, including the upshift.If an up-down asymmetry is indeed revealed by this analysis, what does it reflect? Our tentative hypothesis is that fixation upwards and fixation downwards reflect separate modes of vision. Fixation downwards is used for manipulated objects, as in photopic vision. Hence vision during downward fixations is more concerned with details than during upward fixations.

Disclosures: **O. Spivak:** None. **P. Thier:** None. **S. Barash:** None.

Poster

055. Eye Movements I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

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Topic: E.01. Eye Movements

Support: Intramural Research Program of NEI

FP7-PEOPLE-2010-IRSES CERVISO 269263

Title: GABAergic dysfunction in the olivary-cerebellar-brainstem network causes eye oscillations and body tremor

Authors: E. PRETEGIANI[1], F. ROSINI[2], R. ROCCHI[2], F. GINANNESCHI[2], A. RUFA[2], *L. M. OPTICAN[1];
[1]Natl. Eye Inst., Bethesda, MD; [2]Dept. of Medical, Surgical, and Neurolog. Sci., Univ. of Siena, Siena, Italy

Abstract: Eye and body oscillations are shared features of several neurological diseases. The pathophysiology of ocular oscillations may be simpler to explain, which then might suggest why other motor systems oscillate. Opsoclonus and flutter are ocular oscillations consisting of continuous, involuntary, conjugate saccades without intersaccadic intervals. Two main hypotheses have been proposed for their pathomechanism: reduction of glycinergic inhibition generating oscillations in the positive feedback loop between saccadic brainstem burst neurons, and disinhibition of cerebellar fastigial nuclei inducing unwanted saccades through excitatory projections to the burst neurons. However, both theories remain controversial because they were neither confirmed by clinical findings nor simulated by models. We propose that ocular and body oscillations might be generated by a GABAergic failure in a complicated circuit involving the

cerebellum (vermis and fastigial nuclei), the inferior olives and the brain stem saccade pre-motor neurons (excitatory and inhibitory burst neurons, and omnipause neurons). The GABA dysfunction of the olivary-cerebellar-brainstem network leads to transitory fastigial deficiency, causing saccadic hypermetria and macrosaccadic oscillations. Associated impairment of omnipause neurons eliminates intersaccadic intervals, producing saccadic oscillations. Fastigial deficiency also causes olivary hyperactivity, creating an instability that induces eye and limb tremor. The olivary-cerebellar dysfunction may also explain combined forms of ocular and somatic tremor. For example, involvement of different parts of the IO could explain the difference in oscillation frequency between eye (firing rate of neurons in dorsal cap of Kooy > 30 Hz) and limb (firing rate of neurons in principal olive, 4-8 Hz). The simulations reproduce the findings of eye movement recordings in patients with opsoclonus/flutter, including a novel spindle-shaped asymmetric saccadic oscillation associated with ocular and body tremor.

Disclosures: E. Pretegiani: None. **F. Rosini:** None. **R. Rocchi:** None. **F. Ginanneschi:** None. **A. Rufa:** None. **L.M. Optican:** None.

Poster

055. Eye Movements I

Location: Halls B-H

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Topic: E.01. Eye Movements

Support: CNRS

FRM

Fondation de France, Berthe Fouassier

ERC (grant Position)

Title: Bilateral fastigial control of the horizontal amplitude of saccades: evidence from studying oblique and vertical saccades after unilateral inactivation

Authors: *J. J. QUINET, L. GOFFART;
Inst. De Neurosciences De La Timone, CNRS, Marseille, France

Abstract: The caudal fastigial nuclei (cFN) are supposed to adjust the horizontal amplitude of saccades by their anatomical projections to the excitatory and inhibitory burst neurons located in the left and right pontine and medullary reticular formation (RF). Unilateral inactivation of cFN with muscimol alters the horizontal component of fixational and regular saccades, made with or

without a concurrent movement of the head: its amplitude is increased for all ipsilesional saccades and reduced for all contralesional saccades, whereas vertical saccades are deviated horizontally toward the inactivated side (ipsipulsion) with a magnitude that increases with saccade duration.
We studied in 3 head restrained monkeys how this inactivation alters the coupling between the horizontal and vertical components during oblique saccades by 1) comparing the effects on horizontal saccades with the effects on the horizontal component of oblique saccades, 2) examining whether the changes in the horizontal component of oblique saccades are associated with changes in the vertical component and 3) further testing vertical saccades. The results show that when a vertical component is added to the horizontal one, the magnitude of the horizontal hypermetria of ipsilesional saccades is consistently increased. While their duration is increased during oblique saccades, changes appear in the dynamics of the vertical component. For contralesional oblique saccades, more pronounced hypometria of the horizontal component were observed but rarely associated with concomitant changes in the vertical component. Under particular testing conditions, we were able to make observations which suggest that the burst emitted by saccade-related neurons in the opposite (intact) cFN also participate in the ipsipulsion and the horizontal hypermetria that alter the trajectory of vertical and oblique saccades.
In conclusion, after unilateral cFN inactivation, the hypermetria that affects the horizontal component of ipsilesional saccades is not due to an absent “brake”. It is the consequence of a reduced influence from inhibitory burst neurons located in the medullary RF contralateral to the inactivated cFN, together with an enhanced (and unopposed) influence from excitatory burst neurons in ipsilateral pontine RF as a result from bursting activity in the opposite (not inactivated) cFN. These results are more consistent with a “bilateral” rather than “biphasic” (accelerate-decelerate) fastigial control of saccades. The firing of saccade related neurons in both cFN regulates the dynamic equilibrium between the inhibitory and excitatory burst input to the agonist motoneurons in the abducens nucleus.

Disclosures: **J.J. Quinet:** None. **L. Goffart:** None.

Poster

055. Eye Movements I

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Topic: E.01. Eye Movements

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U54-HD083211

Robin and Richard Patton through the E. Bronson Ingram Chair of Neuroscience

Title: Neuronal diversity in macaque frontal eye field

Authors: *K. LOWE, W. ZINKE, J. D. COSMAN, J. D. SCHALL;
Vanderbilt Univ., Nashville, TN

Abstract: Morphological and connectional anatomy demonstrate the diversity of neuron types in the cerebral cortex. Some of this anatomical diversity relates to biophysical measurements; it has been proposed that excitatory pyramidal cells and inhibitory interneurons could be distinguished based on spike width and spiking statistics. To improve our understanding of cortical function, we are characterizing neuronal diversity with *in vivo* measurements of neurons recorded from frontal eye field (FEF) in macaque monkeys performing memory guided saccades. We applied a set of multivariate classification procedures to identify groups of neurons according to either their biophysical features or response modulation related to stimulus presentation, maintenance, and saccade initiation. A hierarchical cluster analysis based on the spike waveform identified multiple groups that were further subdivided based on spiking statistics (mean firing rate, Fano factor). A separate hierarchical cluster analysis based on response modulations yielded multiple groups of functionally similar neurons. Notably, results demonstrate that the traditional three neuron categories in FEF - visual, visuomovement, and movement-related neurons - underestimate the diversity of functional categories of FEF neurons. We further found that the two independent classification schemes produced overlapping groups; that is, we found a relationship between putative anatomical neuron types and functional neuron types. Supplemented by information about the location of neurons in the cortical neuropil, this approach will be used to identify and provide experimental access to different neuron types and their connections at the mesoscale circuit level, alongside their link to behavior. This will elucidate the link between cognitive outputs and their anatomical implementation, providing a theoretical framework for further circuit-level investigations.

Disclosures: K. Lowe: None. **W. Zinke:** None. **J.D. Cosman:** None. **J.D. Schall:** None.

Poster

055. Eye Movements I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 55.05/BB5

Topic: E.01. Eye Movements

Support: CNRS

Title: Parallel processing for the generation of saccades to simultaneously moving centrifugal targets

Authors: *L. GOFFART;
CNRS, Marseille, France

Abstract: The simultaneous presentation of two static targets in the visual field leads to a saccade which lands on a location situated along a line that connects the two targets. The landing position depends upon the relative salience of the targets. The more salient the target, the stronger its influence on the landing position. A similar process happens during saccades made in response to two sequentially presented targets. Saccades with the shortest latencies land on the location of the first target whereas saccades with longer latencies land closer to the second target location. Obviously, the visuomotor brain does not switch abruptly between two targets; the transition is gradual and made continuously. The spatiotemporal properties of this target selection process were explored further by testing in two monkeys, the saccades made in response to two centrifugal targets moving rapidly and simultaneously in different directions. After the fixation of a central target for a variable interval, two conditions were possible. During the single-target condition, one target moved toward the periphery along the cardinal (horizontal or vertical) or oblique axes. During the double-target condition, the central target was replaced by two identical targets moving away from the center toward the periphery with a constant speed. One target moved along an oblique axis while the other target moved along one of the cardinal axes. The speed of the target moving along the oblique axis was maintained constant (30 deg/s) whereas the speed of the target moving along the cardinal was varied (from 15 to 60 deg/s). The monkeys were free to track anyone of the targets and were rewarded on every trial. The examination of saccades made in response to targets moving in the same visual quadrant show different scatterings of landing positions between the testing conditions. During the double-target condition, the landing positions were scattered in the region of the visual field which is situated between the paths of the targets. Although their distributions were biased toward the slower target, the orientation of the scatters depended upon the speed of the two targets: the landing positions were scattered between lines of isochronous target locations. Thus, in response to two centrifugal targets, the monkeys do not select one of the two targets for its foveation. The first saccade is made toward an intermediate location, a location which corresponds to the average of two commands weighted by their speed (or the duration of retinal exposure). The scattering of endpoints between lines of target isochrony and some curved saccades indicate parallel and continuous visuomotor flows until the saccade is completed.

Disclosures: **L. Goffart:** None.

Poster

055. Eye Movements I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 55.06/BB6

Topic: E.01. Eye Movements

Support: Marie Sklodowska-Curie grant agreement No 642961 (Horizon 2020)

Title: Discrimination of saccade latencies.

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Abstract: Saccadic eye movements are widely used to investigate underlying decision processes and numerous quantitative models have been proposed to account for changes in saccade reaction time (SRT) distributions, often in conjunction with neurophysiological data. Although they are typically short - often ranging from 100 to 400 ms - and conventionally regarded as a consequence of the duration of decision-making process, previous findings showed that reinforcement contingencies modulates SRT distributions (Madelain et al. 2007) which raises the possibility of a voluntary control of SRTs. Because some perception must be necessary for any voluntary response we ask how precisely one can perceive such short reaction time.
We first collected baseline SRTs in four subjects tracking a stepping visual target. For each subject we computed four SRT classes using the four quartiles of the individual baseline SRT distributions (individual example of SRTs class intervals: 80-182ms; 183-212ms; 213-237ms; 238-400ms). Subjects were then trained to discriminate their SRTs: after each saccade they had to classify the saccade latency in a 4AFC task and then received a feedback indicating the correct answer.
Results indicate that, after intensive training, subjects could overall classify their SRT with up to 42% of correct responses, well above chance level (25%). Moreover, data showed that for each of the four latency classes the probabilities of correct responses were systematically higher ($p<0.01$) than for the incorrect ones.
Because these results could depend on volitional control of SRT rather than on an actual perception, the SRT's distributions were manipulated using the overlap paradigm.
Previous research shown that applying a temporal overlap between the fixation point disappearance and the target appearance, the saccadic eye movement latencies increase. Taking advantage of this effect, we used overlap's intervals of 0, 20, 40, 60, 80, 100, 120 and 140 milliseconds in the stepping visual target task, in the 4AFC task described above. In accordance to our previous study, one subject was able to classify his SRTs with up to 51% of correct responses well above chance level.

Altogether these results are the first to indicate that human subjects are able to discriminate their own saccade latencies, albeit imperfectly. The precision and extent of this ability remains to be further probed.

Disclosures: V. Vencato: None. **L. Madelain:** None. **L. Madelain:** None.

Poster

055. Eye Movements I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 55.07/BB7

Topic: E.01. Eye Movements

Title: Adjustments in the Purkinje cell simple spike discharge parallels changes in saccade metrics warranting movement precision despite diminished vigor

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Abstract: The physical properties of our bodies are not constant. Rather they change continuously as a consequence of using them. For instance muscle action inevitably results in muscular fatigue leading to a decline of movement vigor. However, movement vigor may as well result from a decrease of interest in the movement goals. Despite these changes, we are able to maintain a reliable level of precision of our movements by exploiting compensatory mechanisms, the roots of which lie in the cerebellum. This has been established by studying saccadic eye movements of cerebellar patients. Both healthy subjects and patients exhibit a use dependent decrease of saccade velocity. However, only healthy subjects show a compensatory upregulation of duration, preventing a consecutive decline of saccade amplitudes (Golla et al., E. J. Neurosci., 2008). We wondered if changes in saccade duration compensating the declining vigor of eye movements of healthy subjects are reflected in the firing of Purkinje cell simple spikes (PC-SS), the vehicle used by cerebellar cortex to influence extra-cerebellar information streams. This is why we tested two rhesus monkeys on a fatigue inducing repetitive eye movement task, requiring accurate constant amplitude saccades to a fixed peripheral target location at very short inter-saccade intervals. As expected, eye velocity declined over the course of the experiment by 15-20 %. Nevertheless, the required endpoint accuracy was warranted by a fully compensatory upregulation of saccade duration. We recorded the saccade related responses of 60 oculomotor vermal PC-SS and calculated their collective instantaneous firing rate. The behavioral changes were clearly paralleled by changes in the resulting population burst whose amplitude decreased, while the overall duration increased. We also recorded PC-SS (n=60) from 3 rhesus monkeys on a gain decrease adaptation task, in which saccade amplitude is reduced in

response to a consistent intra-saccadic shift of the saccade target back in the direction of the starting position. In accordance with previous findings (Golla et al., see above), the saccade gain reduction could at least partially be explained by a reduction of saccade peak velocity, not compensated by an increase of saccade duration. Again, the changes of the PC-SS population signal paralleled the behavioral changes. These findings clearly indicate that the duration adjustments warranting movement precision despite changes in vigor can be led back to corresponding adjustments of cerebellar control signals. Moreover, they support the notion that gain decrease adaptation is at least to some extent uncompensated fatigue.

Disclosures: **A. Markanday:** None. **Z. Sun:** None. **P. Thier:** None.

Poster

055. Eye Movements I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 55.08/BB8

Topic: E.01. Eye Movements

Support: MEXT, Japan

Title: Correlation between pupil size and self-timed saccade latency in non-human primates

Authors: ***T. W. SUZUKI**[1], J. KUNIMATSU[1,2], M. TANAKA[1];
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Abstract: Our daily experience of passage of time is strongly influenced by internal states, such as arousal, attention and mood. To investigate the underlying mechanism, we recorded pupil diameter, which has a close link with internal factors and neuromodulatory signaling, in monkeys performing the oculomotor version of the time production task. In this task, a visual cue was presented briefly (0.1 s) during fixation. The animals (n = 3) reported the passage of 1 s by making a self-initiated memory-guided saccade to the cue location to obtain a liquid reward. Pupil diameter was measured using the infrared eye tracking system. When we divided the trials into equally-numbered 10 groups according to the pupil diameter measured during the 500-ms before the cue onset, self-timed saccade latency negatively correlated with the pupil size (r_s = –0.90, $p < 10^{-3}$). This correlation cannot be explained solely by facilitation of saccades because no significant correlation was found for visually-triggered saccades (p = 0.33). When the amount of reward was alternated every 10 trials, pupil size and saccade latency altered in the opposite directions, showing a significant negative correlation (r_s = –0.38 ± 0.23, n = 12, $p < 10^{-3}$). Furthermore, sessions with greater modulation of pupil size exhibited larger latency modulation (r_s = –0.80, n = 12, $p < 10^{-2}$). Finally, when animals were trained to produce two different

intervals (short or long) depending on color of the fixation point, we again found a negative correlation between the pupil size and saccade latency within each condition. To assess correlation across short/long conditions, the data for each condition were divided into three groups according to saccade latency, and then the longest latency group in the short condition (S3) and the shortest group in the long condition (L1) were compared. We found that pupil was larger for L1 than S3 (paired *t*-test, $n = 10$, $p < 10^{-2}$), while saccade latency was longer for L1 than S3 ($p < 0.05$). Thus, although trial-by-trial variation of pupil size accounted for the variation of self-timed saccade latency, the pupil size did not correlate with actual saccade timing in different conditions. These results suggest that the pupil-linked brain state, which parallels the level of noradrenergic neuronal activity (Joshi et al., 2016), may regulate the trial-by-trial variability of subjective passage of time.

Disclosures: **T.W. Suzuki:** None. **J. Kunimatsu:** None. **M. Tanaka:** None.

Poster

055. Eye Movements I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 55.09/BB9

Topic: E.01. Eye Movements

Support: Rachel Atkinson Fellowship

Title: The value of simultaneous EOG and EEG recording for measurement of saccade-related brain activity

Authors: ***Y. JIA**, C. W. TYLER;
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Abstract: Introduction. Both to elucidate the cortical mechanism involved in the control of eye movements, and because eye movements cause a large amount of artifacts during electroencephalographic (EEG) recording, it is important to investigate the effects of eye movements on the study of brain activities. The aim of this project is to examine both EEG and electrooculography (EOG) signals during eye movements.
Method. The eye movement target consisted of stimulus squares appearing in one of 25 positions (a 5-by-5 array subtending 20 degrees in each side). Each stimulus was presented for 1.5 seconds, in random order. The participant was asked to saccade onto the presented stimulus and maintain fixation until the next stimulus appeared. EEG and EOG were measured simultaneously with a 128-channel EGI electrode net at a 500 Hz sampling rate. The resulting EEG signals were analyzed using principal component analysis (PCA) separately for saccades around each cardinal

direction (left, up, right, down).
Results. The analysis showed a primary EOG component with a saccade-like waveform measured with anti-symmetry across the facial electrodes for leftward and rightward movements and symmetry for upward and downward movements. These primary components scaled in amplitude and direction with the saccade extent from 5 to 28 degrees. A whole-scalp component with a single negative 50 milliseconds peak at the time of the saccade was found for all directions of eye movement, interpretable as representing the neural substrate for saccadic suppression. A component with an occipital focus peaking about 100 milliseconds after the saccade was interpretable as the cortical response to the fovea landing on the target stimulus. Components with an occipital focus peaking about 100 milliseconds before the saccade were also identifiable, interpretable as the cortical activity generating the saccade signal.
Conclusion. The results show that EOG signals allow accurate analysis of the amplitude and directions of the saccades with a temporal resolution of 500 Hz, which make the EOG from the electrode net a convenient means of measuring eye movements. The findings suggest that the technique allows the measurement of brain responses responsible for, and elicited by, saccadic eye movements.

Disclosures: Y. Jia: None. **C.W. Tyler:** None.

Poster

055. Eye Movements I

Location: Halls B-H

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Support: Prodex (BELSPO, Belgian Federal Government)

IAP VII/19 DYSCO (BELSPO, Belgian Federal Government)

ESA (European Space Agency)

ARC (Actions de Recherche Concertée)

Title: What saccadic behavior can tell us on how we use haptic feedback in collision tasks

Authors: *F. SALMEN[1,2], F. CREVECOEUR[1,2], J.-L. THONNARD[2,3], P. LEFÈVRE[1,2]; [1]ICTEAM, Univ. catholique de Louvain, Louvain-la-Neuve, Belgium; [2]Inst. of Neurosci. (IoNS), Univ. catholique de Louvain, Brussels, Belgium; [3]Physical and Rehabil. Med. Dept., Cliniques Universitaires Saint-Luc, Brussels, Belgium

Abstract: Humans and animals rely on internal representation of the trajectories of moving stimuli, allowing predictive tracking despite transient target disappearance. In collision tasks mainly visual and haptic input influences those predictions. Saccadic eye movements based on the internal models reveal how the different sensorial inputs are integrated.
We studied predictive eye movements in a semi-static (fixed hand, moving target) collision task. Participants interacted with a robotic device (KINARM, BKIN Technologies, Kingston), which applies a force (i.e. haptic feedback of the collision) to the participant's hand. Visual targets were projected on a virtual reality display. Eye movements were recorded with an Eyelink camera (SR Research Ltd., Ottawa, Ontario, Canada).
The participants (N=14) were instructed to perform visual tracking of round-shaped targets of different sizes before and after a collision with a bar representing the hand position. We assumed constant mass density, such that for a given velocity, a higher impact force was experienced for objects greater in size. The target was blanked for a constant period of 800ms immediately after the collision to induce/provoke predictive eye movement. Two different object sizes and velocities resulted in four different impact forces and four different post-collision velocities due to simulated energy loss during the collision. The different conditions corresponded to a factorial design in which visual cues about target size and haptic feedback were provided or not. We then investigated saccadic behavior during target occlusion to address participants' strategies on how to integrate haptic and/or visual feedback.
Our results show that participants adjusted their gaze correctly to different target velocities after the collision in all conditions. Providing only haptic feedback, we found a weaker spatial modulation (≈ 70%) of first saccades regarding to target position after occlusion (linear regressions, $p<0.005$), whereas the spatial modulation after later saccades were similar for all conditions. Also first saccade latencies were significantly longer (KS test, $D>0.15$, $p<0.005$) when only haptic feedback was provided compared to visual and/or combined feedback.
These results show that predictive tracking of the target is adjusted in conditions in which haptic feedback is available in addition to internal predictions from visual information. The increase in saccade latency associated with haptic feedback, and the reduced compensation of the first saccade sheds light on the time course of integration of haptic feedback in the internal estimate of the target location.

Disclosures: **F. Salmen:** None. **F. Crevecoeur:** None. **J. Thonnard:** None. **P. Lefèvre:** None.

Poster

055. Eye Movements I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 55.11/BB11

Topic: E.01. Eye Movements

Support: DFG EXC 307

Title: Sequential hemifield gating of 8-15 hz behavioral performance oscillations after microsaccades generation

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Abstract: Many visual neuroscience experiments impose gaze fixation to rule out potential influences of eye movements on neural and behavioral results. However, even during periods of attempted gaze fixation, our eyes are in constant motion. Among the types of fixational eye movements that are known to occur are microsaccades, which are tiny saccades (typically < 1° of visual angle) occurring 1-3 times per second. Even though visual processing has already been shown to be modulated close to the time of microsaccades (e.g. Chen et al., Curr. Biol., 2015), it is not clear how microsaccade generation might influence longer-term fluctuations in brain activity and behavior. Here we show that visual processing is significantly affected, and in a hemifield-specific manner, even several hundreds of milliseconds after a microsaccade had occurred. We conducted two psychophysical experiments aiming at testing reaction time (RT) and contrast sensitivity for visual targets presented at different times after microsaccades. In the first experiment, 10 human subjects were instructed to fixate a dot in the middle of a screen. After a random period, the fixation dot disappeared and, simultaneously, a target appeared 5 deg to the right or left of fixation. We investigated whether the relative time and direction of the microsaccade preceding target appearance could influence saccadic RT to the target. Mean RT varied in a rhythmic fashion depending on when the stimulus was presented relative to a preceding microsaccade. Surprisingly, the performance oscillations were sequentially pulsed across hemifields: the first 400 ms following a microsaccade revealed strong oscillations in RT (~15 Hz), but only in the hemifield towards which the microsaccade was directed to; the next 400 ms presented similar (albeit lower-frequency) oscillations (~8 Hz), but this time in the opposite hemifield. Thus, there was sequential hemifield activation of behavioral oscillations that were phase-locked to microsaccade generation. In a second experiment involving 14 subjects, we tested contrast sensitivity long after microsaccades. Subjects fixated a dot in the middle of the screen. At a random time, a faint stimulus was presented 5 deg to the right or left of fixation. Subject performance to detect the stimulus was increased in the hemifield towards which the last microsaccade was directed to, but only during the period in which the ~15 Hz pulse of RT modulations occurred in the first experiment. Taken together, our results suggest that microsaccades have prolonged effects on behavior, and that across-hemisphere phase-resetting of brain activity by microsaccades proceeds in a pulsed, sequential manner.

Disclosures: J. Bellet: None. **Z.M. Hafed:** None.

Poster

055. Eye Movements I

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Topic: E.01. Eye Movements

Support: German Research Foundation, Collaborative Research Centre SFB/TRR 135: Cardinal Mechanisms of Perception

Title: Classification of COMT Val^{158}Met genotype by oculomotor behavior

Authors: *J. BILLINO, J. HENNIG, K. GEGENFURTNER;
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Abstract: The neural circuits involved in oculomotor control are extremely well described; however, neuromodulation of eye movements is still hardly understood. Dopamine in particular has been discussed as a functionally significant neurotransmitter because of pronounced oculomotor deficits observed in patients with psychiatric or neurological diseases for which dysfunctions of dopaminergic neurotransmission are considered as the major pathologic mechanism. The crucial question that has remained to be answered is whether dopaminergic modulation of eye movements can also be seen in healthy human observers. A non-invasive way to investigate behavioural effects of dopamine in humans is provided by functional genetic polymorphisms. The COMT Val^{158}Met polymorphism represents probably the best documented dopaminergic polymorphism and its functional mechanism is well-established. Genotype modulates activity of catechol-O-methyltransferase (COMT), an enzyme that plays a major role in dopamine catabolism in prefrontal cortex. The enzyme is supposed to be less active in carriers of the Met-allele who in turn show higher prefrontal dopamine levels. We investigated the functional association between COMT genotype and the performance in two eye movement tasks known to involve substantial top-down control. We measured anticipatory smooth pursuit as well as memory guided saccades in 111 healthy subjects (93 females, age M=23.7, SD=5.1) and determined their individual COMT genotypes (ValVal n=24, ValMet n=55, MetMet n=32). For both tasks we found significant functional associations. Anticipatory pursuit response was most pronounced in individuals with genotypes putatively associated with higher prefrontal dopamine levels, i.e. carriers of the Met allele (anticipatory eye position: $F(2, 107) = 12.25$, $p < .001$, $\eta^2 = .19$). In contrast, higher dopamine levels were detrimentally associated with saccadic accuracy in memory guides saccades ($F(2, 103) = 5.63$, $p = .005$, $\eta^2 = .10$). Most importantly, by using a simple discriminate analysis we were able to segregate Val homozygotes and Met homozygotes successfully with an accuracy of 88% based on performance in both eye movement tasks. This result highlights the importance of selecting the proper combination of paradigms. Each single task explained rather little variance, but combined they got close to perfection. Our approach

emphasizes the value of systematic variability and individual differences for uncovering functional processes that would elude investigation otherwise.

Disclosures: **J. Billino:** None. **J. Hennig:** None. **K. Gegenfurtner:** None.

Poster

055. Eye Movements I

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Program#/Poster#: 55.13/BB13

Topic: E.01. Eye Movements

Title: Compound effects of transcranial magnetic stimulation on the oculomotor system

Authors: ***I. G. CAMERON**[1], A. CRETU[2,4], F. STRUIK[2], I. TONI[2,3,5];
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Abstract: Transcranial magnetic stimulation (TMS) is used extensively to focally perturb function in a brain region in order to infer that region's causal contribution. This approach largely ignores the fact that TMS can influence distal nodes in a network, possibly triggering compensatory responses in those nodes. Here we use a well-defined circuit, the cortical oculomotor system, to qualify distal network-level changes triggered by a focal TMS intervention. This study tested 24 participants across 4 sessions. The first session used fMRI to localize cortical oculomotor regions in each participant, and guide the stereotactic administration of TMS in sessions 2-4. Across the four sessions, participants performed pro- and anti-saccades to visual stimuli along the horizontal meridian, while their gaze was monitored at 500 Hz. Sessions 2-4 used TMS interventions according to this rationale: first, inhibitory continuous theta-burst stimulation (cTBS) was applied (40 s) to the dorsolateral prefrontal cortex (DLPFC), frontal eye fields (FEF), or a control region (primary somatosensory cortex, S1), in the right hemisphere. Second, while the oculomotor network was still perturbed (50 min post-cTBS), single TMS pulses were applied to the right parietal eye fields (PEF) at variable times during task performance. Because FEF and PEF have complementary functions related to visuo-motor behavior, in particular during the performance of anti-saccades, we hypothesized cTBS to FEF would impair visuo-motor effects of anti-saccade behavior, and single-pulses to PEF might compound this effect. However, if the oculomotor network compensates for FEF inhibition following cTBS, then single-pulses to PEF following cTBS on FEF might lead to smaller deficits than single-pulses to PEF following cTBS on the control region (S1). Furthermore, we

hypothesized that cTBS to DLPFC would impair executive control aspects of anti-saccades, but not drive a compensatory effect in PEF.
Contralesional anti-saccades had reduced amplitude, with invariant reaction times and error rates, following cTBS over FEF or DLPFC (as compared to cTBS over S1). After cTBS over S1, single-pulses over PEF also reduced anti-saccade amplitudes. Preliminary data suggests that combining cTBS over FEF and single-pulses over PEF led to a larger reduction in contralesional anti-saccade amplitudes than when that combination involved S1 cTBS and PEF single-pulses.

Disclosures: **I.G. Cameron:** None. **A. Cretu:** None. **F. Struik:** None. **I. Toni:** None.

Poster

055. Eye Movements I

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Strategic Research Programme in Neuroscience Karolinska Institutet

Parkinsonfonden

Title: Dopamine modulates sensorimotor transformation in the optic tectum

Authors: *J. PÉREZ-FERNANDEZ[1], A. KARDAMAKIS[2], B. ROBERTSON[2], S. GRILLNER[2];
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Abstract: The optic tectum (superior colliculus in mammals) shows well conserved features through vertebrate evolution, and controls gaze movements through excitatory output neurons projecting to the brainstem. In all vertebrates the optic tectum integrates different sensory modalities (which are species dependent), and when two stimuli from different senses coincide in space and time a response enhancement occurs increasing event detection reliability. In lamprey, visual and electrosensory information is integrated at the level of single output cells of the deep layer, where direct excitatory inputs are combined to reinforce the motor command. Sensory inputs also evoke strong inhibition following the excitatory input, which arises in the superficial layer interneurons and continually resets the output cells allowing for spatial and temporal

stimuli discrimination. This sensorimotor transformation is also modulated by dopamine from the nucleus of the posterior tuberculum, the homologous region of the mammalian substantia nigra pars compacta (SNc). The dopaminergic system was already well developed at the dawn of vertebrate evolution, and the basal ganglia in general, and the SNc connectivity in particular, are very similar from lamprey to mammals. One striking feature is that the SNc in lamprey, as in mammals, sends direct dopaminergic projections to motor command centers, including the diencephalic and mesencephalic motor regions and the output layer of the optic tectum. Here, we explore how dopamine modulates motor responses in the optic tectum. D1 and D2 receptor expressing cells are two different subpopulations, and dopamine has a differential modulation of their excitability, enhancing the excitability of D1 expressing cells, and decreasing the excitability of those expressing the D2 receptor. This differential modulation affects their responsiveness to the multisensory inputs regulating their motor responses. We have developed a novel preparation keeping the eyes with the brain and rostral segments of the spinal cord, allowing us to apply different visual paradigms coupled to electrophysiological recordings. Local injections of dopamine agonists in the optic tectum have a strong effect in tectal motor responses, including eye movements, but also escape and orienting movements, as reflected in ventral root activity. Our results show that dopamine performs a complex modulation in the optic tectum and, given the high degree of conservation of the basal ganglia and the presence of direct dopaminergic projections from the SNc to the superior colliculus in rats, this previously unexplored mechanism is likely to be present also in mammals.

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Poster

055. Eye Movements I

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Program#/Poster#: 55.15/BB15

Topic: E.01. Eye Movements

Title: Effect of maintaining neck flexion on reaction time of memory-guided saccade: An investigation using transcranial magnetic stimulation to the frontal oculomotor field

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Abstract: We applied transcranial magnetic stimulation (TMS) to the frontal oculomotor field, and investigated the effect of maintaining neck flexion on reaction time of memory-guided

saccade. The subjects were 10 healthy young adults. The experimental protocols were approved by our institutional ethics committee. All subjects provided written informed consent after receiving an explanation of the experimental protocols. The motor area of the first dorsal interosseous muscle (FDI) was determined as the site at which a motor evoked potential was elicited by TMS. The position of the frontal eye field in the frontal oculomotor field has been reported at 2 cm anterior or 2-4 cm anterior / 2-4 cm lateral to the motor area of the FDI. Based on a location 2 cm anterior to the motor area of the FDI, the TMS coil was positioned at various points in the anterior-posterior and left-right directions. We determined in advance the position of the frontal oculomotor field at which TMS induced prolongation of the reaction time of the memory-guided saccade. After identifying the frontal oculomotor field, the reaction time was measured with the chin resting on a stand (chin-on condition) and with voluntary maintenance of neck flexion (chin-off condition) at 80 % maximal neck flexion angle, with and without TMS. TMS timing producing the longest prolongation of the reaction time was first roughly identified for 10 ms intervals from 0 to 180 ms after the start signal of the saccade (lights-out of central fixation point). Thereafter, TMS timing was set finely at 2 ms intervals from −20 to +20 ms of the 10 ms interval producing the longest prolongation. The reaction time without TMS was significantly shorter (25.6 ms) for the chin-off condition (231.6 ± 44.6 ms) than the chin-on condition (256.6 ± 45.9 ms). TMS timing producing maximal prolongation of the reaction time was significantly earlier (23.3 ms) for the chin-off condition (102.6 ± 16.8 ms) than the chin-on condition (125.8 ± 13.6 ms). The ratio of the forward shift in TMS timing relative to the shortening of reaction time was 93.2 %. We indicated that information processing time in the neural pathway of the memory-guided saccade before the frontal oculomotor field shortened with maintaining neck flexion.

Disclosures: **K. Kunita:** None. **K. Fujiwara:** None.

Poster

055. Eye Movements I

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Boswell Foundation

Title: Choice bias after lateral intraparietal (LIP) area inactivation predominantly reflects a decision rather than an attention deficit

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Abstract: The posterior parietal cortex (PPC) has been traditionally associated with spatial perception, awareness, and attention; i.e., largely sensory attributes. However, a growing body of evidence indicates that it is also involved in a wider range of brain functions, such as decision-making. In large part this evidence is based on reversible pharmacological inactivation of specific PPC regions. For instance, recent studies showed that the unilateral inactivation of the lateral intraparietal (LIP) area - i.e., the area of PPC which is involved in saccades - leads to a reduction of contralesional choices in oculomotor decisions. Although these findings suggest that LIP is involved in saccade choices, an equally plausible interpretation is that the choice bias reflects deficits in spatial attention rather than in decision-making. To address this concern, we need to determine whether the choice bias after LIP inactivation is effector-specific. We reversibly inactivated part of LIP by locally injecting the GABA-A agonist muscimol, while two monkeys performed memory-guided saccade and reach movements either to a single target (instructed trials) or selected between two targets presented simultaneously in both hemifields (free-choice trials). Reaches were performed using a 2D joystick positioned between the legs of the animals. Eye-fixation was required during the reach trials. Consistent with previous studies, LIP inactivation led to a strong reduction of contralesional choices, but mostly for saccades. On the other hand, the inactivation did not cause any appreciable change in reach choices. No effects found on the reach/saccade movements to single targets presented in either hemifield. The decision bias cannot be driven by attention, since the temporary “lesion” affected predominantly saccade choices. These results extend our previous findings from the inactivation of the parietal reach region (PRR) on internally-guided decisions. In particular, we showed that the PRR inactivation led to a reduction of contralesional choices but only for reaches. On the other hand, saccade choices were not affected by PRR inactivation. This double dissociation provides direct evidence that LIP and PRR are nodes in networks for making saccade and reach decisions, respectively.

Disclosures: V.N. Christopoulos: None. **I. Kagan:** None. **R.A. Andersen:** None.

Poster

055. Eye Movements I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 55.17/BB17

Topic: E.01. Eye Movements

Support: CIHR MOP-89785

Title: Graph theoretical analysis of functional network changes during recovery of saccade selection bias following stroke in nonhuman primates

Authors: *R. ADAM[1], K. JOHNSTON[1], R. HUTCHISON[2], S. EVERLING[1];
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Abstract: Spatial extinction is an attention deficit commonly seen following unilateral stroke in humans and nonhuman primates. It is characterized by impaired detection of a contralesional stimulus when two stimuli are presented simultaneously in both the ipsilesional and contralesional hemifield. This results in a disabling ipsilesional saccade selection bias that often recovers over time. Here, we used graph theory to identify changes in complex network properties during recovery of the saccade selection bias following a right frontal stroke. We created a nonhuman primate model of ischemic stroke using macaque monkeys by injecting endothelin-1, a potent vasoconstrictor, in the right dorsolateral prefrontal cortex and frontal eye field. Saccade selection bias was measured using a free-choice task in which macaque monkeys were presented with two stimuli, one in each hemifield, either simultaneously or at varying stimulus onset asynchronies. Following stroke, the animals exhibited both a profound ipsilesional saccade selection bias and increased contralesional saccadic reaction times that gradually recovered over time. We used resting-state functional MRI (rs-fMRI) at 7T to obtain the blood oxygen level-dependent (BOLD) signal time series at pre-stroke and week 1, 4, 8, and 16 post-stroke. Graph metrics were calculated using the Brain Connectivity Toolbox and compared within-subjects across all rs-fMRI sessions. At week 1 post-stroke, there was decreased mean global efficiency and small-worldness (decreased clustering, increased mean shortest path length). By week 8-16 post-stroke, when the saccade selection bias had recovered, mean global efficiency and small-worldness had increased. At the time of full recovery at the nodal level, right premotor, supplementary motor, TPO, MST, and extrastriate cortex showed decreased betweenness, degree, and local efficiency; whereas right MT and LIP and left premotor, TPO, and extrastriate cortex showed increased betweenness, degree, and local efficiency. Our preliminary findings suggest a pattern of global and local network changes following unilateral stroke that may underlie recovery of the saccade selection bias.

Disclosures: **R. Adam:** None. **K. Johnston:** None. **R. Hutchison:** None. **S. Everling:** None.

Poster

055. Eye Movements I

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Program#/Poster#: 55.18/BB18

Topic: E.01. Eye Movements

Support: NIH grant NS50942

Title: Evolution of a reference frame along a brain pathway: persistently hybrid coordinates of auditory signals in Frontal Eye Fields implicate the Superior Colliculus in computing eye-centered sound location

Authors: *V. C. CARUSO[1], D. S. PAGES[2], M. A. SOMMER[2], J. M. GROH[2];
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Abstract: Cues for determining visual and auditory locations differ. The optics of the eye provide for eye-centered visual signals. Sound location is inferred from cues that depend on the sound's position with respect to the head and ears. The computations necessary to integrate these different cues are a central problem in multisensory and sensorimotor integration. Previous work has shown that auditory signals are transformed into eye-centered coordinates for integration with vision. This process begins at least as early as the inferior colliculus (Groh et al. 2001) and may in fact begin with eye position-dependent displacements of the eardrum (Gruters et al. SfN 2015). The transformation is "completed" by the time the saccade-related bursts of superior colliculus (SC) neurons are generated: these bursts specify sound location in eye-centered coordinates (Lee & Groh 2012). Yet, to date, these are the only known pre-dominantly eye-centered auditory signals in the brain; all other known signals reflect hybrid head- and eye-centered information (IC, A1, LIP). The SC may compute the eye-centered signal in time or it may receive this signal from an as yet unknown source. Since the frontal eye fields (FEF) plays a similar role to the SC in saccade generation, and projects to the SC, we sought to evaluate the reference frame of auditory signals in the FEF in comparison to the SC. Previous work by Russo and Bruce (1994) had established that the FEF's auditory signals vary with eye position, but had not fully determined the reference frame. Information about FEF's auditory code and its evolution in time can resolve whether the computation of eye-centered sound location is broadly present in saccade-programming areas or unique to the SC (and maybe its afferents). We tested the activity of 324 FEF neurons while 2 head-restrained monkeys performed saccades to the locations of sounds from different fixation positions. We dissociated target-related and saccade-related activity in time by delaying the saccade go-cue relatively to target onset. We found that auditory signals were prevalent in the FEF, occurring in as many as 76% of FEF neurons. At sound onset and throughout the saccade preparation, sound location was encoded in hybrid head- and eye-centered coordinates. Only during the saccade burst we observed a modest shift towards eye-centered coordinates (roughly 60% of neurons remained hybrid, while the eye-centered neurons increased from 15% during the sensory period to 30% during the motor period). Overall, the results in the FEF contrast considerably with the results from the SC, and implicate the SC as the main player in the final computation of eye-centered auditory target location.

Disclosures: V.C. Caruso: None. **D.S. Pages:** None. **M.A. Sommer:** None. **J.M. Groh:** None.

Poster

055. Eye Movements I

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Topic: E.01. Eye Movements

Support: MEC-FEDER BFU2012-33975

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FIUS PRJ201402146

Title: Adult cat injured motoneurons recover a normal firing pattern and synaptic inputs after VEGF administration

Authors: P. M. CALVO, A. M. PASTOR, *R. M. DE LA CRUZ;
Dept. de Fisiología, Univ. de Sevilla, Facultad de Biología, Sevilla, Spain

Abstract: Vascular endothelial growth factor (VEGF) was initially characterized by its activity on blood vessels. However, recent evidences indicate that this factor can also act directly on neurons, exerting a neuroprotective role particularly on damaged motoneurons. Nonetheless, to date, there is no available information regarding the functional state in which those motoneurons that has been rescued by VEGF remains, which is of extreme relevance to assess the therapeutical potentiality of this factor. We pursued this objective by recording in the chronic alert cat preparation the discharge activity of axotomized abducens motoneurons (which innervate the extraocular lateral rectus muscle) following VEGF administration. Abducens motoneurons encode eye position and eye velocity during spontaneous and vestibularly-induced eye movements. Axotomy produced severe changes in the firing of these cells that affect all the components of their discharge, with a general reduction in firing activity and a loss of eye-related signals. VEGF was administered peripherally (from the orbit after removing the lateral rectus muscle and cutting the VI nerve) through a home-made devise that allowed to introduce the distal end of the cut VI nerve on a camera through which the factor was delivered (0.2 μg/kg on alternated days). During the axotomy period only vehicle was administered to the sectioned nerve. The administration of VEGF restored completely the tonic-phasic discharge of axotomized motoneurons during different types of eye movements, so that all eye movement parameters in VEGF-treated motoneurons were similar to control and differed significantly from axotomy. Since injured motoneurons re-established a normal discharge activity after VEGF treatment, we were interested in correlate this finding with the synaptic inputs received by these motoneurons in the three conditions (control, axotomy and axotomy plus VEGF). For this purpose we carried out double immunofluorescence at the microscopy confocal level. Motoneurons were identified as choline acetyltransferase (ChAT)-positive. Synaptic boutons

were labeled by synaptophysin and astrocytic profiles by glial fibrillary acidic protein (GFAP). Our results showed that, as compared to control, axotomy was characterized by a significant loss of synaptic boutons in association with a prominent astrocytic reaction, both returning to normal values after VEGF treatment. Therefore, the present findings show, for the first time, that VEGF is a potent physiological neurotrophic and synaptotrophic factor for motoneurons which reinforces the therapeutical potential of VEGF for diseased or injured motoneurons.

Disclosures: P.M. Calvo: None. **A.M. Pastor:** None. **R.M. De La Cruz:** None.

Poster

055. Eye Movements I

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Program#/Poster#: 55.20/CC2

Topic: E.01. Eye Movements

Support: CIHR Grant

Title: Ketamine-induced changes in prefrontal cortex oscillatory activities during a working memory task in monkeys

Authors: *L. MA[1], K. SKOBLENICK[2], K. JOHNSTON[2], S. EVERLING[1,2,3,4];
[1]Dept. of Physiol. and Pharmacol., [2]Dept. of Anat. and Cell Biol., [3]Robarts Res. Inst., [4]Brain and Mind Inst., Univ. of Western Ontario, London, ON, Canada

Abstract: Acute injection of subanesthetic dose of ketamine in non-human primates is an effective model for creating schizophrenia-like symptoms, such as deficits in working memory. We trained three rhesus monkeys to perform a rule-based working memory task involving pro- and anti-saccades, and recorded both spiking activity and local field potentials from the dorsolateral prefrontal cortex both before and after ketamine injection. Previously we reported that ketamine injection negatively impacted the maintenance and the application of the correct task rule, possibly via a reduction in the signal-to-noise ratio in both single unit and ensemble activities. Comparing to spiking activities, neural oscillations are more easily monitored and better studied in humans, providing an opportunity to test the mechanistic validity of animal models. In healthy humans, the strength of gamma band oscillation is modulated by working memory processes. In patients with schizophrenia, either abnormal enhancement or reduction in gamma band activity depending on the working memory load was correlated with impaired performance (Haenschel et al., 2009). Meanwhile, low frequency oscillations are often attenuated in patients. Studies in rodent models generally reported an enhanced gamma power following treatment with NMDA channel blockade. To quantify the oscillatory activity

specifically associated with working memory, we subtracted the amplitude of gamma activity during inter-trial intervals from the gamma power measured during the delay periods. We found enhanced delay-related low gamma power (30 to 60Hz) accompanied by reduced power in the beta band (13 to 30Hz) during the delay period preceding correct responses following ketamine treatment. To understand how ketamine affects the rule information encoded in oscillatory activity, we calculated the task selectivity, defined as the difference between oscillatory amplitudes associated with the two rules, divided by the sum of these amplitudes across both rules. In two of the three animals, task selectivity was significantly reduced in both beta and low gamma frequency ranges following ketamine injection. These results support an important role of prefrontal oscillatory activities in information processing in working memory.

Disclosures: **L. Ma:** None. **K. Skoblenick:** None. **K. Johnston:** None. **S. Everling:** None.

Poster

055. Eye Movements I

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Topic: E.01. Eye Movements

Support: Innovative Training Network 'Perception and Action in Complex Environment'(PACE) under the Marie Sklodowska-Curie grant agreement N°642961

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Title: Investigating the contribution of M1 in Eye-Hand coordination using TMS

Authors: ***J. MATHEW**, A. EUSEBIO, F. DANION;
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Abstract: Previous literature has shown that the ability to track a moving target with the eye is substantially improved when the target is self-moved as compared to when it is moved by an external agent. To account for this result, it has been proposed that the oculomotor system has access to an estimate of the current hand position by means of a forward model that receives the arm efferent copy. However, direct neurophysiological evidence is needed to support this scheme. The goal of the current study was to explore whether eye motion could possibly be altered by TMS over the hand area of the primary motor cortex (M1) during eye-hand coordination. Practically subjects were asked to track with their eyes a visual target whose horizontal motion was driven by their grip force. When TMS was applied over M1 hand area, involuntary grip force pulse led to transient target jumps. We reasoned that if the output of M1 is

used by the oculomotor system to keep track of the target, TMS on top of disturbing grip force, should also disrupt eye motion. For comparison purposes, the effect of TMS was also monitored when applied while tracking an externally-moved target. In agreement with earlier observations, the current results showed that eye tracking performance is improved when the target is self-moved as compared to when being externally-moved. More importantly, our data suggest that eye motion is poorly influenced by TMS over M1. Indeed when the target was externally-moved, TMS did not disrupt eye motion. Furthermore during Eye-Hand coordination TMS induced an increase in eye-tracking error, but a very similar observation was noticed when using SHAM mimicking the visual effect of TMS on target motion. Overall the results of this TMS study suggest that the output of M1 has limited contribution to eye tracking performance during Eye-Hand coordination.

Disclosures: **J. Mathew:** None. **A. Eusebio:** None. **F. Danion:** None.

Poster

055. Eye Movements I

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Support: Russian Academic Excellence Project '5-100'

RFBR (the projects № 12-04-00719)

Title: Parameters of the presaccadic EEG potentials in the experimental scheme with distracters

Authors: ***V. MOISEEVA**[1,2], M. SLAVUTSKAYA[2], V. SHULGOVSKIY[2], N. FONSOVA[2]; [1]HSE, Moskva, Russian Federation; [2]Lomonosov MSU, Moscow, Russian Federation

Abstract: For successful goal-directed behaviour, it's crucial to attend relevant stimuli in the visual field while ignoring distractor elements. The oculomotor system is a good model for the study of this competition between different elements. The goal of this research was to analyse spatial-temporal parameters of saccades and presaccadic EEG-potentials at the simultaneous presentation of the target and distracting stimuli to the leading and unleading eye. The complex of the positive and negative potentials was revealed in the saccade latent period. Latency of all components was shorter upon presentation of stimuli to the left, unleading eye, that may indicate the earlier saccade preparation. At the same time LP saccades were longer in this conditions ($p<0.05$). The results show that early potentials N1 and P1 were higher in amplitude and dominated in the controlateral parietal-occipital areas. It can be reflection of visual sensory

processing. The amplitude of the later negative potential N2 at the stimulation of the right eye increased in the case when target stimulus was at the same location than at the previous realisation. It's possible that N2 component is connected with processes of preliminary extracting of motor program from memory together with attention processes. N2 amplitude was higher when the distance between target and distracting stimuli was 15 degrees in comparison with the minimal distance 5 degrees. It's corresponded with LP data. The findings show an active role of attention and decision-making processes in saccade programing.

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Poster

055. Eye Movements I

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Topic: E.01. Eye Movements

Support: DBT Grant

Title: Simultaneous analysis of local field potential and spikes to understand the visuo-motor transformation in monkey frontal eye field

Authors: ***N. SENDHILNATHAN**[1,2], D. BASU[2], A. MURTHY[2];
[1]Columbia Univ. Dept. of Neurosci., New York, NY; [2]Ctr. for Neurosci., Indian Inst. of Sci., Bangalore, India

Abstract: The Frontal Eye Field (FEF) is one of several nodes in the oculomotor system that helps transforms a visual input into a saccadic motor commnad. In this study, Local Field Potentials (LFP) collected from the FEF, along with simultaneously recorded spike data, were used to characterize visuomotor cells of the FEF and study their contribution to the visuomotor transformation computation. We used a memory guided saccade to temporally dissociate the visual epoch from the saccadic epoch and study the processes that link them in time. A comparison of the LFP and spike signals during the visual epoch revealed that LFPs typically preceded the spikes and were more broadly tuned suggesting that the LFPs may reflect input into the FEF. In contrast, we observed that the spikes generated during the saccade epoch preceded the LFP signal suggesting that saccade related activity is generated de novo in FEF. Using the frequency domain information of the LFP, we observed that the gamma band activity increased in power only during the memory epoch. Nevertheless, gamma activity was spatially tuned and predicted the subsequent saccadic reaction time of the animal. These evidences suggest that LFP

signals, particularly in the gamma band, may represent a neural correlate of the visuomotor transformation process that is local to FEF. Taken together these results together make a valuable addition to the mechanism and the process of visuomotor transformation in FEF.

Disclosures: N. Sendhilnathan: None. **D. Basu:** None. **A. Murthy:** None.

Poster

055. Eye Movements I

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Topic: E.01. Eye Movements

Support: NIH/NEI R01 EY022290

P30 EY03039

Title: Oculomotor plant dynamics during quick and slow phase movements in alert rhesus monkeys.

Authors: *K. SCHULTZ[1], J. M. MILLER[2], P. D. GAMLIN[1,2];
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Abstract: An oculomotor plant model valid for a broad range of velocities is essential for resolving the transformation from ocular motorneuron (OMN) activity through extraocular muscle (EOM) force to eye position. A simple linear model is generally applied to the relationship between OMN firing and eye position, but fails to predict eye movement profiles and can even lead to paradoxical behavior (Miller et al. 2011). While OMN activity during eye movements is well known and highly stereotyped, the dynamics of EOM and orbital tissues are still being explored. Most models of the plant are derived from Robinson's (1964) model, which includes a series of viscoelastic Voigt elements with different time constants. Apart from nonlinearities related to stimulation frequency (Anderson et al. 2009) and passive muscle forces (Quaia 2009, 2010), Voigt elements in series continue to be used, despite the fact that muscle force is seldom actually measured, and when it is, the muscles are disinserted and/or the animal is sedated. By using muscle force transducers (MFTs) that do not restrict natural eye movements, we sought to address these weaknesses, as well as another - the restricted range of eye movement velocities used to develop plant models based on EOM force and eye position. MFTs were implanted on the horizontal recti of Rhesus monkeys trained to fixate targets. Forces were recorded during both saccades and horizontal pursuit movements at various frequencies, while eye position was recording using scleral search coils. Model parameters were determined in

Matlab (Simulink). The force profiles during pursuit movements were very similar to the eye position profiles. A simple model using one Voigt element was able to closely predict eye position, whereas multiple Voigt elements with multiple time constants were required to predict saccadic behavior. However, estimating parameters from only saccadic movements led to time constants and compliance values able to predict only quick phases; pursuit movements were poorly modeled, both in general profile and smaller oscillations. Likewise, parameters estimated from pursuit movements failed for saccadic movements. To fit a model for both pursuit and saccadic movements requires that parameters be estimated from both types of movement. This suggests that a wide range of eye movement types is necessary to correctly estimate general model parameters.

Disclosures: **K. Schultz:** None. **J.M. Miller:** None. **P.D. Gamlin:** None.

Poster

055. Eye Movements I

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Title: Action selection after motor adaptation.

Authors: ***L. TEUNISSEN**, F. MAIJ, L. P. J. SELEN, W. P. MEDENDORP;
Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ. Nijmegen, Nijmegen, Netherlands

Abstract: Theories about motor decision-making suggest that actions are selected to achieve a (more) rewarding bodily state while minimizing the cost of performing those actions. To estimate the required cost of different potential actions, information of body and world dynamics derived from a continuously adapting forward model might be essential (Cos et al. 2014; see Pélisson et al. 2010 and Shadmehr et al. 2010 for reviews of saccadic and reach adaptation respectively). Therefore, we hypothesize that adaptation of the forward model biases the selection of actions towards the action with the relatively lower cost after adaptation. We investigated this hypothesis for both saccades and reaching movements by asking participants to either make free choice saccades or reaches to one of two (equally rewarding) targets, placed at

equal distance from the fixation location. We varied the targets' stimulus onset asynchrony (SOA) to psychometrically determine the SOA at which both targets were chosen equally often as a quantitative measure of target preference. Next, we performed saccadic adaptation or we modified the visuomotor gain for reach adaptation for one of the two targets and again determined the preferred target psychometrically. The adaptation was thus used to visually have similar amplitudes before the decision was made but to induce different eventual movement amplitudes in the post-adaptation free choice stage of the experiment. Preliminary results suggest a shift in target preference after adaptation, suggesting that visuomotor adaptation affects the process of action selection. Further experiments are currently performed and analyzed to see how changes in the forward model are reflected in action preferences for both eye and arm movements. **References:** Cos, I., Duque, J., & Cisek, P. (2014). Rapid prediction of biomechanical costs during action decisions. *J Neurophysiol*, *112*(6), 1256-1266. Pélisson, D., Alahyane, N., Panouillères, M., & Tilikete, C. (2010). Sensorimotor adaptation of saccadic eye movements. *Neurosci Biobehav Rev*, *34*(8), 1103-20. Shadmehr, R., Smith, M. A., & Krakauer, J. W. (2010). Error correction, sensory prediction, and adaptation in motor control. *Annu Rev Neurosci*, *33*, 89-108.

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Poster

055. Eye Movements I

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Topic: E.01. Eye Movements

Support: NIH Grant R01NS078311

Title: A shared mechanism for control of vigor in saccades, reaching, and head movements

Authors: *T. REPPERT[1], A. RAMAMOORTHY[1], O. KOMOGORTSEV[2], R. SHADMEHR[1]; [1]Johns Hopkins Univ., Baltimore, MD; [2]Texas State Univ., San Marcos, TX

Abstract: Some people consistently move faster than others. For example, some individuals move their eyes with high speed, while others make saccades of the same amplitude with lower speed. These inter-individual differences are reproducible across days and months of recordings. Do individuals that move their eyes with high speed also move other body parts rapidly? When vigor of one movement increases, does it coincide with increased vigor of other kinds of movements? Let us define vigor as the relationship between peak speed and amplitude of

movements for one individual with respect to the mean relationship for the population. For example, a subject has high saccade vigor if their speed-amplitude curve lies above the population mean. We asked subjects (n=286) to complete two sessions during which they made reflexive saccades to targets presented on a computer screen. The target positions were spaced randomly between +/-15 deg horizontally, and +/-9 deg vertically. We found a wide range of saccade vigor across the population. Importantly, subjects who exhibited high vigor for horizontal saccades also exhibited high vigor for vertical saccades. We then asked whether vigor is conserved across movement modalities. We recorded simultaneous eye, head, and reaching movements in subjects (n=25) as they completed a natural pointing task. Subjects were presented with visual targets, and then reached and touched that target. The movement included motion of the eye, head, and arm, with amplitudes ranging from 5-50 deg in 11 subjects, and 30-50 deg in 14 subjects. We found that vigor of head movements and reaching movements were strongly correlated: individuals who exhibited fast reaching movements also exhibited fast head movements. However vigor of saccades was only weakly related to that of head movements and reaching. During the task, each subject exhibited natural variability in their vigor. Given a subject's average vigor of movements for each modality, we studied within-subject changes in vigor about this mean. We found that within a subject, trial-to-trial changes in vigor of saccades were correlated with changes in vigor of both head movements and reaching movements. Specifically, vigor of all three movement modalities increased abruptly following a set break, and then decreased exponentially within-set. Therefore, our results suggest two ideas: 1) movement vigor may be a trait, conserved across head and reaching movements; 2) within-subject change in vigor of one type of movement (saccades) is a predictor of change in vigor of other types of movements (head and arm).

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Poster

055. Eye Movements I

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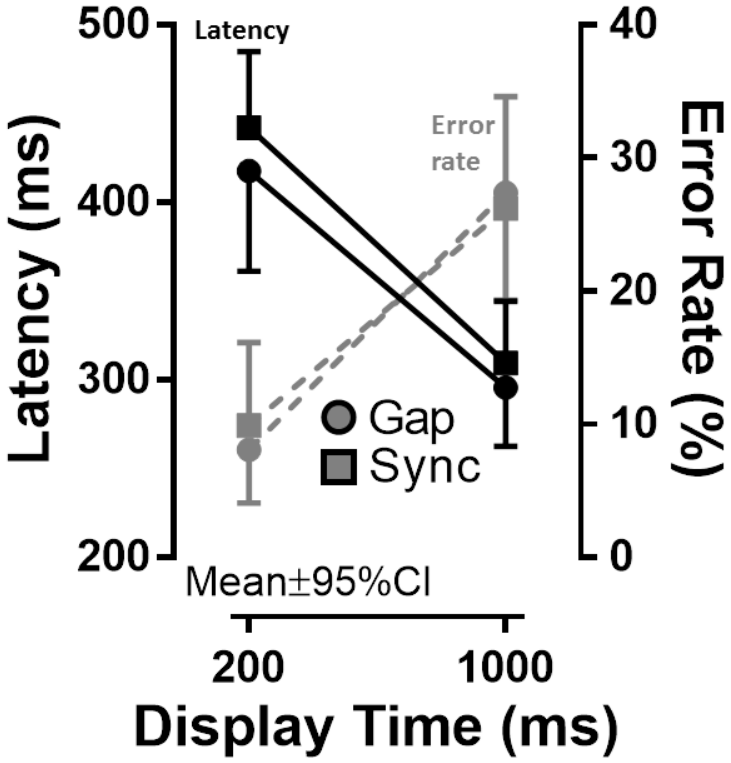
Program#/Poster#: 55.27/CC9

Topic: E.01. Eye Movements

Title: The gap effect on performance in the minimally delayed oculomotor response (MDOR) task

Authors: *P. C. KNOX[1], F. D. A. WOLOHAN[2], E. HEMING DE-ALLIE[1];
[1]Univ. Liverpool, Liverpool, United Kingdom; [2]Psychology, Edge Hill Univ., Liverpool, United Kingdom

Abstract: The antisaccade (AS) task, often used to investigate inhibitory control, involves other aspects of processing (eg attention, working memory). The MDOR task, in which participants inhibit saccades to target onsets and instead saccade to target offsets, generates an error rate (saccades to onsets) and a modulation in saccade latency (Wolohan & Knox, 2014, Exp Brain Res 232:3949). AS error rates are higher in gap AS tasks. Are MDOR error rates similarly affected by gaps and overlaps? In experiment 1, 20 healthy adult participants completed blocks of gap or synchronous MDOR tasks. After a randomised fixation time (1-1.5s), the fixation target was extinguished and a saccade target appeared 5^{o} to either left/right for either 200ms or 1000ms (both randomised) either immediately (synchronous task) or after a gap of 200ms. Participants were instructed to maintain fixation centrally and saccade to the target position on target offset. Eye movements were recorded using an infrared eye tracker; latency and amplitude of primary saccades was measured. Saccades occurring <80ms post target offset, directed at the target, were classed as errors. In experiment 2, 10 participants completed overlap (central fixation target remained throughout the trial) and synchronous tasks. The presence of a gap had no influence on saccade latency to offsets (gap vs synchronous tasks: $F_{1,19}=1.6$; $p=0.2$) or error rate ($F_{1,19}=0.26$; $p=0.6$); display time affected both (latency: $F_{1,19}=70$; error rate: $F_{1,19}=43$; both $p<0.0001$; Figure). In experiment 2, the same display time modulation was observed, there was no difference between overlap and synchronous latency, but error rates were lower in overlap tasks ($F_{1,9}=5$; $p=0.053$). For all trial types, most errors were inhibition failures (ie saccades to onsets). The lack of a gap effect on error rate or latency reflects the lack of competition (present in AS tasks) between the reflexive and the voluntary responses. The reduction of error rates in the overlap task may indicate facilitated inhibition, and a potential dissociation between processes determining latency and error rates.



Disclosures: **P.C. Knox:** None. **F.D.A. Wolohan:** None. **E. Heming De-allie:** None.

Poster

055. Eye Movements I

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Research to Prevent Blindness

Title: Neural mechanisms associated with normal and pathological vergence.

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Abstract: Convergence insufficiency is a common disorder, characterized by impaired vergence eye movements and difficulty with close work, such as reading. Clinically, convergence insufficiency is often detected as an exophoria that is greater at near than at far. Because there is no animal model for this disorder, little is known about the neurological abnormalities that may underlie these symptoms. We have conducted preliminary studies in two monkeys, one with normal convergence and one with a naturally occurring impairment of disparity vergence (CI1). The normal animal easily converged on a scene placed at 10 cm distant (>15° of convergence). However, our impaired animal was only able to achieve 12° of convergence on the near scene for a few seconds at a time, despite extensive training. This vergence response did not decrease when one eye was patched. Disparity vergence was tested using a Risley prism placed in front of one eye while the animal fixated a red laser spot surrounded by a random gray-and-white checkerboard pattern at a distance of 57cm. The animal showed an unstable convergence response (maximum 6°) that increased with prism correction, up to 12 prism diopters (PD). By comparison, the normal monkey (N1) was able to achieve stable, appropriate convergence up to 26 PD. Single unit recording data were collected from 10 near response cells and 8 far response cells in the supraoculomotor area (SOA) while monkey CI1 fixated red plus-shaped LEDs at 12 different distances, in five different directions. After three minutes of recording, neurons were then tested while the animal fixated the visual scene at near (10 cm). Vergence rate-position curves were plotted for all neurons. Although the firing rates were clearly related to vergence angle the mean absolute value of the slope was substantially lower than we observed for a sample of 17 SOA neurons recorded from monkey N1 (Monkey CI1 = 2.78; Monkey N1 = 5.53). In addition, instantaneous firing rates for monkey CI1 were often highly variable, even when the vergence angle was not changing. This was not the case for our normal monkey N1. Our preliminary data suggest that impaired disparity vergence may be associated with aberrant tuning in near response cells.

Disclosures: M.M. Walton: None. **M.J. Mustari:** None.

Poster

055. Eye Movements I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 55.29/CC11

Topic: E.01. Eye Movements

Title: Individual differences in the speed of oculomotor processing

Authors: *B. PARSONS, C. GAMBACORTA, I. LIU, R. B. IVRY;
UC Berkeley, Berkeley, CA

Abstract: Eye movement behavior differs widely between individuals, but is relatively consistent and stable within subjects across a variety of viewing tasks. To investigate the factors underlying these idiosyncratic patterns in visual scanning, twenty subjects were tested on a battery of experimental tasks covering two broad psychological constructs, motor initiation and attentional orienting. We used a novel task, alternating voluntary saccades (AVS), to assess the speed of sequential oculomotor movements with minimal demands on processing related to perception or decision-making. In the AVS, participants made a series of saccades between two targets at a rapid rate, with the primary dependent variable being fixation duration (between successive saccades). Participants completed a series of other tasks used to study eye movements and/or attention. These included a delayed saccade task, fixation stability while maintaining gaze on a small fixation marker, visual search, and the attentional blink task. Mean fixation duration in the AVS was strongly correlated with delayed saccade reaction time, variance in fixation stability, and fixation duration during visual search. In contrast, AVS fixation duration was not correlated with the size of the attentional blink (cost to detect the second of two targets at a short inter-target interval relative to a long inter-target interval), or with accuracy in detecting the first target. The results confirm the existence of stable individual eye movement measures and indicate that the underlying constraint may be associated with individual differences in the speed of motor implementation, rather than attentional factors (i.e., time to shift attention). Whether the motor implementation variability between individuals is related to processes associated with fixation release or saccade initiation remains an open question. This emphasis on motoric constraints in gaze behavior stands in contrast to dominant eye movement control models, which stress exogenous factors and the rate of evidence accumulation.

Disclosures: **B. Parsons:** None. **C. Gambacorta:** None. **I. Liu:** None. **R.B. Ivry:** None.

Poster

055. Eye Movements I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 55.30/DD1

Topic: E.01. Eye Movements

Support: National Institute of Information and Communications Technology (NICT)

"Development of BMI Technologies for Clinical Application" SRPBS by MEXT

JSPS KAKENHI Grant Number 15K16011

Tateishi science and technology foundation

Title: EEG denoising and decoding of smooth pursuit eye movements by using Extra-Dipole Method

Authors: *K.-I. MORISHIGE[1,2], M.-A. SATO[2], M. KAWATO[3];
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Abstract: Eye movements may produce electrical changes that is measured as eye artifacts with EEG data at scalp electrodes. This artifact can be orders of magnitude larger than the signal from the brain, thus making cortical current estimation extremely difficult. In order to remove the eye artifacts, we used Extra-Dipole Method, which is based on hierarchical Bayesian method and simultaneously estimates the cortical and extra-brain source currents while placing dipoles not only on cortical surfaces but also on extra-brain sources. We varied the values for prior information for cortical surface and the eye current source dipoles, and their optimal values for estimation were systematically explored. For this two criteria were deemed necessary. First, the prior and posterior current variances should be the same. Second, the spatial patterns of fMRI activities and estimated current power should be highly correlated. In our previous studies we applied this method to MEG data. Here it is applied to EEG data. EEG is more difficult than MEG because the scalp electrical potential is the combination of different electric conductivities in various shells. The three-shell boundary element method (BEM) model derived from the MRI data set was employed. We calculated the current intensities from the estimated mean cortical currents during tasks. The current intensities were increased in the cortical regions of the lateral occipital temporal cortex (LOTC), the intraparietal cortex (IPC), the precentral cortex (PreCC), and the medial superior frontal cortex (MSFC). These areas are related to the saccadic and smooth pursuit eye movements. By introducing proper prior information, our approach can reasonably remove the effect of artifacts from contaminated EEG signals. To confirm the performance of our denoising method, we reconstructed the target velocities from the estimated cortical currents using a sparse linear regression (SLiR) method. First, the dataset was divided into to ten subsets. For each subset, the optimal sparseness parameter for linear regression model was determined by using an inner 10-fold nested cross-validation. Given this parameter, a regression model was trained using nine of the ten datasets and performance was evaluated on the remaining set. Test datasets for a typical subject demonstrated a good performance (Pearson correlation coefficients: 0.94; determination coefficient: 0.88), and weight values were mainly distributed on the cortical regions that related to the smooth pursuit eye movements. These results indicated that our method isolate cortical activities and eye artifacts from contaminated EEG data by using the Extra-Dipole Method.

Disclosures: **K. Morishige:** None. **M. Sato:** None. **M. Kawato:** None.

Poster

056. Reaching Control: Action and Sensation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 56.01/DD2

Topic: E.04. Voluntary Movements

Support: NSERC Discovery Grant

CIHR doctoral fellowship to CM

Title: Are vestibular contributions to online reach execution processed by an internal model of the limb?

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Abstract: To reach accurately, estimates of body motion are required to compensate for spatial displacement of the limb relative to a reach goal as well as for additional forces imposed on it during body motion. Vestibular signals are important contributors to such estimates. However, the mechanisms by which they contribute to reaching remain poorly understood. Recently, we used galvanic vestibular stimulation (GVS) to selectively activate the vestibular sensors during reaching for different head-re-body orientations and showed that vestibular signals contributing to online reach execution have been appropriately transformed from a head- to a body-centered reference frame (Moreau-Debord et al., J Neurophysiol, 2014). The goal of the current study was to explore whether such transformed vestibular signals are processed by an internal model of limb biomechanics. To address this question, we used GVS to simulate body rotation as human subjects reached in different horizontal plane directions. Model simulations were used to predict how trajectories would be perturbed if subjects used vestibular signals to compensate either 1) purely kinematically for the expected spatial displacement of the arm or 2) both kinematically and dynamically, taking into account the additional forces that would be imposed on the arm by the simulated rotation and its biomechanical properties. If vestibular signals contribute to purely kinematic compensations then GVS is predicted to elicit deviations perpendicular to the desired trajectory that are symmetric for reaches to the left and to the right of body midline. In contrast, if vestibular contributions take into account limb dynamics then when reaching with the right arm, leftward reaches should elicit larger deviations than reaches to the right. To test these predictions, 12 subjects made 20 cm reaching movements while seated with their head inclined forward and their right arm supported in the horizontal plane by a robotic exoskeleton (KINARM, BKIN). Reaches were made in darkness to remembered targets in three directions (straight forward, 60^{o} to the left, 60^{o} to the right). GVS of unpredictable polarity (3 mA pulse) was applied during reaching in 17% of trials chosen pseudo-randomly. Average perpendicular trajectory deviations for forward and leftward reaches were similar in amplitude and significantly

larger than those for rightward reaches, consistent with the predictions for a compensatory mechanism that takes into account both reach kinematics and dynamics. The results suggest that the mechanisms by which vestibular signals contribute to on-line reach execution indeed take into account the biomechanical properties of the limb.

Disclosures: C. Martin: None. **D. Lucien:** None. **P. Lapierre:** None. **A.M. Green:** None.

Poster

056. Reaching Control: Action and Sensation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 56.02/DD3

Topic: E.04. Voluntary Movements

Support: JSPS Grant-in-Aid (KAKENHI) 14430103

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Title: Functional tuning of rubromotoneuronal cells in the forelimb movement in a macaque monkey

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[1]Dept. of Neurophysiol., Natl. Ctr. of Neurol. and Psychiatry, Kodaira, Japan; [2]Ctr. for Neurosci. Studies, Queens Univ., Kingston, ON, Canada

Abstract: Rubromotoneuronal (RM) cells consist of the descending tract that has a monosynaptic contact with spinal motoneurons. As the contribution of RM cells to generating electromyographic (EMG) activity remain elusive, we sought functional significance of RM cells on the basis of analysis of spatiotemporal profiles of its neuronal activity, i.e., synaptic linkages (SL) with muscles (post synaptic effects: PSEs) and functional linkage (FL): a broad temporal correlations with muscles (cross-correlations) in a behaving monkey, as studied in premotor (PreM) neurons in the spinal cord (Takei & Seki 2013). RM cells were identified based on PSEs using spike-triggered averaging; we recorded the single-unit activities from magnocellular part of red nucleus neurons concurrently with EMG activities from the 26 forelimbs muscles while a macaque monkey performed a sequence of whole-limb movements (reach-to-grasp of an object, precision grip, and self-feeding).
The identified 88 RM cells were characterized with divergent SLs with forelimb muscle with a

preference on forearm extensors and the muscles spanning proximal joints, and discharge of bursts in timings where a variety of muscles were recruited.
These RM cells were further subject to cross-correlation analysis with the same 26 muscles with a time windows ranging between 1s before and after the neuronal activity. A pair with a significant cross correlation validated with Monte Carlo simulation was defined as having a FL. We compiled pairs with SLs and FLs among 2288 (88 (RM cells) x 26 (muscles)) pairs and examined their overlap in population.
Out of whole pairs, FL pairs had a greater proportion (784/2288) than that of SL population (327/2288), with positive correlations being predominant (positive:negative = 652:132 out of 784), as contrasted with relatively even proportions of SLs (excitatory:inhibitory = 168:159 out of 327). It is notable that a large number of incongruities between SLs and FLs were found; overlapped pairs (SL∧FL) were counted as 153, whereas the number FL-exclusive pairs (!SL∧FL) was 673 and the number of SL-exclusive pairs (SL∧!FL) was 216, indicating 41.6% of SL pairs were overlapped with FL pairs. Further, out of 153 overlapped pairs, congruent pairs (i.e., positive FL/excitatory SL or negative FL /inhibitory SL pairs) were counted as 76. The disparity of SLs and FLs in RM cells was markedly contrasted with coherent SLs and FLs in spinal PreM interneurons (ca. 80% overlap), suggesting a different functional role of RM neurons than spinal synergistic control.

Disclosures: **T. Oya:** None. **T. Takei:** None. **K. Seki:** None.

Poster

056. Reaching Control: Action and Sensation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 56.03/DD4

Topic: E.04. Voluntary Movements

Support: Strategic Research Program for Brain Sciences

Medtronic Japan External Research Institute

Brain and Information Science on SHITSUKAN (material perception)

Title: Temporal dynamics of interaction between efference copy and sensory feedback in the primary somatosensory cortex

Authors: ***T. UMEDA**[1], Y. NISHIHARA[2,3,4], M. SUZUKI[2,3,4], Y. YAMANISHI[2], T. ISA[2,3], Y. NISHIMURA[2,3];

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Abstract: The brain deal with anticipation of the sensory consequences during stereo-typed voluntary movement. Primary motor cortex (MI) is thought to be source of this anticipations by generating an internal copy of motor output that alerts somatosensory cortex (SI) to change their response to peripheral afferent input, termed efference copy. However, there is no clear evidence how the interaction of inputs from MI and peripheral afferent generates activity of the SI. In this study, we calculated activity of the SI from activity of the MI and peripheral afferents using a decoding algorithm to clarify signal processing in the SI. We simultaneously recorded electrocorticogram (ECoG) signals from cortical areas including the SI and MI, activity of population of dorsal root ganglion (DRG) neurons which conveys activity of peripheral afferents, electromyography of 11 muscles and kinematics of forelimb joints of two monkeys in voluntary reaching and grasping movements. During voluntary reaching-and-grasping task in awake, high gamma activation in the MI and SI preceded movement onset, discharge of DRG assemble and muscle activities. High gamma activity of the SI after movement onset could be predicted from activity of the DRG neuronal ensemble, but not just prior to movement onset. This results implies SI activity contains not only the information from peripheral afferent inputs but also from other source. Activity of the SI in prior to movement onset could be predicted by including high gamma activity of the MI in addition to DRG neurons into the decoding model. Furthermore, prediction of SI activity from activity of the MI and DRG neurons is more accurate than those from activity of premotor cortex and DRG neurons. The results suggest that the SI receives efference copy which represents sensory information in anticipation of upcoming movement from the MI before the actual feedback signal from the periphery. Thus, efference copy from the MI can affect processing of the following feedback signal in the SI.

Disclosures: **T. Umeda:** None. **Y. Nishihara:** None. **M. Suzuki:** None. **Y. Yamanishi:** None. **T. Isa:** None. **Y. Nishimura:** None.

Poster

056. Reaching Control: Action and Sensation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 56.04/DD5

Topic: E.04. Voluntary Movements

Title: Distinct temporal evolution for visuomotor feedback of the hand and target

Authors: *D. W. FRANKLIN;
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Abstract: Visual feedback of the hand and target guides goal-directed reaching movements. However, the magnitude of visuomotor responses to visual shifts of hand location are not consistent throughout the movement, but evolve temporally, peaking in the middle and falling as the hand nears the target (Dimitriou et al., 2013). Recently we showed that the two visual feedback systems of hand and target motion elicit partially independent responses that are integrated in later stages of the sensorimotor system (Franklin et al., 2016), thereby suggesting that the evolution of feedback could differ in these two systems. Here we examined to what degree the temporal evolution of the visuomotor feedback gain of the target differs from the visuomotor feedback gain of the hand. Participants performed reaching movements to a target while grasping a robotic manipulandum. Visual feedback of both the hand position (cursor) and target position was provided in the plane of movement using a virtual reality setup. On random trials (probe trials), the cursor or target was momentarily laterally displaced visually before returning to the actual hand or target position. On all probe trials the hand trajectory was constrained by a mechanical channel in order to measure the resulting feedback force. The perturbations occurred at thirteen different times prior to movement initiation, during movement, or after movement completion. The involuntary force responses to both target and cursor shifts exhibited a strong temporal evolution, and were not present before or after the movement. Consistent differences in the temporal pattern of hand and target responses further demonstrate independent control of visual feedback systems for hand and target motion.
References: Dimitriou M, Wolpert DM, Franklin DW (2013) The Temporal Evolution of Feedback Gains Rapidly Update to Task Demands. J Neurosci 33:10898-10909. Franklin DW, Reichenbach A, Franklin S, Diedrichsen J (2016) Temporal Evolution of Spatial Computations for Visuomotor Control. J Neurosci 36:2329-2341.

Disclosures: D.W. Franklin: None.

Poster

056. Reaching Control: Action and Sensation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 56.05/DD6

Topic: E.04. Voluntary Movements

Support: Piper Health Solutions Seed Grant

NSF Integrative Graduate Education and Research Traineeship

Title: Gravitational effects on proprioceptive sensitivity

Authors: *J. D. KLEIN[1,2,3], B. WHITSELL[4,1], M. PATEL[1,5], P. ARTEMIADIS[4,1], C. A. BUNEO[6,1];
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Abstract: Proprioception is the sense of body position, movement, force and effort. Loss of proprioception can affect planning and control of limb and body movements, negatively impacting activities of daily living and quality of life. Assessments employing planar robots have shown that proprioceptive sensitivity is dependent upon the location of the limb within a horizontal plane (Wilson et al, 2010). Little is known however about proprioceptive sensitivity in the vertical plane. Previous studies have suggested that proprioception plays a key role in anticipating arm configuration dependent effects of gravity (Proske & Gandevia, 2005) suggesting that proprioceptive sensitivity could differ for arm displacements performed with and against the direction of the gravitational vector. To test this hypothesis we developed a novel experimental paradigm employing a 7-DoF anthropomorphic robot arm (LWR4+, KUKA Inc.), which enables reliable testing of arm proprioception along arbitrary paths in 3d space, including vertical motion.A participant's right arm was coupled to a trough held by the robot that stabilized the wrist and forearm, allowing for changes in configuration only at the elbow and shoulder. Sensitivity to imposed displacements of the endpoint of the arm were evaluated using a "same-different" task, where participant's hands were moved 1-4cm away from a recently visited reference position. The proportion of trials where subjects responded "different" when the stimuli were different ("hit rate"), and where they responded "different" when the stimuli were the same, ("false alarm rate"), were used to calculate d', a measure of sensitivity derived from signal detection theory. Data were obtained from 20 subjects, 5 in the upward direction and 16 in the downward direction (one subject was tested in both directions). In both directions sensitivity (d') increased monotonically as the distance from the reference location increased. Sensitivity was generally more variable across subjects for the downward direction and beyond 1 cm median sensitivity was also reduced for the downward direction with respect to the upward direction. A Mann-Whitney U test comparing sensitivity between the upward and downward directions at each displacement distance revealed that sensitivity was significantly different for the 2 cm displacement but not for other distances ($p<0.05$). These data suggest that the ability to estimate the position and velocity of the limb via proprioception might be enhanced for movements performed against gravity, which has important implications for understanding the multisensory planning and control of reaching movements performed in 3d space.

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Poster

056. Reaching Control: Action and Sensation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 56.06/DD7

Topic: E.04. Voluntary Movements

Support: CREST, JST

Cooperative Research Program at the Primate Research Institute, Kyoto University

KAKENHI 70728162

Title: Multisynaptic projections from the basal nucleus of the amygdala to the ventral premotor cortex in macaque monkeys

Authors: *H. ISHIDA[1], K.-I. INOUE[2], M. TAKADA[2], E. HOSHI[1];
[1]Frontal Lobe Function Project, Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan; [2]Systems Neurosci. Section, Primate Res. Institute, Kyoto Univ., Aichi, Japan

Abstract: The forelimb region of the ventral premotor cortex (PMv) plays a central roll in reaching, grasping, and eating food. In such feeding behavior, it is crucial to evaluate a target to reach for. The amygdala has been implicated in this process. Amygdalar neurons code the visual-gustatory valence of food. Dysfunctions of the amygdala induce a compulsion to reach, grasp, and bring to mouth food, regardless of its value. These facts suggest that PMv uses a valence signal about the target derived from the amygdala for planning and executing feeding behavior. Since no direct connectivity between the amygdala and PMv has been reported, the structural basis of their interaction still remains elusive.
In order to identify possible multisynaptic projections from the amygdala to PMv in macaque monkeys, we employed retrograde transneuronal labeling with rabies virus (CVS-11). The survival time was set to allow the second-order neuron labeling across one synapse. Histological analysis of the distribution pattern of amygdalar neuron labeling after rabies injections into PMv revealed that PMv receives disynaptic inputs primarily from the basal nucleus; mostly (67.2% of the total labeled neurons in the amygdala) from the intermediate subdivision, less frequently (13.4%) from the
magnocellular and parvicellular subdivisions, and rarely (1.5%) from the accessory basal nucleus. It was also found that PMv receives additional disynaptic inputs from the central nucleus (10.5%), the dorsal portion of the lateral nucleus (5.9%) and other nuclei (1.5%).
The present results provide the first evidence for the anatomical linkage from the amygdala to PMv. The following structures may mediate the disynaptic projections from the amygdala to the forelimb region of PMv: (1) the medial cortical areas including the presupplementary motor area and cingulate motor areas; (2) the lateral cortical areas including the precentral opercular cortex

and posterior insular cortex; and (3) the cholinergic cell group 4 (Ch4) in the basal forebrain. The PMv could receive the valence signals about target objects derived from the amygdala via these pathways. Dysfunctions of the pathways might underlie Klüver-Bucy syndrome in which animals behave as if they were ignorant about the value of target objects.

Disclosures: H. Ishida: None. **K. Inoue:** None. **M. Takada:** None. **E. Hoshi:** None.

Poster

056. Reaching Control: Action and Sensation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 56.07/DD8

Topic: E.04. Voluntary Movements

Title: Effects of the vertical-horizontal illusion on visual perceptions and manual estimations

Authors: S. YAN[1], *J. M. HONDZINSKI[2];
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Abstract: Visual illusions often provide important clues for understanding perceptual mechanisms associated with sight, which can influence motor output. This illusion refers to the overestimation of the vertical segment when compared to a bisected horizontal segment of the same length. The bisected segment (horizontal) looks shorter than the bisecting segment (vertical). Similarly, the vertical segment also appears to be longer than the horizontal segment when the horizontal segment is not bisected. With discrepancies in the literature we chose to determine if perceptions and manual estimations of healthy young adults were influenced by the vertical-horizontal illusion of varied bisected segments. In the perceptual task, subjects were presented with the inverted "T" (T), modified inverted "T" (MT), or "L" configurations with horizontal and vertical segments equal in length or with 10-15% longer vertical segments, or with 10-15% longer horizontal segments. After viewing a figure, subjects reported which segment in the figure looked longer or if the segments looked equal. In the motor task, subjects were presented with vertically or horizontally oriented reference segments with a very long perpendicular segment attached. Subjects were instructed to touch the figure at the intersection of these two segments, then again at a distance on the long segment so that the length from first touch to second touch matched the length of the reference segment. Perceptual accuracy varied to a large extent across subjects. However, subjects were mostly affected by inverted "T" figures in the perceptual task. Illusory configurations with longer horizontal segments or identical segment lengths had the strongest illusory effects. In the motor task, subject's manual estimations of vertical segment lengths were larger than the estimations of horizontal lengths to

suggest vertical-horizontal illusory effects on manual estimations. These data offer insights to the vertical-horizontal illusion effects on perception and action and suggest that varying the bisection of the illusion is strongest when it occurs centrally.

Disclosures: S. Yan: None. **J.M. Hondzinski:** None.

Poster

056. Reaching Control: Action and Sensation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 56.08/DD9

Topic: E.04. Voluntary Movements

Support: MEXT/AMED SRPBS (BMI)

MEXT KAKENHI 26112004

Title: Modulation of muscle synergy activation during arm movements in patients with hemiparesis

Authors: *T. KAWASE[1], A. NISHIMURA[2], A. NISHIMOTO[2], F. LIU[2], Y. KIM[1], H. KAMBARA[1], N. YOSHIMURA[1], Y. KOIKE[1];
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Abstract: Previous studies have suggested that damage to central nervous system may alter muscle synergies, coordinated activities of multiple muscles that organize modules for motor control. To investigate the alternation following the damage will facilitate more effective design of neurorehabilitation technologies. Most of the studies have focused on muscle synergies of the patients recruited during simple tasks, such as isometric force generation or reaching movements, and contributed significant insight into the research field. However, few studies have investigated muscle synergies of the patients related to complex movements in daily activities. In this study, we investigated how the muscle synergies were modulated during a peg transfer task in patients with hemiparesis toward the realization of effective neurorehabilitation systems covering complex movements.
Seven patients with hemiparesis (Fugl-Meyer upper arm score 19-58) picked a peg on 3×3 grid points on a board and placed it out of the board sequentially by using their affected arms. Six able-bodied subjects also performed the same task. We estimated a set of muscle synergies for each subject by applying nonnegative matrix factorization to electromyographic signals measured during the task, and classified the synergies into 14 classes by using a hierarchical

clustering method. For each class of the synergies, we calculated mean activation coefficients of the synergies during a movement corresponding to each peg position in the patients and the able-bodied subjects.
We found 10 of 14 classes of the synergies were activated during the movements in both of the patients and the able-bodied subjects. The activation coefficients in 4 classes were well explained by coordinates of the peg positions in both the groups (linear regression model, $R^2 > 0.7$), but in 3 classes, different coefficients of the coordinates were derived in the two groups (cosine similarity of the coefficients < 0.7). One of the classes showed almost opposite effect of the coordinates in the two groups (cosine similarity of the coefficients < −0.9).
These results suggested the patients modulated activation levels of the muscle synergies according to the peg position, but the manner of the modulation was differed from the able-bodied subjects. The results may be useful for development of new neurorehabilitation technologies.

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Poster

056. Reaching Control: Action and Sensation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 56.09/DD10

Topic: E.04. Voluntary Movements

Support: ISF Grant 1787/13

GIF Grant I-1224-396.13/2012

Title: Thalamocortical mechanisms controlling motor timing in behaving primates

Authors: ***A. NASHEF**, O. COHEN, Y. PRUT;
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Abstract: The timing of actions is considered to be dictated by cerebellar output that is relayed to the motor cortex via the motor thalamus. This hypothesis is consistent with the finding that cerebellar patients exhibit poorly timed and uncoordinated actions. We investigated the mechanisms by which the cerebellar-thalamo-cortical (CTC) system dictates temporal properties of motor cortical activity and the events that emerge when information flow through this pathway is temporarily interrupted.
Monkeys were trained to perform a 2-D reaching task that required tight control of motor timing.

A cortical chamber was implanted above the motor cortex and stimulating electrodes were chronically implanted in the ipsilateral superior cerebellar peduncle (SCP). Neural activity was recorded from primary motor (M1, n=252) and premotor areas (PM, n=131).
Single pulse SCP stimuli efficiently recruited neurons in both M1 and PM (77% and 68% respectively) producing an early excitation followed by a prolonged inhibition. Cortical response in M1 occurred earlier than in premotor cortex (2.9 vs. 3.6 ms, $p < 0.01$) and had a shorter duration, whereas the subsequent inhibition was significantly longer (34.6 vs. 26.5 ms, $p < 0.01$).
Persistent high frequency SCP stimulation (HFS) led to a significant increase in reaction time (RT; -144ms vs. -189.3ms in control; $p<0.005$) and movement time (MT; 447.2ms vs. 369.6ms in control; $p<0.001$). In addition, the path travelled from center position to the peripheral target became more variable and generally longer (3.8cm vs. 3.5cm in control; $p<0.001$). Finally, these changes were more prominent for targets that required a coordinated elbow-shoulder movement.
These behavioral changes were accompanied by changes in neural activity. We computed the preferred direction (PD) of single cortical cells and their phasic-tonic index (PTI) which measured their tendency to fire in a tonic vs. phasic manner. Single cortical cells maintained their PD during HFS trials but their PTI decreased significantly ($p < 0.005$), consistent with a shift from a phasic to tonic response pattern.
These results suggest that the CTC evokes an extensive excitatory-inhibitory motor cortical volley that is temporally organized across M1 and PM areas.
Interfering with the flow of information in this pathway produces motor deficits similar to those found in cerebellar ataxia. The neural correlate of these behavioral changes is the loss of phasic firing at movement onset. It is thus suggested that CTC system controls the timing and coordination of voluntary movements by shaping the response pattern of single cortical cells independently of their spatial properties.

Disclosures: **A. Nashef:** None. **O. Cohen:** None. **Y. Prut:** None.

Poster

056. Reaching Control: Action and Sensation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 56.10/DD11

Topic: E.04. Voluntary Movements

Support: EU FP7 604063 HealthPAC

NWO-VICI 453-11-001

Title: Task-dependent vestibular feedback corrections in reaching

Authors: *J. KEYSER, L. P. J. SELEN, W. P. MEDENDORP;
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Abstract: When reaching for an object during self-motion, our motor system should appropriately integrate vestibular signals to compensate for the intervening motion and its induced inertial forces. We asked whether the brain processes these vestibular signals automatically with the aim to preserve hand trajectory in space or more flexibly, correcting trajectories only in task-relevant spatial dimensions.
Using a robotic manipulandum, we tested subjects (n=24) making reaches to either a narrow (2 cm) or a wide (60 cm) target in front of them. Subjects reached to the target without instruction on where to hit it. In 20% of reaches, subjects received galvanic vestibular stimulation (GVS) and shutter glasses prevented visual feedback. In GVS trials, the current was proportional to hand speed, but clipped at 3 mA, with either positive polarity (cathode left) or negative polarity (cathode right).
We found that the same vestibular stimulation led to smaller trajectory corrections for the wide target compared to the narrow target. We interpret this reduced compensation as a task-dependent modulation of vestibular feedback responses, tuned to minimally interfere with the task-irrelevant dimension of the reach (Todorov and Jordan, 2002). These flexible vestibular feedback corrections mimic the sophistication seen in feedback responses to mechanical (Pruszynski et al., 2008) or visual (Knill et al., 2011) perturbations of limb position.
References:
Knill, David C., Amulya Bondada, and Manu Chhabra. “Flexible, Task-Dependent Use of Sensory Feedback to Control Hand Movements.” *The Journal of Neuroscience* 31, no. 4 (January 26, 2011): 1219-37. doi:10.1523/JNEUROSCI.3522-09.2011.
Pruszynski, J. Andrew, Isaac Kurtzer, and Stephen H. Scott. “Rapid Motor Responses Are Appropriately Tuned to the Metrics of a Visuospatial Task.” *Journal of Neurophysiology* 100, no. 1 (July 1, 2008): 224-38. doi:10.1152/jn.90262.2008.
Todorov, Emanuel, and Michael I. Jordan. “Optimal Feedback Control as a Theory of Motor Coordination.” *Nature Neuroscience* 5, no. 11 (November 2002): 1226-35. doi:10.1038/nn963.

Disclosures: J. Keyser: None. **L.P.J. Selen:** None. **W.P. Medendorp:** None.

Poster

056. Reaching Control: Action and Sensation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 56.11/DD12

Topic: E.04. Voluntary Movements

Support: JSPS KAKENHI 15H03062

JSPS KAKENHI 15K12363

Title: Effect of handedness on unilateral and bilateral reaching movements under the influence of the mirror illusion

Authors: *K. YAMANAKA;
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Abstract: The mirror illusion can be used to induce systematic errors for reaching movements made with the unseen hand behind the mirror. However, it remains unclear whether the reaching errors induced by the mirror illusion differ depending on 1) movement of the contralateral hand seen in the mirror (unilateral or bilateral reaching) and 2) participant's handedness (right-hander or left-hander). The aim of this study is to investigate the performance of the unilateral and bilateral reaching under the influence of the mirror illusion in right- and left-handed individuals. Six right-handed and six left-handed female volunteers conducted reaching tasks under the influence of the mirror illusion. Participant initially placed one hand in front of, and the other hand behind, a mirror placed vertically in the mid-sagittal plane on the table. She asked to move the hand seen in the mirror to the directions toward or away from the mirror, or not to move it. After 10 s of exposure to the mirror reflection, participant reached to unseen target situated 15 cm forward with their unseen hand behind the mirror (unilateral reaching) or both hands simultaneously (bilateral reaching). We record the reaching trajectories and endpoint errors made with the tip of index finger of the unseen hand using by VICON motion capturing system. As a result, regardless of whether the mirror faced to right or left side, reaching errors in horizontal direction were larger in the bilateral reaching than in the unilateral reaching. These results suggest that participants can detect their visual-proprioceptive conflicts in unilateral reaching condition more easily, while they remain insensitive to the conflict in the bilateral reaching condition. Next, when the mirror faced to the right side, unseen left-hand reaching errors in anteroposterior direction were larger in the right-handers than in the left-handers. In contrast, when the mirror faced to the left side, there was no difference in unseen right-hand reaching errors between right- and left-handers. These results suggest that right-handers are difficult to control their non-dominant left hand under the influence of the mirror illusion while left-handers can control their non-dominant right hand even under the influence of the mirror illusion.

Disclosures: K. Yamanaka: None.

Poster

056. Reaching Control: Action and Sensation

Location: Halls B-H

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Program#/Poster#: 56.12/DD13

Topic: E.04. Voluntary Movements

Support: NIH CoBRE P20GM109098

NIH/NIGMS U54GM104942

Title: Sensorimotor transformation during reaching investigated with transcranial magnetic stimulation and biomechanical simulations

Authors: *R. L. HARDESTY, JR[1,2], E. OLESH[2], B. POLLARD[2], P. H. ELLAWAY[3], V. GRITSENKO[2];
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Abstract: Coordinated movement of the multi-segmental musculoskeletal system requires the nervous system to employ or compensate for diverse dynamic properties. This is achieved by multisensory integration and internal predictive circuits shaped by experience. While the importance of this information has been extensively documented, the specific roles of forward motor planning and sensory feedback to the formation of motor commands is not entirely understood.
We investigated the role of cortical input to the formation of motor commands during reaching by healthy young human subjects. The subjects pointed to virtual targets placed in locations that created distinct dynamic characteristics, such that the movement was largely passive (#1), interaction torques were resistive with increasing gravitational load (#2), and interaction torques were assistive with decreasing gravitational load (#3). A wearable headset (Oculus Rift) and the WorldViz (Vizard) system was used to create the virtual environment and provide visual feedback of performance. At randomized time periods during the reaching movement, the primary motor cortex was noninvasively stimulated using transcranial magnetic stimulation. During movement electromyography (EMG, MotionLab Systems) of 12 muscles of the arm and kinematics (PhaseSpace) were recorded. Kinematics was used to calculate joint torques and simulate sensory feedback. Motor evoked potentials (MEPs) were integrated and normalized to background EMG activity measured in non-TMS control trials. Sensory feedback was simulated using published models and an OpenSim model of the human arm to estimate muscle lengths. The data was analyzed with hierarchal clustering of multiple correlations to derive quantitative representations of imbedded relationships between motion biomechanics and neural signals. Preliminary results suggest that corticospinal signals measured with MEPs have strong relationships with task-specific dynamic biomechanical variables, while simulated sensory

signals have strong relationships with kinematic biomechanical variables. This suggests that sensorimotor transformation includes task-specific changes of the modality of sensory feedback signals into a dynamic domain.

Disclosures: R.L. Hardesty: None. **E. Olesh:** None. **B. Pollard:** None. **P.H. Ellaway:** None. **V. Gritsenko:** None.

Poster

056. Reaching Control: Action and Sensation

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 56.13/DD14

Topic: E.04. Voluntary Movements

Support: NIH T32HD055180

Title: Neural and kinematic effects of increased reliance on visual feedback in prosthesis users

Authors: *J. T. JOHNSON, L. A. WHEATON;
Applied Physiol., Georgia Inst. of Technol., Atlanta, GA

Abstract: Reach and grasp is one of the most fundamental tasks performed by humans. When sensory feedback from a limb is lost due to disease or amputation, compensatory processes such as increased reliance on vision are thought to be used to ameliorate the deficit. These compensatory processes may come at the cost of increased cognitive load as well as inefficiencies in execution of motor tasks, both of which may contribute to the high rate of abandonment of upper extremity prostheses. To date, no formal studies have been conducted to demonstrate the impact of increased visual reliance in prosthesis users. The purpose of this study was to assess the neural and kinematic differences in a reach and grasp task between intact subjects and intact subjects using an upper extremity prosthesis simulator when vision is occluded. Subjects were divided into four groups; (1) intact using vision, (2) intact with occluded vision, (3) fictively amputated using vision, and (4) fictively amputated with occluded vision. EEG and kinematic data were gathered while the subjects performed a repetitive reach and grasp task. EEG differences in neural behavior as measured by task-related potential, as well as differences in kinematics as measured by harmonicity and smoothness of movement were compared between the four groups with intact/fictive amputation and vision/occluded vision as factors. The results of this study can help drive improvements in rehabilitative and therapeutic practices based on the effect of vision in conjunction with a prosthetic device.

Disclosures: J.T. Johnson: None. **L.A. Wheaton:** None.

Poster

056. Reaching Control: Action and Sensation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 56.14/DD15

Topic: E.04. Voluntary Movements

Support: DFG-Grant Fi1567/4-2: Reference frames for reaching movements to somatosensory targets [02/2015-09/2017]

Title: Gaze-dependent coding of proprioceptive reach targets is influenced by effector movement and availability of online information

Authors: *S. MUELLER[1], K. FIEHLER[2];
[1]Justus-Liebig Univ. Giessen, Giessen, Germany; [2]Justus-Liebig-University Giessen, Giessen, Germany

Abstract: In previous research, we demonstrated that the spatial coding of proprioceptive reach targets depends on the presence of an effector movement (Mueller & Fiehler, 2014, 2016). In these studies, participants were asked to reach (in total darkness) with their right hand to the location in space where they had felt a touch on a fingertip of their left hand. In the moved condition, they had to actively move their left hand forward to the target location followed by a touch on one of their fingertips, and then had to move their hand back to the start before reaching to the remembered location of the felt touch in space. In the stationary condition, the left hand was kept at the target location throughout the trial. Thus, the stationary condition did not contain a movement of the target effector; however, online proprioceptive information about the target location was available that was absent in the moved condition. Evidence for gaze-dependent coding reflected by reach errors opposite to gaze direction was obtained in the moved but not in the stationary condition.
In the present experiment, we were interested in whether the observed switch from gaze-independent to gaze-dependent coding is due to the effector movement or due to the availability of online proprioceptive information about the target during reaching. We asked participants to reach to a proprioceptive-tactile target while gaze was varied relative to the target's location. In addition to the moved and the stationary condition, we conducted a moved-online condition in which participants performed the same movement with their target hand as in the moved condition but instead of reaching to the remembered proprioceptive target location, they reached to the current location of their fingertip that had been touched.
We examined whether reach errors varied as a function of gaze relative to target between the three conditions. Indeed, we found a significant interaction showing gaze-dependent reach errors in the moved and moved-online conditions but not in the stationary condition. However, the gaze effects in the moved-online condition were less pronounced than in the moved condition

suggesting that both, the effector movement and the availability of proprioceptive online information about the target location influence the contribution of gaze-dependent coding in proprioceptive reaching.

Disclosures: **S. Mueller:** None. **K. Fiehler:** None.

Poster

056. Reaching Control: Action and Sensation

Location: Halls B-H

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Program#/Poster#: 56.15/DD16

Topic: E.04. Voluntary Movements

Title: Differential sensory control of the reach and the grasp in 12-month-old human infants: Increased visual guidance of the reach relative to the grasp in a skilled reach-to-eat task

Authors: *J. M. KARL, A. WILSON;
Thompson Rivers Univ., Kamloops, BC, Canada

Abstract: The ability to reach out and grasp an object is generally believed to develop under visual control and to be mature by about 12 months of age. An alternate view, however, is that the Reach and the Grasp are separate movements, mediated by separate neural pathways that initially develop under haptic control with vision acquiring subsequent control later in development. A hypothesis that has emerged from this latter view is that the Reach and the Grasp come under visual control at different developmental rates. Specifically, it is proposed that visual control of the Reach may developmentally precede visual control of the Grasp. To test this hypothesis we filmed 12-month-old infants and healthy adults as they reached, grasped, and ate ring-shaped pieces of cereal. Adults were separated into a Vision condition (V) and a No Vision condition (NV) whereas infants always had Vision. The participants' movements were video recorded from bottom-up, face-on, and reach-side views and offline frame-by-frame video analysis was used to analyze two measures of Reach accuracy (Maximum Height of the Index Knuckle and Variability of the Reach Trajectory) and two measures of Grasp accuracy (Index-Thumb Aperture at First Contact with the Cereal and Time from First Contact to Final Grasp), with increased accuracy on each of these measures reflecting a greater influence of visual input on movement execution. Preliminary results suggest that the accuracy of the Reach of 12-month-old infants is more similar to that of sighted adults, whereas the accuracy of the Grasp is more similar to that of unsighted adults. These results lend support to the idea that the Reach and the Grasp follow different developmental trajectories such that visual guidance of the Reach is more prominent than visual guidance of the Grasp by 12 months of age.

Disclosures: **J.M. Karl:** None. **A. Wilson:** None.

Poster

056. Reaching Control: Action and Sensation

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Topic: E.04. Voluntary Movements

Support: DoD Grant W81XWH1310496

Title: Former unilateral amputees exhibit bilateral differences in the control of reach to grasp actions

Authors: ***D. MATTOS**[1], N. BAUNE[1], B. PHILIP[1], K. VALYEAR[2], C. KAUFMAN[3], S. FREY[1];
[1]Occup. Therapy, Washington Univ. Sch. of Med., Saint Louis, MO; [2]Psychology Dept., Univ. of Bangor, Bangor, United Kingdom; [3]Christine M. Kleinert Inst., Louisville, KY

Abstract: Upper limb amputation is associated with reorganization of the somatosensory system, where changes are detected in the cerebral hemispheres contra- and ipsi-lateral to the trauma. Some of these changes persist even years after the reversal of amputation through hand replantation or transplantation. We used high spatiotemporal-resolution motion analysis to test the hypothesis that, as a result of limitations in somatosensation, these individuals are heavily dependent on visual feedback of the limb to coordinate reach-to-grasp actions. This study included six control subjects, two hand replants, and one hand transplant recipient (the latter evaluated longitudinally). All were right-handed and, with the exception of one of the replants, had their left hand affected. Subjects grasped cubes of three different sizes, with (light) and without (dark) vision of the hand. Importantly, objects were luminescent and thus always visible. We measured: the peak (PGA) and timing (tPGA) of the grip aperture (measured between index finger and thumb), and the peak (PTV) and timing (tPTV) of the transport velocity of the hand. The values for the right and left hands of the patients were compared to the 95% CI for the mean of the control group, matched by hand dominance. *Controls*. The PGA and tPGA scaled with the object size in both vision conditions. The PGA was smaller in the dark, with a larger decrease in the left than in the right hand. The PTV was ~15% larger for the dominant right [51.77 ± (SD) 3.28 cm/s, CI: 48.33 - 55.21] vs. the left hand (45.12 ± 2.30 cm/s, 42.71 - 47.54), and ~10% larger in the dark (50.75 ± 4.36 cm/s, 46.17 - 55.33) than in the light (46.15 ± 3.29 cm/s, 42.69 - 49.60). *Patients.* As with controls, PGA scaled with object size and was also smaller in the dark. However, when the smaller objects were grasped with the affected hand, patients did not scale the PGA to the same extent as the controls. The PTV was higher than the 95% CI of controls

with the *affected* and *unaffected* limb and for both vision conditions. In contrast to controls, in the affected hand, the values of PTV of all patients were similar in the light and dark. For the unaffected hands of the two replants, the PTV was higher in the dark vs. light, while in the transplant, the PTV was similar in both conditions. Although our hypothesis was not supported, we observed unexpected differences in performance with the unaffected limb in all three patients. While further work is needed, we speculate that these changes in the sensorimotor control of the healthy limb may be attributable to documented bilateral reorganizational changes within sensory and/or motor cortex following unilateral deafferentation.

Disclosures: D. Mattos: None. **N. Baune:** None. **B. Philip:** None. **K. Valyear:** None. **C. Kaufman:** None. **S. Frey:** None.

Poster

056. Reaching Control: Action and Sensation

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Topic: E.04. Voluntary Movements

Support: NIH NS035103

NIH F32 NS093721

Title: Intracortical microstimulation maps of motor, somatosensory, and posterior parietal cortex in macaque monkeys

Authors: *M. K. BALDWIN[1], D. F. COOKE[2], A. B. GOLDRING[3], L. KRUBITZER[1]; [1]Ctr. for Neurosci., Univ. of California Davis, Davis, CA; [2]Ctr. for Neurosci., Unviersity of California Davis, Davis, CA; [3]Ctr. for Neurosci., Univ. of California, Davis, CA

Abstract: Long-train intracortical microstimulation (ICMS) has been used in a number of primates and recently tree shrews to reveal movement maps in both motor and posterior parietal cortex; however, the full extent, location, and number of those maps, and the characteristics of the movements elicited using ICMS have not been fully explored in macaque monkeys. In the current study, long-train ICMS was used to reveal the organization of movement maps within a large portion of the primary motor cortex as well as the parietal lobe. Our results revealed roughly topographic motor maps throughout primary motor (M1), as well as areas 1, 2, 5, and 7. Movements involving the forelimb, and specifically movements of digits were represented over the largest area of cortex, especially within posterior parietal cortex. Further, movements usually involved multiple digits or forelimb joints and resembled natural hand movement typical of

macaques, including precision grips and full hand grasps. Representations of similar grasp movements were observed in areas 1, 2, 5L, and 7b. Taken together we find that much of motor and parietal cortex contains representations of ethologically relevant hand movements. Further, movements can be evoked from a larger extent of parietal cortex than was previously believed.

Disclosures: M.K. Baldwin: None. **D.F. Cooke:** None. **A.B. Goldring:** None. **L. Krubitzer:** None.

Poster

056. Reaching Control: Action and Sensation

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Topic: E.04. Voluntary Movements

Support: DFG IRTG Brain Act 1901

SFB/TRR 135

Title: Neural correlates of perceiving audiovisual consequences of voluntary movements

Authors: *B. ARIKAN[1], B. VAN KEMENADE[2], K. PODRANSKI[2], O. STEINSTRÄTER[2], B. STRAUBE[2], T. KIRCHER[2];
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Abstract: Theories of motor-control suggest an internal forward model where an efference copy signal is used to predict the consequences of voluntary movements. This mechanism leads to an attenuation of the sensory input mainly to save resources for the processing of unexpected stimuli. Sensory attenuation has been observed for auditory, somatosensory and visual modalities both on a behavioral and neural level, however no research has been done yet on the common experience where a self-generated movement leads to multiple sensory consequences. We aimed to investigate neural mechanisms involved in the prediction of voluntary movements leading to unimodal vs bimodal sensory consequences. In addition, we aimed to manipulate the influence of predictability on attenuation by assessing the processing of temporal discrepancies in the movement-consequence relationship. Participants performed hand movements gripping the handle of a custom-made device inside the scanner while seeing an online movie of their hand as they moved. We systematically introduced temporal delays to the recorded movie, and asked participants to judge whether there was a delay between their hand and the online movie. In order to account for the influence of efference copy, we introduced a passive condition where the handle was moved automatically. Half of the trials involved an auditory tone coupled to the

movement onset, corresponding to bimodal sensory feedback condition. Preliminary imaging analyses showed reduced activation in areas linked to somatosensory, visual and auditory processing in the active compared to the passive condition, suggesting suppressed activity for the sensory consequences of voluntary movements, both for unimodal and bimodal consequences. This is in line with the behavioral finding showing better delay detection the passive condition. These preliminary results support the hypothesis that efference copy-related predictive mechanisms contribute to BOLD suppression in auditory, visual and somatosensory cortices. Further analyses are planned to assess the influence of subjective experience of delay detection, and the contribution of multisensory feedback in this process.

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Poster

056. Reaching Control: Action and Sensation

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Topic: E.04. Voluntary Movements

Support: NIH EY021252

NSF 1329829

NSF 0953652

Title: Ultrasound imaging of upper limb muscle velocity during passive movements and involuntary reflex responses

Authors: ***E. MCKENNA**, N. AKHLAGHI, P. OTTO, M. HARRIS-LOVE, W. M. JOINER, S. SIKDAR;
George Mason Univ., Fairfax, VA

Abstract: Ultrasound imaging can be used to visualize and quantify the mechanical deformations (contraction and relaxation velocity) of different muscle compartments in deep tissue. The sensing of muscle activation through mechanical rather than electrical changes may provide complementary insight into changing muscle properties during movement that are not readily observed with standard surface electromyography (EMG) sensors. Specifically, it is difficult to characterize passive movements using EMG, but these movements can be distinguished with ultrasound imaging. There is also a clinical need to separate these passive motions from involuntary muscle activation in certain disease states such as spasticity where

there is an abnormal velocity-dependent resistance to passive stretch. We tested the ability to distinguish these respective motions in unimpaired healthy subjects by examining the changes in mechanical and electrical activity during passive arm reaching movements and subsequent involuntary stretch reflex responses. Using a robotic manipulandum, subjects made passive 10 cm horizontal planar movements with the right arm at 8 different movement durations (200, 250, 300, 350 400, 600, 800, and 1000 ms). We tested two directions of motion: passive flexion of the triceps moving the limb away from the body (90^o) and passive flexion of the biceps moving the limb towards the body (270^o). We made simultaneous measurements of the biceps and triceps muscle velocity, surface EMG, subject applied force and manipulandum joint velocity, and made several observations based on these data. First, there was good correlation between ultrasound based muscle velocity and joint velocity measures for purely passive movements, with minimal EMG activity and force. Second, as the movement speed increased ($\leq$ 400 ms duration) we observed involuntary stretch reflexes which were confirmed using surface EMG and force measures. Additionally, the stretch reflex response was readily observed in the ultrasound data and the measured muscle velocity showed higher variability, which was modulated by motion speed. This quantification and discrimination of passive and involuntary muscle activation patterns through mechanical deformations could potential help differentiate healthy muscle mechanical properties from abnormal states, such as the activation disorders that result from spasticity.

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Poster

056. Reaching Control: Action and Sensation

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Program#/Poster#: 56.20/EE4

Topic: E.04. Voluntary Movements

Support: VR 2011–3128

Title: Muscle spindle discharges from lower leg muscles during active ankle movements in humans

Authors: *E. JAROCKA, B. B. EDIN;
Umea Univ., Umea, Sweden

Abstract: We have developed a novel paradigm that for the first time allows stable recordings from unitary human muscle spindle afferents from all lower leg muscles actuating the ankle joint

during active, unconstrained movements of the foot within most of its physiological range. The recordings were obtained from the sciatic nerve ca 5 cm proximal to the popliteal crease with the participants lying prone with the left knee flexed and the lower leg in a vertical position.
The foot was attached to a custom-made contraption which provided a stable position of the lower leg but allowed unconstrained movements in the ankle joint. We recorded force/torque and the 3D position of the ankle joint and surface EMG from the eight accessible lower leg muscles. Muscle length changes for all ankle joint muscles but plantaris were calculated from the joint angles using OpenSim.
The participants controlled the position of a cursor on a video screen by dorsal/plantar flexion and inversion/eversion of the ankle joint. During microneurography they performed a target-chasing task in which they moved the cursor into a displayed target as quickly as possible. As soon as the target had been reached, the trial was over and a new target appeared.
We have recorded from >40 muscle spindle afferents (ca 75% Ia) originating from almost all 12 muscles of interest during >9,400 target-to-target movements. The movement range was about ±20 deg in dorsal/plantar flexion and inversion/eversion corresponding to about ±5 % muscle length changes. The target hit rate varied between 30-60/min and for most afferents >75 movements were recorded.
All afferents responded to passive elongation as has been described repeatedly in the past. During active movements, however, no muscle spindle afferent showed a simple relationship between its discharge and the kinematics of its parent muscle. Nevertheless, during the target-chasing task, the vast majority of spindle afferents encoded muscle stretch velocity whereas only a handful encoded muscle length albeit poorly. In a subset of afferents, recordings during the task were obtained also with loading in the dorsal/plantar direction but it affected discharge rates only marginally. The preliminary analyses indicate that the spindle discharges did not depend on the direction of the ankle joint movement, e.g., with similar muscle stretch during dorsal flexion combined with inversion or eversion, the spindle discharges from the gastrocnemius seemed indistinguishable.
In conclusion, muscle spindle responses during active ankle joint movements cannot be extrapolated from their responses during passive movements.

Disclosures: **E. Jarocka:** None. **B.B. Edin:** None.

Poster

056. Reaching Control: Action and Sensation

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Program#/Poster#: 56.21/EE5

Topic: E.04. Voluntary Movements

Support: CIHR MOP106662

Title: Using robotics to assess sense of effort in the upper extremity

Authors: L. LOGAN[1], J. SEMRAU[1], S. H. SCOTT[3], *S. P. DUKELOW[2];
[2]Clin. Neurosciences, [1]Univ. of Calgary, Calgary, AB, Canada; [3]Queen's Univ., Kingston, ON, Canada

Abstract: Sense of effort, a component of proprioception, is our perception of the amount of effort required to execute actions (e.g., knowing how hard to squeeze a fruit to determine its ripeness). Given its importance in performing activities of daily living, we developed a robotic task to assess sense of effort. We aimed to investigate sense of effort in the upper limb for 1) different levels of effort and 2) different limb positions in healthy subjects. We hypothesized that subjects would accurately match the effort required to resist a force in one arm with their opposite arm. Further, we hypothesized that they would perform best with their arms in the same position. In the task, a robot applied a submaximal torque (2/3/4Nm) at the elbow. Individuals were asked to match the effort of this torque with the opposite elbow. Their reference arm remained in a constant position (30°/shoulder and 90°/elbow), while their matching arm was tested at a 30° at the shoulder and 75/90/105° at the elbow (0° is full extension). We performed preliminary testing in five healthy individuals in both flexion and extension.
The mean difference between torques in both arms (matched minus reference) across all trials was -0.21±1.03 Nm. The middle torque, 3Nm, was matched most accurately (2.77±0.87Nm). Subjects overestimated loads of lower magnitude (2Nm: 2.33±0.84Nm) and underestimated loads of higher magnitude (4Nm: 3.27±1.10Nm). Subjects also performed better in the more proximal workspace. To quantify this, we computed the ratio of the reference torque matched (matched torque/reference torque). In the 90° position, this ratio was 0.97±0.35 when matching elbow flexors and 0.95±0.29 for extensors. In the 105° position, it was 0.95±0.39 (flexors) and 0.99±0.31 (extensors). Subjects were less accurate in the more distal position (75°). Subjects' matches exceeded the reference torque when matching in flexion (1.15±0.46) and fell below the reference for extension (0.80±0.30).
In conclusion, our preliminary results show that individuals matched elbow torques with reasonable accuracy, but demonstrated variability in performance. Perceived sense of effort was most accurate in positions closer to the body and at the 3Nm torque level. Thus, similar to position sense, sense of effort may be more accurate in the proximal workspace. This work provides a baseline for future studies, and provides an important first step towards the development of a robotic tool to detect deficits in sense of effort in individuals with stroke and brain injury.

Disclosures: **L. Logan:** None. **J. Semrau:** None. **S.H. Scott:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Scott is the Chief Scientific Officer for the company (BKIN Technologies Ltd.) that manufactures the robot used in the present study.. **S.P. Dukelow:** None.

Poster

056. Reaching Control: Action and Sensation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 56.22/EE6

Topic: E.04. Voluntary Movements

Support: MotMotLearn: 637488

Title: Explorative motor learning is decision-making with motor noise

Authors: *X. CHEN, J. GALEA;
Sch. of Psychology, Univ. of Birmingham, Birmingham, United Kingdom

Abstract: The ability of humans to learn a new motor task through behavioural exploration has received considerable recent attention (Dam et al., 2013; Wu et al, 2014). This learning has been characterised as reinforcement learning in which participants use a reward prediction error to learn actions that optimise future reward. One interesting question raised by these studies concerns the contribution of exploration and motor noise to motor learning (Therrien et al., 2015). Our current work shows that explorative motor learning can be conceptualised as a decision making process adapted to motor noise.
We investigated participants' learning performance in a motor reaching task and a decision-making analogue. The tasks were isomorphic with the exception that the motor task was subject to motor noise, whereas the decision making task was not. Specifically, in the motor task, participants had to discover the shapes of 24 hidden trajectories, each of which was characterised by two parameters: direction and curvature. Participants had 25 reaching attempts to find the optimal trajectory, but the only feedback they received was a monetary score that reflected the proximity of their attempt to the target. Importantly, we also took an independent measurement of each participant's motor noise. In the decision-making task, participants had to discover a hidden target cell within a grid that reflected the direction and curvature parameters. Although the feedback function was identical as that used in the motor task, participants did not make a reaching movement but simply selected a cell (thus removing the influence of motor noise).
First, we modelled the decision making task by formulating the learning problem as a Partially Observable Markov Decision Process. The optimal control strategy derived, which governs model behaviour, was a consequence of maximising the cumulative reward. The model was able to explain 91±6% of participants' decision-making behaviour. Next, we applied this model (with the same estimated parameters) to the motor task by adding each participant's motor noise. In other words, we defined the learning observed in the motor task as a decision making process with motor noise. In 80% of the participants (remaining 20% did not perform the motor task correctly) this model was able to explain 79±11% of participant's behaviour during the motor learning task.

This indicates that explorative motor learning could be cast within the same broad theoretical framework used to describe decision-making, such as loss aversion and a propensity to be modulated by levels of dopamine and serotonin in the brain.

Disclosures: X. Chen: None. **J. Galea:** None.

Poster

056. Reaching Control: Action and Sensation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 56.23/EE7

Topic: E.04. Voluntary Movements

Title: Rapid visuomotor corrections in reaching are modulated by gaze position

Authors: *A. J. DE BROUWER, T. JARVIS, J. P. GALLIVAN, J. R. FLANAGAN; Queen's Univ., Kingston, ON, Canada

Abstract: Goal-directed movements are guided by continuous visual feedback about the hands and the target object. This visual feedback supports rapid, automatic corrections to perturbations in hand and/or target position. Here, we examine how the system corrects for lateral visual perturbations in the position of the cursor representing the hand during unimanual and bimanual reaching. Two narrow or two wide targets were always presented and participants were required to fixate one or the other target. We replicated the finding that corrective forces are stronger for narrow than for wide targets, consistent with the framework of optimal feedback control. We found that corrections were stronger during unimanual reaches compared to bimanual reaches. This suggests that there may be limited resources for the processing of visuomotor errors, such that greater resources can be called upon when only one hand is reaching and, as a consequence, the location at which a perturbation could occur is certain. We also found that corrections were stronger when the perturbed hand was directed towards the fixated target compared to the non-fixated target and that the effect of target width on correction strength was stronger when the perturbed hand was directed towards the fixated target. These results suggest that fixating reach targets may confer an advantage in terms of visuomotor error processing.

Disclosures: A.J. De Brouwer: None. **T. Jarvis:** None. **J.P. Gallivan:** None. **J.R. Flanagan:** None.

Poster

056. Reaching Control: Action and Sensation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 56.24/EE8

Topic: E.04. Voluntary Movements

Support: CIN Pool Project 2014-03: Investigating Body Representation Distortions in Patient Populations using Biometric Self-Avatars in Virtual Reality

Title: Objects vs. hand: the effect of knuckle misconceptions on localization task distortions

Authors: *A. SAULTON, H. BÜLTHOFF, S. DE LA ROSA;
Max Planck Inst. For Biol. Cybernetics, Tuebingen, Germany

Abstract: Stored representations of body size and shape as derived from somatosensation are considered to be critical components of perception and action. Recent research has shown the presence of large hand distortions in proprioceptive localization tasks consisting of an overestimation of hand width and an underestimation of finger length. Those results were interpreted as reflecting specific somatosensory perceptual distortion bound to a body model underlying position sense. One important prerequisite to this interpretation is that measured localization task distortions actually stem from body representation. In this study, we re-examine hand distortions underlying positon sense and investigate whether these distortions are body specific or due to non-perceptual factors, e.g. conceptual knowledge. Participants made localization judgments regarding the spatial position of various landmarks on occluded items including their own hand. Our results show that larger hand distortions in localization tasks are likely to be induced by participants' incorrect conceptual knowledge about hand landmarks rather than proprioceptive or somatosensory influences. Moreover, we show that once we account for such incorrect conceptual knowledge, hand distortions in localization tasks are statistically similar to those of other objects. These results suggest that localization task distortions are not specific to the hand and call for caution when interpreting localization task distortions in terms of body specific effects.

Disclosures: **A. Saulton:** None. **H. Bülthoff:** None. **S. de la Rosa:** None.

Poster

056. Reaching Control: Action and Sensation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 56.25/EE9

Topic: E.04. Voluntary Movements

Support: Wellcome Trust Grant 101002/Z/13/Z

Title: Facilitation of short latency responses in human arm muscles by stimuli targeting the reticular formation

Authors: *I. S. GLOVER, S. N. BAKER;
Inst. of Neurosci., Newcastle Upon Tyne, United Kingdom

Abstract: Background: Previous studies have demonstrated that visual stimuli can elicit arm muscle activity at latencies of 75-100ms, with these rapid visual reactions (RVRs) being time-locked to target appearance rather than movement onset (Pruszynski et al., 2010). To investigate the hypothesis that RVRs are mediated by the reticulospinal tract (RST), we combined the RVR-eliciting task with stimuli thought to target the reticular formation: median nerve stimulation, vestibular stimulation and startle. **Methods:** Intramuscular and surface electrode recordings were made from the deltoid and pectoralis major of 16 subjects performing a reaching task in which they were required to make fast movements toward targets appearing within a virtual reality set up. Median nerve stimulation was applied at twice motor threshold at -200, -100, -50, 0, 50 ms relative to target appearance (n=8). In separate experiments, subjects received vestibular stimulation (4mA, 20ms) or startle noises at -150, -100, -75, -50, 0 ms relative to target appearance (n=8). **Results:** Median nerve stimulation, vestibular stimulation and startle all increased the magnitude of RVRs up to 3-fold; this facilitation was significant across all tested stimulus latencies and was observed with both intramuscular and surface recordings. The RVRs showed spatial tuning and were largest in trials with the fastest reaction times. **Discussion:** The short latency of RVRs and their facilitation with stimuli targeting the reticular formation suggest that the RST may contribute to the generation of rapid movements toward visual stimuli.
References: Pruszynski JA, King GL, Boisse L, Scott SH, Flanagan JR, Munoz DP. (2010). Eur J Neurosci. 32(6):1049-57

Disclosures: I.S. Glover: None. **S.N. Baker:** None.

Poster

056. Reaching Control: Action and Sensation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 56.26/EE10

Topic: E.04. Voluntary Movements

Support: NSERC

Title: Absolute haptic cues mediate pantomime-grasping only when egocentric visual cues are delayed

Authors: *S. DAVARPANAH JAZI[1], J. CHAN[1], M. HEATH[1,2];
[1]Kinesiology, [2]Grad. Program in Neurosci., Univ. of Western Ontario, London, ON, Canada

Abstract: Pantomime-grasping entails a movement with dissociated stimulus-response relations and/or responses towards physically removed target objects. Unlike *naturalistic* grasps that are mediated via a target's absolute visual properties and specified in egocentric reference frames, relative and allocentric visual cues support pantomime-grasping. Notably, however, our group recently demonstrated that providing haptic feedback (i.e., of a physically removed target) following a pantomime-grasp supports an absolute visuo-haptic calibration. In the current study we examined specific sensory and spatial requirements necessary to support a visuo-haptic calibration during pantomime-grasping. To that end, in a series of experiments participants pantomime-grasped differently-sized target objects while receiving haptic feedback (i.e., through physical touch) following response completion. Notably, the target's spatial location as well as online limb and target vision were manipulated. Results showed that an absolute visuo-haptic calibration process is limited to situations wherein a spatially-overlapping pantomime-grasp is performed following a visually-based memory delay. In accounting for our results we have drawn upon the maximum likelihood estimator model's (MLE) tenet that multisensory cues integrate in an optimal fashion with processing weighted towards the more reliable sense. As such, we propose that the decay of visual cues renders an increased weighting and salience of haptic signals and thus, supports an absolute visuo-haptic calibration.

Disclosures: S. Davarpanah Jazi: None. **J. Chan:** None. **M. Heath:** None.

Poster

056. Reaching Control: Action and Sensation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 56.27/EE11

Topic: E.04. Voluntary Movements

Support: Grant-in-Aid for JSPS Fellows

Title: Motor adaptation to experimentally extended bills in pigeons.

Authors: *H. MATSUI[1], E.-I. IZAWA[2];
[1]Grad. Sch. of Human Relations, Keio Univ., Shinagawa-ku, Japan; [2]Psychology, Keio Univ., Minato-ku, Tokyo, Japan

Abstract: Feeding behavior in primates consists of two motor components such as arm-reaching to a target food and hand-grasping it, which are visually controlled. Analogously, pecking in birds is composed of neck-reaching and bill-grasping. However, pigeons are known to close their eyes during pecking, suggesting no visual control. Previous studies revealed that pigeons show a brief pause at a close distance to a target, called as fixation, before initiating pecking, suggesting a feedforward control: during fixation, the timing of bill-grasping is predicted based on the perceived distance to the target to generate an appropriate motor command and the command is executed without visual control during pecking. However, it remains unknown what learning mechanisms operate to integrate between the visually perceived distance and the motor control of the timing of bill-grasping. In this study, we hypothesized that the learning is driven by the error between the predicted timing of grasping and the actual timing of physical contact with the target as a somatosensory feedback signal in pecking of pigeons. We examined whether pigeons could adapt their pecking behavior to experimentally extended pseudo-bills. The extended pseudo-bill required pigeons to adjust the timing of bill-grasping since the distance to contact with the target is shorten for the extended bill length. In this study, pigeons' pecking foods (i.e., hempseeds) on a platform was high-speed video-recorded and compared kinematic profiles between before and after pseudo-bill attachment and also after its removal. If pigeons can adapt pecking to the extended bills, the motor adjustment to the bill extension is expected to remain even after its removal. After introducing 10 sessions of 2-43 feeding trials with bill extension, we found that the timing of bill-grasping was temporally advanced in all the 3 pigeons. As expected, the advanced timing of bill-grasping remained even after the bill-extension removal. This bill-grasping adjustment did not occur on the first day of the pseudo-bill attachment, suggesting the lack of visual contribution to the pecking adaptation. These results suggest that pecking in pigeons is controlled by a feedforward system involving an error-based learning mechanism with a somatososory feedback signal.

Disclosures: H. Matsui: None. **E. Izawa:** None.

Poster

057. Reaching Movements: Movement Control and Strategy

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 57.01/EE12

Topic: E.04. Voluntary Movements

Support: CIHR MOP97944, MOP142220

GRSNC-FRQS

Title: “Change of mind” (COM) of reach direction from one target to another during reach is more frequent and corrects for more initial target choice errors in speed than precision regimes.

Authors: *S. DUROCHER, J. BEAULAC, L. AIT-ALI, J. F. KALASKA;
Neurosciences, Univ. De Montreal, Montreal, QC, Canada

Abstract: Ten subjects did a "Choose-and-Go" (CG) task in which they decided if a checkerboard-like Decision Cue (DC) had more Blue or Yellow squares and then reached to the one of two targets whose color (B,Y) matched the DC’s dominant color. In each trial, they viewed 1 of 120 DCs with a wide range of total and relative numbers of B&Y squares, from 30/0-100/0 B or Y (strong color bias, evidence for only one target) to 30/30-100/100 B & Y squares (i.e., zero color bias, equal evidence for both targets). Subjects were told to be either precise about their target choices at the cost of longer response times (RTs; Precision regime, P), or fast at the cost of more choice errors (Speed regime, S) in 260-trial blocks on different days. Each subject performed 6000-7000 trials of the CG task in each of the P and S regimes over several daily sessions. In the P regime (Durocher & Kalaska SfN 2015) RTs became progressively longer and error choices increased as DC color bias decreased. Subjects’ RTs were mainly determined by the difference in the number of Y&B squares in the DCs rather than the total numbers of Y&B squares supporting each target. We report here that subjects’ RTs were systematically shorter for all DCs in the S regime, especially for DCs with low color biases (mean RTs for all DCs: P 910±30ms; S 509±23ms). However, overall target-choice success rates decreased only modestly from P to S regimes (mean: P 79.5±1.8%; S 77.3±0.7%). In most trials, subjects began to reach to the correct target, more often overall in the P regime (mean: P 5053±215; S 4843±248), and less often as DC color bias decreased in both regimes. Also, subjects usually reached directly to one target, more often in the P regime (mean: P 95.9±2.3%; S 87.1±5.3%), with similar success rates across regimes (mean: P 80.1±2.1%; S 78.3±1.4%). In other trials, subjects started toward one target but then reversed direction to the other (“change of mind”; COM). COMs were more frequent in the S regime (mean: P 4.1±2.3%; S 12.9±5.2%). COM frequency increased as DC color bias decreased in both regimes, and occurred for all color biases in the S regime but mainly at low biases in the P regime. COMs corrected wrong initial target choices with similar overall frequency in both regimes (mean: P 65.9±6.8%; S

70.7±7.1%), and showed nearly identical increases in the rate of correction for wrong initial choices as DC color bias increased. These findings show that success rates of COM trials are similar in P and S regimes and lower than in non-COM trials. Initial target choices are less accurate in the S regime of the CG task, but COMs correct more reaches with wrong initial choices in the S than the P regime, resulting in nearly similar overall task success rates.

Disclosures: **S. Durocher:** None. **J. Beaulac:** None. **L. Ait-Ali:** None. **J.F. Kalaska:** None.

Poster

057. Reaching Movements: Movement Control and Strategy

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 57.02/EE13

Topic: E.04. Voluntary Movements

Support: Department of Occupational Therapy, University of Pittsburgh, PA

Title: Interaction torque control deficits in patients with stroke

Authors: ***A. SETHI**[1], S. RAJ[2], N. DOUNSKAIA[3];
[1]Occup. Therapy, Univ. of Pittsburgh, Pittsburgh, PA; [2]Bioengineering, Univ. of PIttsburgh, Pittsburgh, PA; [3]Arizona State Univ., Phenix, AZ

Abstract: Introduction: Among the 795,000 individuals who sustain a stroke annually in the United States, 85% exhibit impairments in function of one (or affected) upper extremity (UE), which limits their ability to perform daily tasks such as eating. Current treatments have limited effectiveness, possibly because these treatments may not improve control and coordination of joint motions required to produce efficient UE movements. Movements of healthy adults are characterized by effective incorporation in joint control of interaction torque (IT) caused by mechanical interactions among limb segments during their motion. Research suggests that the skillful use of IT for movement production relies on sophisticated feedforward and feedback mechanisms implemented through activity of multiple brain areas. It is therefore can be hypothesized that stroke disrupts the ability to exploit IT for movement production, thus causing dyscoordinated UE movements. Here, we tested this hypothesis with the use of a kinetic analysis applied to shoulder, elbow, and wrist motions during reaching for a can of soda.
Methods: Five individuals with mild impairments after stroke and six age-matched healthy controls participated in the study. The task was to reach and grasp a soda can. Controls reached with their dominant arm, while patients used their affected arm. At the beginning of each trial, the arm rested on the knee in the neutral position. The can was placed on a table in front of the subject in the parasagittal plane of the shoulder of the used arm. The can position ensured motion

of the entire arm in the parasagittal plane. Arm movements were recorded with a VICON motion capture system. Net torque (NT), muscle torque (MT), GT, and IT were calculated at the shoulder, elbow, and wrist. At each joint, NT=IT+GT+MT. We computed the percentage contribution of IT to NT for each joint in both the acceleration (acc) and deceleration (dec) phases of the hand motion.
Results: In both the acceleration and deceleration phases, the percentage contributions at the shoulder {[Acc ($U = 2$; $p = .01$); Dec ($U = 0 = 2$; $p = .004$) and elbow [Acc ($U = 0$, $p = .004$); Dec ($U = 0$, $p = .004$)]} were significantly higher in healthy controls than patients with stroke. However, the percentage contribution was not significantly different at the wrist between both groups ($U = 14$, $p = .93$).
Conclusion: Though stroke patients were able to complete the task, a lower IT contribution suggests inefficient movement control at the shoulder and elbow joints as compared to healthy controls. This finding supports the hypothesis that stroke disrupts the ability to effectively use IT for joint rotations during UE movements.

Disclosures: **A. Sethi:** None. **S. Raj:** None. **N. Dounskaia:** None.

Poster

057. Reaching Movements: Movement Control and Strategy

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 57.03/EE14

Topic: E.04. Voluntary Movements

Support: NIH-R01-HD045639

NIH-R01-HD081346

NIH-R01HD087089

NSF-EAGER 1548514

Title: Predictability and effort in complex object control

Authors: ***P. MAURICE**[1], F. YE[2], C. J. HASSON[3], D. STERNAD[4];
[1]Biol., [2]Electrical and Computer Engin., [3]Physical Therapy, [4]Biology, Electrical and Computer Engineering, Physics, Northeastern Univ., Boston, MA

Abstract: Manipulation of complex objects or tool use is ubiquitous in everyday life and has given humans an evolutionary advantage. Yet, there has been little research on interaction with dynamic objects, and control principles for such actions remain elusive. To gain insight into the

control of complex motor skills, such as carrying a cup of coffee, the present study examines the strategies that humans choose when manipulating an object with nonlinear internal dynamics. The under-actuated system renders the temporal evolution complex and even chaotic, hence unpredictable. Humans then have to continuously correct and adapt their behavior, which is physically and cognitively tiring. Extending previous research, we hypothesize that humans develop strategies that make the hand-object interactions predictable to avoid excessive sensorimotor processing, even if such strategies require higher interaction forces. To test this hypothesis, a simplified version of moving a cup of coffee back and forth between two targets was implemented in a virtual environment, using a cart-and-pendulum model to mimic coffee sloshing in a cup. Analysis of the mathematical model reveals that the amplitude, frequency and relative cart/pendulum phase strongly influence the force profile and the predictability of the hand-object interaction required to perform the motion. Importantly, optimal predictability did not coincide with low force profiles. Two experiments were conducted in which subjects rhythmically manipulated a robotic manipulandum to displace the virtual cup (cart) containing a rolling ball (pendulum). In Experiment 1, subjects (n=8) were paced at the pendulum resonance frequency, but were free to choose the amplitude of the motion. In Experiment 2, subjects (n=9) were free to choose the frequency of the oscillations, whereas the amplitude was imposed. Results of Experiment 1 showed that oscillation amplitude increased with practice, leading to more predictable object dynamics, while there was no significant change in effort. In Experiment 2, two different strategies appeared, respectively with high and low frequency oscillations, likely the resonance modes of the coupled hand-cup system. Both strategies corresponded to a highly predictable behavior, and again, not to low force profiles. These results show that in complex object manipulation, humans do not prioritize mechanical efficiency, but rather seek strategies where interactions are predictable. This conclusion contradicts common expectations from studies on unrestrained movements and manipulation of simple rigid objects, and highlight that different principles rule the manipulation of complex objects.

Disclosures: **P. Maurice:** None. **F. Ye:** None. **C.J. Hasson:** None. **D. Sternad:** None.

Poster

057. Reaching Movements: Movement Control and Strategy

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 57.04/EE15

Topic: E.04. Voluntary Movements

Support: NWO-VICI: 453-11-001

Title: The role of the vestibular system in eye-hand coordination

Authors: *L. OOSTWOUD WIJDENES[1], J. MASSELINK[2,1], W. P. MEDENDORP[1];
[1]Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ. Nijmegen, Nijmegen, Netherlands; [2]Inst. of Psychology, Univ. of Muenster, Münster, Germany

Abstract: The world offers many potential targets to direct our eye- or hand movements towards. If the body and the world are stationary, which of these potential targets is selected depends on the expected reward associated with the targets and on the cost of moving to the targets. Experiments that disentangled the response preference for eye and hand movement showed that when the eye and hand move to the same target in a coordinated fashion, target selection resembles the preference of hand-only target selection (Horstmann and Hoffmann, 2005). Thus in stationary environments, hand target selection guides the eye movement.
In every day life we often select targets while our body is moving. Body motion is registered by our visual, proprioceptive and vestibular sensors. Recent results from our lab show that vestibular information about body acceleration affects target selection for eye and hand movements. For eye movements, we found a preference for targets that are in the direction of body acceleration. However, for hand movements, data suggests that at the moment of reach execution there is a preference for targets that are in the direction opposite to body acceleration. Thus it seems that the vestibular effect on eye movements is diametrically opposite to the effect on hand movements. Here we ask how combined eye- and hand movements are coordinated under passive full-body translation.
Participants are seated in a vestibular sled that moves them in a sinusoidal fashion along the inter-aural axis. After central fixation of the eye and hand, they are presented with two targets, 10 degrees of visual angle to the left and right of central fixation. The targets are presented at different phases of the sled motion. By dynamically updating the difference in onset time between the two targets, we measure response preference for the eye only, hand only, and a combined condition with eye and hand movements.
Experiments are currently underway and the results analysed according to following hypotheses. If response preferences in the combined effectors condition resemble the preferences in the eye only condition, this suggests that the eye is leading the hand. Vice versa, if response preferences resemble the hand only condition, as is observed in the stationary situation, this suggests that the hand is leading the eye. A third possibility is that at the critical peak acceleration phases, opposing targets are selected for eye and hand movements. This would suggest that the vestibular effect on target selection is response effector specific, even when the two effectors move at the same time. Possibly, cost and reward are optimized for eye and hand separately.

Disclosures: L. Oostwoud Wijdenes: None. **J. Masselink:** None. **W.P. Medendorp:** None.

Poster

057. Reaching Movements: Movement Control and Strategy

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 57.05/EE16

Topic: E.04. Voluntary Movements

Title: Speed and accuracy in reaching during full-body reaching tasks

Authors: *S. T. LEITKAM, M. E. APPLEGATE, A. HOYNACKE, J. S. THOMAS;
Ohio Univ., Athens, OH

Abstract: Fitts' Law states for a reaching target, the maximum speed and accuracy at which the target can be reached is governed by the size and distance of the target from the end-effector. Few have investigated if these relationships hold true in full-body reaches requiring movement of the trunk and head. Accordingly, 20 healthy participants performed full body reaches to virtual cylindrical targets at four target locations that necessitated lumbar excursions of 0°, 15°, 30°, 60°, with three indices of difficulty (ID=3, 5, 7). For each target location, the size of the cylinder was adjusted to maintain ID according to Fitts' Law. The targets appeared in random order oriented to face the participants who were instructed to reach the targets as quickly and accurately as possible. Participants reached to each target location and ID combination five times.

Movement accuracy was analyzed by comparing the position of the end-effector at the time of the first peak anterior excursion with the position of the center of the target. The distance between the two points was separated into three measures with respect to the target: overshoot error (through the plane of the target), vertical/anterior error ("up" axis in the target plane), and lateral error (horizontal axis). Movement time (MT) was the time between first movement of the hand and first peak excursion after the target appeared. The error measures and MT were analyzed using a mixed-model MANOVA with ID and target location as within subject factors and sex as the between subjects factor.

There was no main of effect of ID on any of the error measures. There were main effects of target location on overshoot error ($p<0.05$) and vertical/anterior error ($p<0.05$). Overshoot error decreased as target height decreased, indicating less movement past the target for trials that required large lumbar excursions. Vertical/anterior error increased in the direction of the participant as target height decreased, indicating that for trials that required large lumbar excursions, participants did not reach out as far for the target. Main effects for MT were found for both ID ($p<0.05$) and target location ($p<0.05$). The movement times were greater for larger ID and for lower targets.

While MT increased as ID increased (consistent with Fitts' Law), MT increased and accuracy decreased as a function of target location when controlling for ID (inconsistent with Fitts' Law). This indicates there are neural or motor control factors influencing full-body reaching tasks that

are not present in other systems that are well-defined by Fitts' Law. Maintaining balance or changing visual frame of reference may override the classic speed-accuracy tradeoff.

Disclosures: S.T. Leitkam: None. **M.E. Applegate:** None. **A. Hoynacke:** None. **J.S. Thomas:** None.

Poster

057. Reaching Movements: Movement Control and Strategy

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ETH Scientific Equipment Grant 0-43824-13

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ERC Grant 294115

Title: Challenging rat motor skills: development of a two-choice forelimb task

Authors: *A. C. MOSBERGER[1], O. LAMBERCY[2], N. HEIRI[1], N. BJELOPOLJAK[1], R. GASSERT[2], M. E. SCHWAB[1];
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Abstract: Many rodent studies of action selection use two-choice tasks requiring the animal to perform a specific motor response in one of two different locations within a behavioral setup (e.g., left vs. right nose-poke, left vs. right lever press, left vs. right runway). In these tasks, the kinematics of the movements themselves are similar or identical for both responses, and the choice is about WHERE to respond. Here, we aim to investigate the choice of HOW to respond in a given context. More precisely, we study the selection of different skilled actions directed toward the same object. Our paradigm models the interaction with specific objects using upper limb movements in rodents. To achieve this, we have developed a novel task requiring the selection of one of two skilled forelimb movements using a three-degree-of-freedom robotic manipulandum (Vigaru et al. 2013, Lambercy et al. 2015). We train the rats to perform different skilled forelimb motor tasks while recording the precise position of the manipulandum during the forelimb interaction. Animals learn to perform two skilled forelimb movements, i.e. rotation and pulling of the manipulandum, associated with different auditory, visual, and object-based cues;

and go through different stages of training to achieve high success rates in both tasks. Subsequently, animals are asked to respond to the specific cues with either rotation or pulling movements in sessions where both trials are presented in an intermixed design. The detailed kinematic recordings of the interaction with the manipulandum allow us to detect and separate wrongly executed but correctly intended movements vs. wrongly intended but correctly executed movements. This analysis gives a new and unique perspective on rodent skilled action selection. We are currently investigating the impact of different cue modalities on the animals' choice behavior. Furthermore, we are performing extracellular electrophysiological recordings in the dorsolateral striatum to identify neuronal signatures of choice and movement execution.
Lambercy O, Schubring-Giese M, Vigaru B, Gassert R, Luft AR, Hosp JA. 2015. Sub-processes of motor learning revealed by a robotic manipulandum for rodents. Behav Brain Res 278: 569-76
Vigaru BC, Lambercy O, Schubring-Giese M, Hosp JA, Schneider M, et al. 2013. A robotic platform to assess, guide and perturb rat forelimb movements. IEEE transactions on neural systems and rehabilitation engineering : a publication of the IEEE Engineering in Medicine and Biology Society 21: 796-805

Disclosures: **A.C. Mosberger:** None. **O. Lambercy:** None. **N. Heiri:** None. **N. Bjelopoljak:** None. **R. Gassert:** None. **M.E. Schwab:** None.

Poster

057. Reaching Movements: Movement Control and Strategy

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 57.07/EE18

Topic: E.04. Voluntary Movements

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Title: Spatially selective graded modulation of neural motor planning activity suggests biased competition between task rules in action selection

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Abstract: Prior to choice, neurons in dorsal premotor cortex (PMd) and parietal reach region (PRR) can simultaneously encode two alternative spatial locations when monkeys have equal

preference for reaching towards them. Such dual spatial encoding occurs even when competing spatial transformation rules determine the two potential reach goals relative to the position of single spatially incongruent visual cue, suggesting co-encoding of competing potential motor goals. Here, we test if and how PMd and PRR neurons encode two potential reach goals when monkeys showed graded preferences for the two motor goals. Particularly, we ask if the spatial profile of neural modulation is compatible with known models of biased competition.
Two rhesus monkeys had to determine the correct reach goal from two instructive cues. A pre-cue consisted of two differently colored triangles which appeared at one of the four cardinal directions from the center (e.g. top) and which pointed to two opposite directions (clockwise and counterclockwise). The pre-cue indicated the two possible goals at 90 deg CW/CCW from the pre-cue. Triangle sizes could differ and represented rule validity. A delayed rule cue indicated the valid rule if its color matched one of the pre-cue triangles, or, indicated that both options were rewarded with equal probability (free-choice) if it was white.
Results show that graded planability of movements due to the pre-cue induced a graded bias in subsequent free-choice behavior, despite balanced expected values at the time of commitment. The spatial selectivity of PMd and PRR neurons during motor planning was modulated according to the subsequent choice bias, showing bimodal neural spatial selectivity in unbiased trials, increasing neural responses with increasing preference of the animal for the preferred direction of the neuron, decreasing responses for the opposite-to-preferred direction and no bias-dependency for neurons with preferred direction orthogonal to the potential reach directions. Existing biased-competition models with center-surround inhibition at the level of spatial encoding seem not sufficient to explain the observed spatial pattern of bias-induced response modulations. Thus, based on our previous dynamic neural field model (Klaes et al. 2012), we propose that biased competition in rule-guided action selection is implemented via a strong competition between pools of neurons which encode the conjunction of a spatial cue and a learned transformation rule, as they exist in visuomotor association areas. Particularly, we postulate the competition between neurons which share the same spatial preference but which have different rule preference.

Disclosures: **L. Suriya-Arunroj:** None. **A. Gail:** None.

Poster

057. Reaching Movements: Movement Control and Strategy

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Topic: E.04. Voluntary Movements

Support: Natural Sciences and Engineering Research Council of Canada

Title: Interception of virtual dynamic objects in atypical gravitational accelerations

Authors: *B. J. CHANG, K. M. STUBBS, D. J. QUINLAN, J. C. CULHAM;
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Abstract: In many sports, we often intercept dynamic objects with various aerodynamic properties and acceleration vectors. Due to non-gravitational forces such as Magnus forces and air resistance in sports like baseball and ultimate frisbee, these objects often have a downward acceleration vector that differs from Earth's native acceleration ($g = 9.81 m/s^2$). How we compensate for these accelerations and intercept these objects remains unknown. That said, we know that expert groups such as jugglers can attend to and intercept multiple objects at different accelerations. In this study, we examine how untrained individuals intercept dynamic virtual objects at different accelerations. We used a back-projected setup allowing participants to directly interact with a large screen. Optotrak (NDI, Waterloo, Canada) infrared emitting diodes (IREDs) were used to calibrate the projected image to real space as well as to track the hand movements. We tested 3 accelerations at a regular and inverted set up in six conditions (-1.5, -1.0, -0.5, 0.5, 1, and 1.5 g). In each condition, participants observed a virtual circle follow a parabolic trajectory velocity at different accelerations. Participants were instructed to intercept the object with their index finger within an allotted space. Preliminary results showed that across all accelerations, inverted (negative) accelerations elicited both slower reaction times as well as an initiation of movement later in the objects trajectory compared to non-inverted (positive) acceleration conditions. These results suggest that additional cognitive processing is required for accelerations which are not often observed in our natural environment. Furthermore, higher gravitational accelerations generally elicited quicker reaction times in both the inverted and non-inverted conditions. In the inverted condition, participants initiated their movement later in the trajectory with greater acceleration. However, this trend was not observed in the non-inverted condition. We are currently expanding this research to include jugglers as an expert group.

Disclosures: B.J. Chang: None. **K.M. Stubbs:** None. **D.J. Quinlan:** None. **J.C. Culham:** None.

Poster

057. Reaching Movements: Movement Control and Strategy

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Topic: E.04. Voluntary Movements

Support: Beatrice Menne Haggerty Center for Research on Brain Injury and Repair in Stroke

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Title: Strength vs precision: What does the mouse automated reach task assess?

Authors: *A. BECKER[1], D. BETZ[2], M. P. GOLDBERG[1];
[1]Neurol. and Neurotherapeutics, UT Southwestern Med. Ctr., Dallas, TX; [2]Univ. of Texas at Dallas, Dallas, TX

Abstract: Rodent models of stroke recovery have often failed to translate into useful treatment. Research translation might be improved by developing behavioral tests in rodents that address functionally similar impairments to those of humans. We recently developed a behavioral assessment of functional stroke recovery in mice called the automated reach task. This task requires the use of distal forelimb musculature, the precision of which often suffers in the context of human ischemic stroke. We have previously demonstrated that focal photothrombotic cortical stroke of the forelimb motor area of mice produces a deficit in performance of this task that can be automatically quantified and lasts much longer than in many typical mouse behavioral assays, mirroring the chronic human condition. We asked a further question: is the deficit caused by a loss of precision in distal muscle control, as is the case in the human condition, or to loss of strength alone? To answer this question, we modified the task by lowering strength requirements while leaving precision requirements high and analyzed post-stroke performance of mice in the modified task. We find evidence that reach task performance is sensitive to loss of precision.

Disclosures: A. Becker: None. **D. Betz:** None. **M.P. Goldberg:** None.

Poster

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Topic: E.04. Voluntary Movements

Support: NIH Grant R01NS084948

Title: Recalibration, heuristics, and learning de novo: On the multiple processes of sensorimotor learning and the role of the medial temporal lobe

Authors: *S. D. MCDOUGLE, N. B. TURK-BROWNE, J. A. TAYLOR;
Princeton Univ., Princeton, NJ

Abstract: It has been over 50 years since seminal work with patient HM (Milner, 1962) led to the codification of explicit and implicit memory systems. Although this work and many other studies have revealed surprising learning of novel sensorimotor tasks (e.g., mirror drawing, rotary pursuit) after medial temporal lobe damage, a detailed examination of how such patients learn — and also why they can display subtle impairments — has been lacking. Recent work has revealed that multiple learning processes contribute to sensorimotor adaptation, including cerebellar-based recalibration (Shadmehr et al., 2010), use-dependent plasticity (Diedrichsen et al., 2010; Verstynen & Sabes, 2011), cognitive strategies/heuristics (Taylor et al., 2014), and the apparent *de novo* learning of entirely new control policies (Telgen et al., 2014). Two seemingly similar sensorimotor tasks — visuomotor rotations and mirror reversals — involve surprisingly different mixtures of these learning processes. Rotation tasks rely on both slow, cerebellar-dependent recalibration (Tseng et al., 2007) and a fast, explicit process that provides rapid, flexible "good enough" solutions to the task, such as "re-aiming" (McDougle et al., 2015). In contrast, mirror reversal is thought to induce the creation of an entirely new control policy from scratch, since recalibration would only amplify the error (Telgen et al., 2014). Here, in healthy controls, we confirm this dissociation, showing that performance in rotation tasks is aided by iterative error-based recalibration, whereas mirror reversal must rely on learning a novel control policy. Importantly, both tasks engage explicit learning, but the latter requires outside coaching for subjects to utilize the appropriate strategy. Furthermore, we replicate the findings from HM in a patient with complete bilateral hippocampal loss, LSJ. Her learning in mirror reversal tasks is comparable to naive age-matched controls, suggesting a preserved ability to learn a new control policy over the course of several days. Controls who are instructed about the nature of the mirror reversal show rapid explicit learning, whereas LSJ is unaided by such instruction. Consistent with this failure of explicit learning, her performance in rotation tasks is slower than controls, reflecting the operation of only an iterative calibration process. These findings confirm the presence of multiple learning processes in sensorimotor tasks and illustrate a more nuanced, process-oriented role for the medial temporal lobe in motor learning.

Disclosures: **S.D. McDougle:** None. **N.B. Turk-Browne:** None. **J.A. Taylor:** None.

Poster

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Title: Exploring structure-specific knowledge in a visuomotor adaptation task

Authors: *K. BOND, J. A. TAYLOR;
Princeton Univ., Princeton, NJ

Abstract: Structural learning is a meta-learning phenomenon evidenced by an accelerated learning rate for novel tasks sharing the same structure as the training task. Exposure to pseudorandomly varying visuomotor perturbations of a fixed structure facilitates the rate at which novel, yet isostructural, perturbations are learned (Braun et al., 2009). Previous investigations suggest that this effect is driven by the implicit extraction of invariant task features, thereby reducing the dimensionality of the hypothesis space (Genewein et al., 2015). However, we have shown that participants can actively counter rotations with explicit strategies, demonstrating explicit structural knowledge of the perturbation (Bond and Taylor 2015). We sought to determine whether participants truly extract task structure (i.e., rotational structure) or use simple heuristics to solve perturbations.

We exposed separate groups of participants to pseudorandom perturbations drawn from either a rotational structure or a complex structure (consisting of a combined rotation, scaling, and shear perturbation) prior to exposing them to a 'consistent' rotation at the end of training (test phase). To probe explicit knowledge of the task conditions, participants were instructed to report their intended aiming direction via a touchscreen monitor prior to reaching.

In contrast to previous results, we found that the rotation and random groups countered the consistent rotation in the test phase equally well ($p = 0.2$). Furthermore, for the rotation group, performance was not attributable to an implicit internal model ($p = 0.07$), but rather explicit aiming strategy ($p < .001$). We examined reported aiming locations in the exposure phase and found that participants in the rotational structure group aimed in what appeared to be a rotation-appropriate way. Indeed, aiming error slowly improved over time. The random structure group failed to show these benefits, suggesting that a relatively simple structure is needed for effective aiming strategies to develop. Despite these differences, aiming strategies in both groups shared some structural similarities. This may reflect either a general heuristic or a higher-order task structure, such as the distribution of training targets.

Given these results, it's likely that there are multiple sources of meta-learning in a visuomotor adaptation task. We suggest that one of these sources may be able to extract structural information from exposure to a variable perturbation sequence and refine aiming strategy, and that another may be more consistent with a generalized aiming heuristic.

Disclosures: K. Bond: None. **J.A. Taylor:** None.

Poster

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Topic: E.04. Voluntary Movements

Support: NIH Grant NS084948

Title: Assessing explicit strategies (or knowledge?) in force field adaptation experiments

Authors: *J. A. TAYLOR, S. D. MCDOUGLE;
Psychology, Princeton Univ., Princeton, NJ

Abstract: It has been assumed that learning in sensorimotor adaptation tasks is largely implicit, involving the recalibration of an internal model. However, a growing body of research has shown that explicit aiming strategies contribute substantially to learning, at least in the subdomain of visuomotor rotation tasks (for review, see McDougle et al., 2016). Furthermore, the time course of explicit and implicit learning resemble, respectively, the oft-cited "fast" and "slow" processes first outlined in force field adaptation tasks (Smith et al., 2006; McDougle et al., 2015). While there are clues that explicit learning plays a role in force field adaptation, demonstrating this has proven difficult because the metric usually used as a proxy for strategy use (e.g., aiming direction) is not in the same dimension as the actual learning metric (e.g., lateral force; McDougle et al., 2015). Here, we rectified this mismatch by introducing a technique designed to quantify explicit strategies in force field adaptation tasks. Participants learned to first counter viscous force fields with their right hand in a perturbation rebound paradigm, which introduces a force field in one direction for a long period before reversing direction for a shorter period. During a subset of learning trials, participants performed movements with their left hand in an "error-clamp," which constrains lateral movements of the limb. To assay explicit knowledge of the force field, one group of participants were instructed to use their left hand to mimic what they were doing to counter the forces with their right hand (Instruction group). A control group (No-Instruction) also moved their left hand in the error clamp, but were not instructed about what they should do beyond simply moving to the target. Critically, for the Instruction group we found that various metrics (e.g., max force, adaptation index, etc.) of the left hand closely matched those of the right hand. In the No-Instruction group this was not the case, suggesting that our results were not due to implicit transfer. Consistent with our predictions, the time course of the left hand forces resembled the time course of the fast/explicit process, rapidly ramping up to counter the initial force field, then quickly countering the opposing force field, and, finally, showing a complete lack of implicit rebound in a subsequent error clamp block. These data suggest that even in complex sensorimotor adaptation tasks, explicit strategies may play a surprisingly central role.

Disclosures: **J.A. Taylor:** None. **S.D. McDougle:** None.

Poster

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Topic: E.04. Voluntary Movements

Support: R01AG041878

Title: Measuring implicit adaptation to task-irrelevant clamped visual feedback

Authors: ***J. R. MOREHEAD**, M. A. SMITH;
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Abstract: Humans display a remarkable capacity for acquiring and modifying motor skills. When there are changes in the environment that alter the movement outcome relative to the command, a type of motor learning termed sensorimotor adaptation occurs. This adaptation retunes the mapping between desired movements and motor commands so that a given command will again achieve its desired outcome.

This is thought to be driven by an error between the predicted sensory feedback and the actual feedback. However, this learning is typically studied in the context of a behavioral task where the perturbation affects both errors between predicted sensory feedback and the actual feedback and errors in task performance. This makes it difficult to differentiate between adaptive responses to these two types of errors.

Here we set out to study visuomotor adaptation in the absence of task performance errors by exposing participants to a visual motion stimulus that matched the radial extent of their hand but was otherwise clamped to a fixed heading angle and offset from the target. We made this feedback task-irrelevant by informing participants of how the motion was manipulated and asking them to ignore it while moving their unseen hand to the target on each trial.

Previous work with this manipulation (Morehead et al., 2014) showed saturation of the initial rate of adaptation for clamp offsets greater than 7.5°, resulting in invariance of aftereffect size at 12°. The present experiments addressed two key limitations of the previous data set: we had a smaller visual feedback delay (25ms vs. 90ms), and many more trials (240 cycles of 8 targets vs 30 cycles) to ensure adaptation indeed reached asymptote. First, we found similar adaptation between clamp offsets of 7.5° or 30°, consistent with previous work. However, the low-latency setup resulted in an initial adaptation function that was 40% greater than the high latency data, and continued to climb over 70 cycles to an asymptote 100% higher than previously observed (25°).

We performed an additional experiment to explore whether further learning is possible after the initial asymptote. After an exposure block of 190 target cycles with 7.5° clamped feedback, we either expanded the clamp size to 22.5° or reversed it to -7.5° for an additional 240 cycles. The expanded clamp group showed no change in performance, while the reversal group showed a ~40° change in the direction of reaches relative to the target. These results rule out several reasons for learning to asymptote, such as a habituation to the clamped feedback. We plan to further study other potential mechanisms, such as an angular limit for implicit adaptation or equilibrium between learning and forgetting.

Disclosures: J.R. Morehead: None. **M.A. Smith:** None.

Poster

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Topic: E.04. Voluntary Movements

Support: NIH Grant R01 AG041878

Title: Motor memories are confined to distinct channels with differing stability across time and experience

Authors: *A. E. BRENNAN, M. A. SMITH;
Harvard Univ., Cambridge, MA

Abstract: The fundamental goal of motor practice is generally to form long term stable memories. Indeed, when we practice a sport or a musical instrument, we train not to perform better during the practice session itself, but to improve the practiced skill set for the future. However, the link between short term learning that happens during a practice session and long term retention of that learning is not well understood.
Here, we study that link by dissecting adaptation from training into three distinct components, then examining their retention over 24h. First, we estimate temporally labile (TL) learning as the adaptation that decays during a 60s time delay; the remaining adaptation is temporally stable (TS). Sing et al (ACMC 2009) found that TL and TS adaptation correspond to the fast and slow learning processes, respectively, as identified by Smith et al (2006). Second, we dissect TS adaptation into a decay susceptible (DS) component that decays with the experience of consecutive error clamp (EC) trials that eliminate lateral errors, and a decay resistant (DR) component that does not.
We trained participants for 200 force field trials, isolated DR adaptation by erasing both TL

(using 60s) and DS adaptation (using 500 EC trials), then examined retention after 24h. We expected this measurement to reveal DR retention in the absence of the other two components, but a multiple linear regression of individual differences in 24h retention onto TL, DS, and DR adaptation revealed contributions from both DS and DR adaptation. DS adaptation was 17% recovered after 24h despite being erased after training; DR adaptation was 73% retained.
We next examined whether 24h retention is susceptible to decay with EC exposure. In particular, consolidation could cause the recovered DS adaptation to become resistant to decay; conversely, DR adaptation might become susceptible to decay after retrieval via a process called reconsolidation (Walker et al, 2003). We found that the 24h memory was indeed susceptible to decay. Moreover we found an intriguing double dissociation whereby day 2 decay was specifically predicted by day 1 DS adaptation, whereas the day 2 memory that survived decay was specifically predicted by day 1 DR adaptation. That is, DS adaptation remained susceptible to decay on day 2 while DR adaptation remained resistant to decay.
Thus we find distinct channels of motor memory formation that vary in stability across time and experience, with little evidence of memories changing channels - that memories in a fragile channel can move to a more stable channel in line with a consolidation process or that memories in a stable channel can move to a more fragile channel in line with a reconsolidation process.

Disclosures: **A.E. Brennan:** None. **M.A. Smith:** None.

Poster

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Topic: E.04. Voluntary Movements

Support: NIA (R01 AG041878)

Title: Implicit learning compensates for low-fidelity explicit strategy

Authors: *Y. R. MIYAMOTO[1], S. WANG[2], M. SMITH[1];
[1]Harvard Univ., Cambridge, MA; [2]Harbin Inst. of Technol., Harbin, Heilongjiang Province, China

Abstract: Recent studies investigating the contribution of an explicit strategy alongside implicit learning during visuomotor adaptation (e.g. Mazzoni & Krakauer 2006, Taylor et al 2014) have found that implicit adaptation and explicit strategy both contribute to improved task performance, and it has generally been assumed that implicit and explicit responses are both beneficial. Here we investigate the fidelity of implicit and explicit responses by training

participants on an aim-report visuomotor rotation task that tracks explicit strategy and implicit learning throughout the course of training. We coupled this task with a novel sum-of-sines perturbation sequence that continually varies the amount of visuomotor rotation from trial-to-trial, allowing us to parse implicit and explicit responses into discrete frequency-based components that are appropriate vs inappropriate for the task. We found that implicit adaptation and strategy both show large task-appropriate and task-inappropriate components. Task-appropriate strategy and implicit responses followed each other closely, with a strong positive correlation for both subject-averaged and individual data (r_{avg} = +0.86; r_{indv} = +0.47, $p < 0.001$). In contrast, task-inappropriate strategy and implicit responses mirrored one another, thus largely cancelling each other (r_{avg} = -0.71; r_{indv} = -0.36, $p < 0.001$). Consequently, the combined learning response displays only a small inappropriate component (with a variance less than 10-fold smaller than for inappropriate strategy or implicit responses). This suggests that either (a) implicit responses actively compensate for inappropriate strategy, or (b) strategy actively compensates for inappropriate implicit responses. If one response actively compensated for the other from one trial to the next, the compensatory response would systematically lag the compensated one. We thus performed a Granger causality based lag analysis that revealed that implicit responses inappropriate to the task systematically lag behind the corresponding strategy responses ($p<0.01$), indicating that implicit adaptation systematically compensates for inappropriate strategic performance, rather than the reverse. Furthermore, individuals with higher levels of strategy exhibited systematically higher levels of performance error ($r = 0.42$, $p = 0.02$), suggesting that increased reliance on strategy learning reduces the effectiveness of the overall adaptive response. These results reveal the higher fidelity of implicit learning mechanisms and suggest benefits for training procedures that rely on implicit mechanisms over explicit cognitive strategies.

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Poster

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Topic: E.04. Voluntary Movements

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Title: Intentional and automatic motor rhythm control in human and monkey

Authors: *S. MIYACHI;
Primate Res. Institute, Kyoto Univ., Inuyama, Japan

Abstract: Rhythm is a fundamental factor of the voluntary movement. We can synchronize movements with external rhythms. On the other hand, we often find ourselves moving to some rhythm without intention. To elucidate the characteristics of the intentional and automatic control of motor rhythm, effects of isochronous cue rhythm on the reaction time (RT) in the repetitive button press tasks were examined in the human and monkey. In the first experiment, 18 human subjects were tested in two tasks. In both tasks, subjects responded to the repeatedly presented audio-visual cues by pressing a button. In the "rhythmic" task, the cue was presented 5 times/trial with a constant interonset interval (IOI, 900 ms). The subjects well synchronized the button presses with the cue onsets; RT was < 150 ms for 93% of the 2nd-5th presses. In the "random" task, the cue was presented 11 times/trial. Three IOIs (900/600/1200, 900/600/1500, or 900/1200/1500 ms) were pseudo-randomly mixed in each block of trials. The subjects were encouraged to respond as quick as possible. In some trials, the cue was presented rhythmically with 900-ms IOI for 3-5 times (2-4 IOIs). Such unexpected cue rhythm shortened the RT: 196, 188, 188, and 185 ms after the 1st-4th consecutive 900-ms IOIs (median, $p < 0.01$, Friedman test). Moreover, if a longer IOI (1200 or 1500 ms) was preceded by the repetition of 900-ms IOI, the response was delayed ($p < 0.05$). The delay would reflect the suppression of the unwanted rhythmic movement. The response after 600-ms IOI was not delayed by the preceding repetition of 900-ms IOI; attention to the cue may be independent of the motor rhythm.
In the next experiments, a monkey (*macaca fuscata*) was trained on the rhythmic and random tasks. In both tasks, the cue was presented 7 times/trial. In the rhythmic task, the IOI was 800 ms. Liquid reward was given if the button was pressed within +/-260 ms from the cue onset. The amount of reward was maximized at RT = 0. The median RT for the 1st press was 198 ms, which was hastened progressively to the minimum of -56 ms at the 4th press, then gradually delayed to -16 ms at the 7th press. In the random task, IOIs of 800, 1200, 1600 ms were pseudo-randomly mixed. Each response before 300 ms from the cue onset was rewarded. The reward was maximized if RT = 0, but presses before the cue onset was not rewarded. RT after 800-ms IOI was progressively shortened if the same IOI was repeated 2-4 times ($p < 0.01$). The RT after a 1200-ms IOI was prolonged if preceded by repeating 800-ms IOIs ($p < 0.01$). The present data suggest that monkeys can synchronize movement with isochronous rhythm, and that motor rhythm can be induced by external rhythm even if it was not intended.

Disclosures: S. Miyachi: None.

Poster

057. Reaching Movements: Movement Control and Strategy

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Topic: E.04. Voluntary Movements

Title: Kinematically similar basketball free throws have surprisingly different muscle contraction velocity profiles

Authors: *D. A. HAGEN[1], S. CAJA[1], S. CHAKRAVARTHI[2], F. J. VALERO-CUEVAS[1,3]; [1]Dept. of Biomed. Engin., [2]Dept. of Electrical Engin., [3]Div. of Biokinesiology & Physical Therapy, USC, Los Angeles, CA

Abstract: The pursuit of the perfect basketball shot relies heavily on practice, with only those capable of consistently accurate throws becoming professional athletes. Why is it then that practice or mimicry do not suffice to accomplish a professional level of accuracy? Recent work re-emphasizes that the neural control of limb movements is in fact overdetermined: the rotations of a few joints determine the length changes in all muscles [1]. As pointed out by Sherrington (*Exp. Physiol.* 1913), movement trajectories can be disrupted if even one muscle undergoing eccentric contraction fails to silence its stretch reflex appropriately. Thus throws requiring higher eccentric velocities likely require more accurate and time-critical alpha-gamma control, and are thus more prone to disruption and therefore variability. Also, higher concentric velocities reduce muscle power output. Therefore, we investigated whether kinematically similar throws can exhibit large differences in eccentric and concentric muscle fiber contraction velocities. Using a planar kinematic model of a generalized jump shot with 18 muscle, 10,000 trajectories were calculated for each of the three joint angles. Normalized muscle velocities were calculated using posture-specific moment arms and optimal fiber lengths. Assuming muscles do not go slack, each trajectory was associated with obligatory muscle fiber velocities. We then classified the relative robustness of each trajectory by the sum of squares of maximum eccentric and concentric velocities, *Fig. 1 (Bottom Right),* with throws exhibiting lower velocities likely being more robust. Within the wide range of successful shots there are multiple cases where kinematically similar throws have surprisingly different muscle velocity profiles. This may explain why a "good looking" shot may, in fact, be fundamentally different from a "good" shot. Future work will explore the interplay between these kinematic constraints and the timing and coordination of alpha-gamma co-activation. 1. Valero-Cuevas, FJ, *Fundamentals of Neuromechanics,* Springer-Verlag London, 2016.

Disclosures: D.A. Hagen: None. **S. Caja:** None. **S. Chakravarthi:** None. **F.J. Valero-Cuevas:** None.

Poster

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ISD

MEC

Title: Characterization of hand motor deficits in a marmoset 6-OHDA model of Parkinson's disease

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Abstract: Characterization of motor deficits in animal models of Parkinson's disease (PD) is important for the descriptive accuracy of the impairments observed in PD research. Most studies focus on describing the postural and locomotion parameters observed on the PD animal models. This work describes the use of forelimbs by 2 male marmosets during a reaching task, comparing performance before and after 6-hydroxydopamine (6-OHDA) lesion in the right hemisphere. All procedures were previously approved by the institution's Ethic Commitee for Animal Use (protocol 03/2015). The animals were first trained to reach and grasp marshmallows before induction of PD. During the experimental sessions, they were placed inside a transparent acrylic box (45x45x45cm) and were monitored by two cameras, positioned at the top and at the front of the box. The front wall of the box had 9 holes that gave access to 18 marshmallow pieces (3 for each hole) strategically positioned to evaluate forelimb use and reaching movements. For each animal, 5 sessions before and 5 sessions after 6-OHDA lesion were used to describe the forelimb impairments after the lesion. There was a significant decrease in contralateral motor performance. Specifically, the number of marshmallows captured with the left hand decreased 75.5%. In addition, there was a 165% increase in the number of marshmallows captures with the right hand. An overall reduction in motor performance was also observed, with 19% reduction in the number of marshmallows captured. This study provides a more detailed approach on the motor performance of the forelimbs, in a marmoset model of PD, and provides subsidy for future research that may describe and compare the motor performance of the arms of their DP animal models.

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Poster

057. Reaching Movements: Movement Control and Strategy

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Title: Visual stimulus-locked responses on upper limb muscle are modulated by the upcoming reach trajectory

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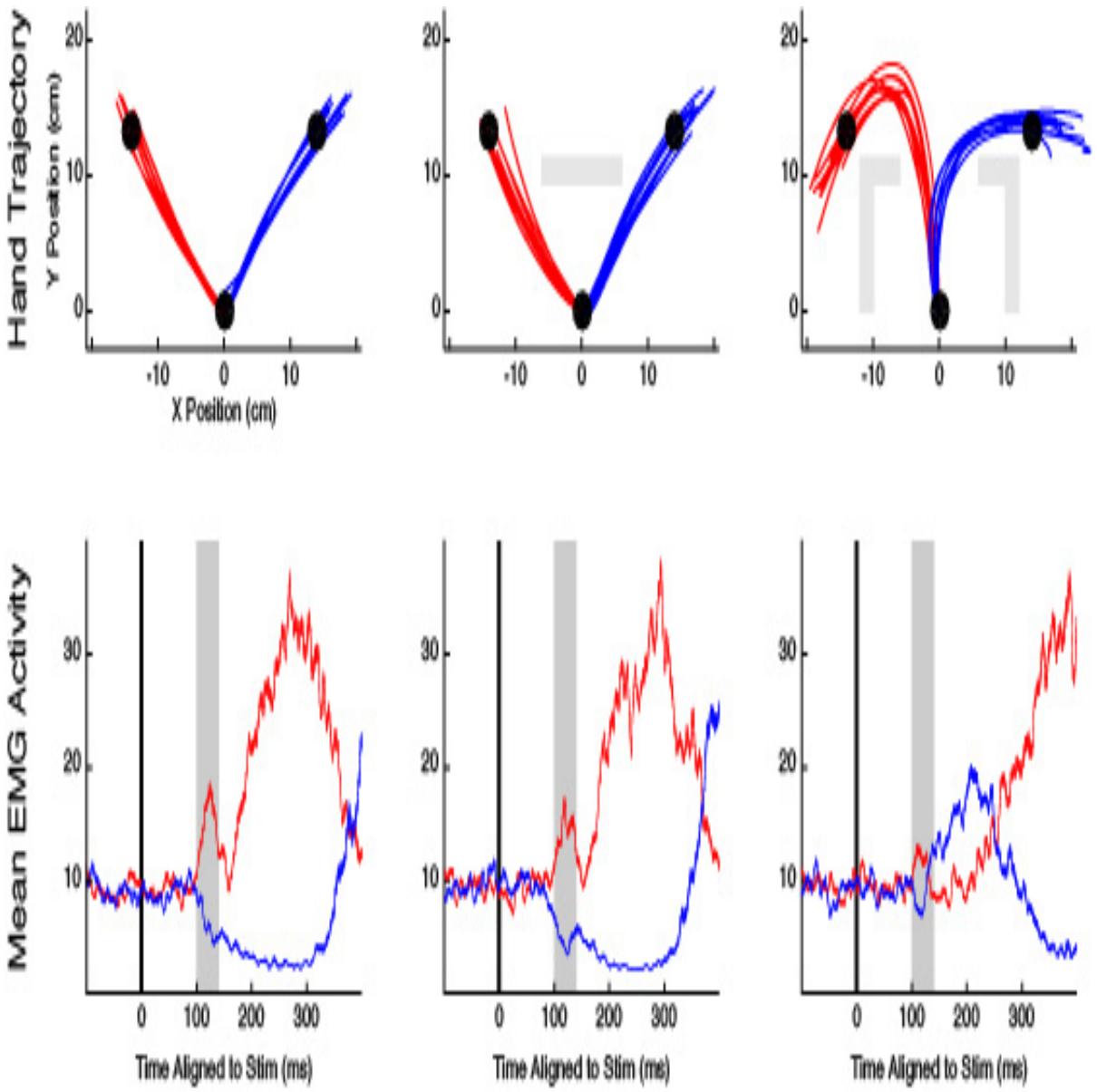
Abstract: Recent studies have reported that visually guided reaches are preceded by a wave of very short-latency electromyographic (EMG) activity in upper limb muscles that is time-locked to the visual stimulus rather than movement onset (Pruszynski et al. *EJN* 2010; Wood et al. *EJN* 2015). Such stimulus-locked responses (SLRs) evolve within ~100 ms, providing a unique opportunity to quantify the earliest phase of a sensory-to-motor transformation. Despite the reflexive-like nature of the SLR, it is endowed with a surprising degree of sophistication. For example, the SLR is represented in an arm-centric reference frame that encodes visual stimulus location relative to the limb rather than to the eye, attesting to a rapid integration of visual and proprioceptive signals. Here, we test whether the SLR reflects only the final position of the limb at the end of the reach (i.e. a goal-based response) or whether it is also modulated by the trajectory of the limb over the entire movement (i.e. a path-based response).

18 healthy people performed horizontal reaches with their right arm to either a leftward (45° counter-clockwise from straight away) or a rightward (45° clockwise from straight away) visual stimulus. We recorded surface EMG activity from the clavicular head of the right pectoralis major muscle. To dissociate goal- versus path-based responses, we introduced visual obstacles that participants were told to avoid. In one condition the obstacle did not impede straight reach trajectories, whereas in another condition the obstacle was positioned so that a curved movement was required to get to the final reach target. In the 14 participants exhibiting an SLR, the magnitude of the SLR differed for straight versus curved trajectories to the same final visual target.

Consequently, even though the SLR evolves at very short stimulus-locked latencies, it also conveys path-based information relating to the planned reach trajectory. This result shows that the earliest wave of muscle recruitment, which we quantify through the SLR, is a surprisingly rich signal that conveys many aspects of the upcoming movement.



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Poster

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Support: Interdepartmental Research Grant, College of Public Health, Temple University

Title: Are common motor modules activated in static and dynamic motor tasks in the human arm?

Authors: *K. D. WILGER, J. ROH;
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Abstract: Our previous studies suggest that activation of a small number of motor modules (a fixed pattern of activation of a group of muscles) underlies isometric (static) force generation. Interestingly, isometric force strengthening has been shown to improve dynamic strength comparable to dynamic training. The finding supports a hypothesis that motor modules are fundamental to both static and dynamic tasks and are an avenue of strength transfer. This study tested the hypothesis by examining modular organization identified in isometric force generation task and dynamic reaching in the human upper extremity. Three neurologically intact adults performed target matches repeatedly in 16 directions equally spaced on the circumference of a circle in the horizontal plane by generating isometric force and by reaching with a manipulandum of a robotic device, respectively. Surface EMG data were recorded from eight key upper arm muscles, preprocessed, and concatenated across trials. A non-negative matrix factorization algorithm identified motor modules and their activation coefficients from the EMG of each motor task. Spatial tuning curves of the averaged motor module activations were examined in two dimensional force and displacement space. Subjects typically expressed four and five modules in the isometric force match and reaching tasks, respectively. During isometric force matches, four motor modules consisted of elbow flexor, elbow extensor, shoulder adduction/flexion, and shoulder abduction/extension patterns. In static force match and reaching, three out of the four modules were common and had similar activation tuning directions: elbow extensor, shoulder adduction/flexion, and shoulder abduction/extension modules. The muscle weights of the static elbow flexor pattern appeared as being split into two modules in the reaching task, resulting in two reaching-specific modules consisting of single activation of the brachioradialis and biceps. The spatial tuning curve of the biceps module in reaching showed no directional tuning to any reaching target, suggesting the activation of the module for compensation for gravity. These findings support that the central nervous system modulates the activation of common and task-specific motor modules to accomplish static force generation and dynamic motor tasks in the human arm.

Disclosures: K.D. Wilger: None. **J. Roh:** None.

Poster

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Support: NSF Grant BCS-1358756

Title: Motor planning flexibly optimizes performance under uncertainty about task goals

Authors: *A. L. WONG, A. M. HAITH;
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Abstract: In an environment full of potential goals, how does the brain determine a single movement to execute? Existing theories posit that when faced with competing potential goals, the motor system prepares a response for each individual goal by generating several distinct movement plans in parallel (Cisek 2007; Erlhagen and Schoner 2002; Stewart et al. 2013). Such theories are founded on the observation that presenting two competing goals often results in a movement that appears to be the average of the responses to the two targets. These intermediate movements are taken as evidence of unintentional interference between the competing plans. In contrast, normative theories suggest that rather than being accidental, intermediate movements are actually deliberately generated to improve task performance (Hudson et al. 2007; Haith et al. 2015). That is, an intermediate movement allows for additional time to process the stimulus by delaying commitment to a specific goal until later in the movement.

According to this interpretation, moving quickly should hinder this strategy because it limits the time available to resolve goal uncertainty and adjust the movement online. To test this hypothesis, we varied the required speed with which participants moved to the target goal under a go-before-you-know paradigm (Chapman et al 2010), which has been shown to reliably encourage the generation of intermediate movements. We found that the motor system generates intermediate movements only when they lead to improved performance, in support of the normative hypothesis. Interestingly, we also observed speed-modulated biases on unambiguous reaches when there was only a single target goal, suggesting that the occurrence of use-dependent biases might also be explained using the same normative framework.

In summary, our findings thus refute the hypothesis of parallel planning and instead suggest that the motor system forms only a single, flexible plan that is optimized to respond in the face of uncertain goals.

Disclosures: A.L. Wong: None. **A.M. Haith:** None.

Poster

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Topic: E.04. Voluntary Movements

Support: NSF Career SES 1352632

Title: Mass decreases movement speed during arm reaching

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Abstract: A decision-making framework suggests that the goal of movement is to maximize utility, represented as the sum of the rewards obtained during movement minus the costs of the movement itself (i.e. effort) [1]. A critical prediction of this hypothesis is that movement characteristics, such as speed, are influenced by rewards obtained from movement as well as the effort required to complete it. Movement speed should increase towards more rewarding targets and decrease when movements require greater effort. Previous research has shown that humans move faster towards more rewarding targets. Here, we asked whether preferred reach speed is also modulated by movement effort. Seated subjects (N = 26) made horizontal reaching movements while grasping a robotic handle. We increased movement effort by adding mass to the handle. Subjects performed reaching movements from a central starting circle to one of four targets displayed on the perimeter of a 10cm invisible circle, centered at the starting circle. The targets appeared at 45o, 135o, 225o, and 315o in pseudorandom order. They were instructed to reach to the target at a self-selected velocity. Each subject performed reaches under four mass conditions (0, 3, 5, and 8 lbs) in blocks of 200 trials, in randomized order. Eight subjects performed an out-and-stop movement. The remaining 18 subjects performed an out-and-back movement without any visual feedback of the cursor to prevent corrective movements. Importantly, subjects were not penalized for accuracy. Results were compared to the predictions of optimal movement duration given a utility in which effort is represented as metabolic cost and increases linearly with mass. With increasing mass, subjects increased both movement duration and reaction time ($p<<0.01$). We also observed that the faster an individual preferred to reach, the less sensitive their reach speed was to increasing mass, a finding that is in line with model predictions ($p<<0.01$). Our results demonstrate that increased effort slows both reaching movements and reaction time, and this can be explained using a framework in which movement utility is represented as the temporally discounted sum of the reward to be obtained minus the effort of the movement itself.

1. Shadmehr et al. (2015). TCMC.

Disclosures: **G. Bruening:** A. Employment/Salary (full or part-time): University of Colorado - Boulder. **M. O'Brien:** A. Employment/Salary (full or part-time): University of Colorado - Boulder. **A. Ahmed:** A. Employment/Salary (full or part-time): University of Colorado - Boulder. **R. Shadmehr:** A. Employment/Salary (full or part-time): Johns Hopkins.

Poster

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Scholarship CAPES

Title: Effect of the side of the brain lesion on arm reaching movements in upright position of stroke individuals

Authors: *R. B. GARBUS[1], A. G. NARDINI[2], S. R. ALOUCHE[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: Individuals with mild post-stroke hemiparesis present several motor impairments that can negatively affect the performance of arm reaching during upright standing. However, it is unclear whether the side of brain lesion affects differently the reaching performance. Therefore, the aim of the present study was to investigate the effect of the side of the damaged hemisphere on the arm reaching during standing. Nineteen individuals with mild post-stroke hemiparesis divided in two groups (nine on the right and ten on the left side) stood in upright position and performed reaching movements with their arm ipsilateral to the brain lesion. Reaches were performed towards a target shown in a monitor placed at a distance of 105% of the upper limb's length and the targets were presented in one of three heights (a center target placed at the eye level or 10 cm upper or 10 cm lower to the center target). Each participant performed 60 trials in 3 blocks of 20 trials. Target location was the same within each block and changed among blocks. The order of target location was randomized across participants. Participants were instructed to reach and touch the center of the target displayed on the monitor as fast as possible after the auditory beep. Time elapsed from the auditory sound until participants' movement initiation (time of movement onset), time spent to complete the task (movement time) and the accuracy measured by the variable errors were computed and compared among target heights and between groups. The results showed an effect of the side of the brain lesion only on the time of movement onset. Individuals with right hemisphere damage took more time to initiate their movements

compared to the left stroke individuals. The height effect was observed on the movement time and variable error. The movement time increased with the target height. On the other hand, greater variable error was observed for the center target (i.e. placed on the eye level). Overall, the results suggest that the effects of the side of lesion are more evident on the movement planning. The performance of the arm reaching movement during upright standing was not dependent on which hemisphere was damaged.

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Poster

057. Reaching Movements: Movement Control and Strategy

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Topic: E.04. Voluntary Movements

Support: NSERC CGS-D

Title: On action intent: behavioural correlates of reach-to-grasp actions

Authors: *J. W. FLINDALL, C. L. R. GONZALEZ;
Kinesiology, Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: Long-train electrical stimulation of the motor and pre-motor cortex has been shown to produce functional movements in anesthetised macaques. Location-specific stimulation may produce either multi-limb defensive or locomotory movements, or context-specific single-limb movements, such as grasp-to-inspect or grasp-to-eat actions. Such grasping movements are always tied to a precise end-goal, regardless of initial limb position. In addition, single-neuron recording studies have shown distinct populations of neurons in the macaque inferior parietal lobule (IPL) that respond uniquely to task-specific grasping actions. These studies show that "grasp-to-eat" and "grasp-to-place" neurons in the IPL selectively fire when their preferred action is performed. This selectivity is maintained irrespective of arm kinematics, end-goal location, target identity, or grip-type. Together, these studies suggest that the macaque motor cortex is organized around the production of functional, goal-oriented movements, rather than the activation of specific muscles. But what about humans? In a series of behavioural studies, we provide evidence that the hand-to-mouth (or "grasp-to-eat") action is not only kinematically distinct from grasp-to-place actions, but that it is left-hemisphere lateralized. In Study 1 we show that smaller maximum grip apertures (MGAs) are produced when grasping to eat cereal items than when grasping to place the same items into a container near the mouth, and that this

difference is lateralized to the right hand. In Studies 2 and 3 we show that this right hand lateralization is not an effect of practice; first, by demonstrating that the lateralization is preserved in left-handers, and second by showing an unexpected pattern of development in children. Studies 4 and 5 tested the specificity of the effect with regards to consumption; they show that the grasped item need not be eaten, nor need it even be edible, provided the action's goal is to bring the target to the mouth. Studies 6 and 7 show that the kinematic asymmetries cannot be attributed to planned mouth movement, nor can they be explained by the (arguably) increased specificity of placement required by the mouth. Altogether, our research suggests that the human motor cortex is also organized around the production of goal-specific actions, and that the hand-to-mouth movement, which almost certainly evolved for the purpose of self-feeding, is lateralized to the left-hemisphere. This hemispheric specialization may have served as the foundation for other lateralized functions such as praxis and speech, as well as the driving force behind species-wide right-hand dominance in humans.

Disclosures: J.W. Flindall: None. **C.L.R. Gonzalez:** None.

Poster

057. Reaching Movements: Movement Control and Strategy

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Topic: E.04. Voluntary Movements

Title: Gravity is used to minimize neural effort for joint coordination during arm movements

Authors: *N. DOUNSKAIA, W. WANG;
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Abstract: How control for gravity is organized during arm movements remains under debate. We tested three alternative interpretations suggested by previous research: (1) that muscular control includes two components, tonic which compensates for gravity and phasic which produces the movement; (2) that there is a tendency to exploit gravity to reduce muscle effort; and (3) that there is a tendency to use a trailing pattern of joint control during which either the shoulder or elbow is rotated actively and the other joint rotates predominantly passively, and to exploit gravity for control of the passively rotated joint. A free-stroke drawing task was performed that required production of center-out strokes within a circle while selecting stroke directions randomly. The circle was positioned in the horizontal, sagittal, and frontal plane. The arm joints freely rotated in space. In each plane, the distribution of the strokes across directions was non-uniform. Directional histograms were built and their peaks were used to identify preferred movement directions. The directional preferences were especially pronounced in the

two vertical planes. The upward directions were most preferred. To test the three interpretations, we used a kinetic analysis that determined the role of gravitational torque in production of movement in the preferred directions. The results supported the third interpretation and provided evidence against the first and second interpretation. The trailing pattern has been associated with reduced neural effort for joint coordination, and therefore, we conclude that the major tendency with respect to gravity is to exploit it for simplification of joint coordination.

Disclosures: N. Dounskaia: None. **W. Wang:** None.

Poster

057. Reaching Movements: Movement Control and Strategy

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Topic: E.04. Voluntary Movements

Support: NSF 1358756

Title: Age-related increases in reaction time: slower preparation or sluggish initiation?

Authors: *R. M. HARDWICK[1], M. COSTELLO[2], K. M. ZACKOWSKI[2], A. M. HAITH[3];
[1]Dept. of Neurol., [2]Kennedy Krieger Inst., [3]Neurol., Johns Hopkins Univ., Baltimore, MD

Abstract: Healthy aging is associated with slower reaction times. While corticospinal conduction velocities decrease marginally with aging, these changes are too small to account for changes in voluntary reaction times, suggesting this slowness is primarily a result of age-related changes in behavior (e.g. being more cautious to ensure the correct response) and/or the brain (e.g. decreased myelination). In a recent study (Haith et al., 2015) we have shown that while the reaction times of younger adults are typically within the range of 200-220ms, they can in fact prepare and execute accurate responses in ~130ms, suggesting they introduce a seemingly unnecessary delay of 80-100ms prior to reacting. Here we examined whether the slower reaction times seen in healthy older individuals are due to a slowing of their ability to prepare movements (e.g. due to reduced speed of information processing), or an increase in the delay between when their movements are prepared and initiated (e.g. due to being more cautious), or a combination of both.

In a visually guided planar reaching task, we measured reaction times and assessed the time actually required for accurate movement preparation in older and younger adults. To measure reaction times, participants were instructed to react as soon as possible to the appearance of a visual target. To assess required preparation time, we tested participants' ability to move accurately at lower-than-normal reaction times through a timed-response paradigm. Participants

made reaching movements at a fixed time during each trial (moving synchronously with the last of four equally spaced tones). Varying the time of target presentation in relation to movement onset allowed us to determine the minimum time required for accurate movement preparation. A preliminary comparison was conducted between a group of healthy older (n=8, mean age=73) and younger (n=8, mean age=27) adults. In line with previous studies, we found that reaction times were longer for the healthy older group. Behavior under timed-response conditions revealed that this age-related increase was attributable to slower movement preparation while the delay between the time required to prepare movements and when they were initiated was remarkably similar across age groups (~90ms). These results indicate that the slower reaction times of healthy older adults are not simply due to an increased hesitancy to respond, but may instead be attributable to a declining ability to rapidly process target location and prepare movements accordingly, potentially due to age related changes in brain structure (e.g. reduced white matter integrity) or function (e.g. compensatory brain activity).

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Poster

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Support: University of South Carolina ASIPRE II Grant

Title: Distinct and common processes underlie impaired motor execution and inhibition following stroke

Authors: *A. HARRISON, C. PERRY, T. SINGH, A. ROSS, S. FRITZ, J. FRIDRIKSSON, T. HERTER;
Univ. of South Carolina, Columbia, SC

Abstract: Many motor skills involve executing movements in response to task-relevant stimuli (motor execution) and inhibiting movements in response to non-relevant stimuli (motor inhibition). Stroke survivors commonly show impaired motor execution, but the extent to which stroke survivors exhibit impaired motor inhibition remains unclear. Here we examine extent to which stroke survivors exhibit impaired motor inhibition and whether motor execution and inhibition are mediated by distinct or common perceptual, cognitive and motor processes. METHODS: We studied 31 stroke survivors and 67 controls who used an upper-limb robotic

device to perform a motor execution/inhibition task, Object Hit and Avoid (OHA). Subjects used virtual paddles to hit away target objects (2 geometric shapes, n=200) and avoid hitting distractor objects (4 different geometric shapes, n=100) that moved towards them in the horizontal plane. Hand and eye kinematics were collected and used to compute measures of *Motor Execution* (percent of targets hit), *Motor Inhibition* (percent of distractors avoided), *Limb Movement Control* (hand speed bias), *Visual Search Efficacy* (percent of objects pursued with the eyes), *Eye-Hand Coordination* (distance between the eyes and hand at the time of target contact), *Visual Recognition Speed* (mean distractor pursuit time), and *Motor Planning Speed* (mean difference between target and distractor pursuit times) RESULTS: Compared to controls, many stroke survivors exhibited impairments of both *Motor Execution* and *Motor Inhibition*. However, these impairments did not exhibit a strong relationship with each other ($r = 0.22$, $p = 0.06$). Both *Motor Execution* and *Motor Inhibition* were correlated with *Eye-Hand Coordination* ($r = 0.62$, $p = 0.0001$; $r = 0.33$, $p = 0.366$) and *Visual Recognition Speed* ($r = -0.55$, $p < 0.0001$; $r = -0.58$, $p < 0.0001$). *Motor Execution* was also uniquely correlated with *Visual Search Efficacy* ($r = 0.75$, $r < 0.0001$) and *Limb Movement Control* ($r = -0.34$, $p < 0.0001$). In contrast, *Motor Inhibition* was uniquely correlated with *Motor Planning Speed* ($r = 0.68$, $p < 0.0001$). CONCLUSION: These results show that motor inhibition is a common impairment following stroke and a combination of distinct and common brain processes contribute to motor execution and inhibition.

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Poster

057. Reaching Movements: Movement Control and Strategy

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Topic: E.04. Voluntary Movements

Title: The striatum encodes the full kinematic details of reaching movements

Authors: *E. A. YTTRI[1], B. PANIGRAHI[2], K. A. MARTIN[2], J. T. DUDMAN[2];
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Abstract: To accomplish a successful action, it is important to select not only what movements to perform, but also when, how fast, and how far to perform those movements. The generation of motor plans requires the coordination of several brain areas. The dorsal striatum, the major input nucleus of the basal ganglia, receives input from several cortical areas, including the motor cortex. Motor cortex is a dynamical system that may represent several possible actions while the basal ganglia is thought to act as a gate on action itself. It is possible that downstream structures

such as the basal ganglia might likewise have regular dynamics associated with movement execution, but this have been missed due to previous technique limitations and that stimulation of the two projection pathways of the basal ganglia induce robust starting or stopping of behavior, respectively. However, we recently demonstrated that selective stimulation of either pathway was sufficient to bidirectionally change a specific parameter, the velocity, of a reach, while leaving other movement parameters unaltered. Additionally, rather than being active at only the initiation and termination of an action, the striatum is active throughout an action, with individual neurons reliably responding at the same phase of movement.
To determine in what aspects of action planning the striatum is involved, we trained mice to perform a goal-directed reaching task while we recorded from populations of striatal neurons. We explored the possibility that striatal ensembles function as dynamical systems using GPFA on these population data. We discovered that the striatum encodes not only start and stop of a reach, but also other control parameters such as velocity, amplitude and direction – a profoundly greater level of detail than previously thought.
If striatal dynamics are causally involved, as our prior experiments have shown, we hypothesize that neuronal activity will lead the representative behavior. Alternatively, a reflexive response or corollary read out of movement would occur concurrently or after the behavior is performed. Utilizing a simple decoder, we were able to predict the full path and velocity of a individual reaching movement over 100 milliseconds before it is performed. Furthermore, stimulation just prior to a reach can alter the current reach. These findings suggest that the basal ganglia is actively involved in driving the kinematics of movements. These results demonstrate that neuronal representations of movement in the basal ganglia are not merely to start or stop movement, but are detailed blueprints for precisely how and when to act.

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Poster

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Title: Impedance modulation as a strategy to control force and movement during object manipulation

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[1]Bioengineering, [2]Neurobio., Univ. of Pittsburgh, Pittsburgh, PA; [3]Mechanical Engin., MIT, Boston, MA

Abstract: Object manipulation necessitates simultaneous control of force and movement. However, the relation between force and movement changes in a way that is specific to the object and the way it is acted upon. The ability to perform manipulation tasks in the face of such changes suggests that flexible and adaptable control of both force and movement takes place in parallel. We studied this control in a task in which five human subjects pulled on a handle that was held stationary until a specified force threshold was reached. The handle was then suddenly released to move along a track and the subjects were required to stop the handle in various target position zones. Targets were spaced at different distances from the release position and we released the handle at different force thresholds. Our main finding was that the subjects varied their arm impedance for different combinations of target distance and force threshold. For the same target distance, arm impedance increased with the force threshold; for the same force threshold, arm impedance increased as the target distance decreased. Surface electromyograms were used to calculate the co-activation of antagonist muscles at the shoulder, wrist, and elbow. Co-activation, combined with arm configuration, was found to be consistently related to arm impedance. This simple task shows that force, movement, and arm impedance can be controlled independently and lays the groundwork for investigating impedance modulation as a strategy for object manipulation.

Disclosures: S.D. Kennedy: None. **N. Hogan:** None. **A.B. Schwartz:** None.

Poster

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Title: Options coding in the movement system

Authors: *J. I. GLASER[1,2], M. G. PERICH[1], P. N. LAWLOR[1], P. RAMKUMAR[1], D. K. WOOD[1], M. A. SEGRAVES[1], L. E. MILLER[1], K. P. KORDING[1];
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Abstract: In general, the state of our body restricts our movement options. When our arm is fully stretched out it can only be moved towards our body, and if our eyes are fully rotated left, we can only move them rightward. The visuomotor system could use this prior knowledge to reduce the range of potential movements during movement selection. We thus studied the encoding of a distribution of potential arm and eye movement options using extracellular recordings from several different areas of monkey cortex. We found strong signatures of options coding in dorsal premotor cortex (PMd) during arm movements (reaching) and in the frontal eye field (FEF) during eye movements (saccades). In both areas, early planning activity averaged across movements represented the distribution of upcoming movements, while late planning activity represented the selected movement. Moreover, by decoding simultaneously recorded neurons in PMd, we showed that the neural population represented upcoming movement options for single reaches. Our results suggest that option representations may be a ubiquitous component of motor planning.

Disclosures: J.I. Glaser: None. **M.G. Perich:** None. **P.N. Lawlor:** None. **P. Ramkumar:** None. **D.K. Wood:** None. **M.A. Segraves:** None. **L.E. Miller:** None. **K.P. Kording:** None.

Poster

058. Neuroprosthetics: Processing Techniques

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 58.01/GG6

Topic: E.05. Brain-Machine Interface

Support: Craig H Neilsen Foundation #296453

Center for Sensorimotor Neural Engineering (CSNE), a National Science Foundation Engineering Research Center (EEC-1028725)

Raymond and Beverly Sackler Scholar

Title: A micro-LED implant for long-term optogenetic stimulation of the rat spinal cord

Authors: *S. E. MONDELLO[1,2,6,3], M. D. SUNSHINE[1,6], A. E. FISCHEDICK[1], P. J. HORNER[2,3], C. T. MORITZ[1,4,6,5];
[1]Rehabil. Med., [2]Dept. of Neurolog. Surgery, [3]Inst. for Stem Cell and Regenerative Med., [4]Dept. of Physiol. & Biophysics, [5]UW Inst. for Neural Engin. (UWIN), Univ. of Washington, Seattle, WA; [6]Ctr. for Sensorimotor Neural Engin. (CSNE), Seattle, WA

Abstract: Optogenetic stimulation permits activation of specific neuron populations without the need to penetrate the brain or spinal cord. While many implantable devices have been developed for optogenetic stimulation of the brain, few have been created that allow for long-term stimulation of the spinal cord in freely moving animals. Rats are an ideal animal model for testing therapeutics to treat disorders of the spinal cord such as chronic pain or spinal cord injury. They are capable of learning numerous tasks that allow for comprehensive assessments of their responses to treatment. Further, they are more genetically similar to humans, potentially accelerating translation. Working with rats, however, requires overcoming obstacles not present in mice. One of the greatest obstacles is the lack of a publicly available optogenetic transgenic rat. This results in the necessity to inject optogenetic viruses into the regions of interest, which in turn leads to less efficient optogenetic activation and requires higher light intensities to elicit a response. In the current study, we developed a high-powered LED implant that permits long-term optogenetic stimulation of freely moving rats. Prior to implantation, terminal *in vivo* heat tests confirmed the safety of the implant at 4Hz frequency using ≤ 5ms pulse widths at light intensities up to 215 mW. Some rats received a hemi-contusion at cervical segment C4, followed by an injection of an optogenetic virus (either AAV1-Camkiiα-Chr2-mcherry, or AAV6-hsyn-Chr2(H134)-eYFP) at ipsilateral cervical segment C6. Following an addition of an 8 week transfection period, rats were then implanted with a high-powered 473 nm surface LED directly over the viral injection site. Some rats underwent similar procedures, but without an injury or an initial viral transfection period. Light-intensity thresholds for eliciting movement were tested weekly for 8 weeks. In some animals, the implant was stimulated for 4.5hrs, 5 days/week to simulate therapeutic treatment. For 2 out of 4 transfected rats, thresholds slowly increased over the course of 8 weeks, while thresholds remained relatively constant for the other two animals. All implants succeeded in evoking optically activated forelimb movements for at least 6-8 weeks following implantation. In combination, these findings indicate that this implant could be used for long-term studies using optogenetics as a therapeutic or restorative technology, or to answer fundamental science questions across a longitudinal timeframe.

Disclosures: **S.E. Mondello:** None. **M.D. Sunshine:** None. **A.E. Fischedick:** None. **P.J. Horner:** None. **C.T. Moritz:** None.

Poster

058. Neuroprosthetics: Processing Techniques

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Program#/Poster#: 58.02/GG7

Topic: E.05. Brain-Machine Interface

Support: Center for Sensorimotor Neural Engineering (CSNE), a National Science Foundation Engineering Research Center (EEC-1028725).

Washington Research Foundation, University of Washington Institute for Neuroengineering (UWIN)

Title: Stimulation strategies to convey sensory information directly to the cortex via intracortical microstimulation (ICMS)

Authors: *D. A. BJANES[1,2,5], A. L. FAIRHALL[3,2,5], C. T. MORITZ[3,4,2,5];
[1]Electrical Engin., [2]Inst. for Neuroengineering (UWIN), [3]Physiol. & Biophysics, [4]Rehabil. Med., Univ. of Washington, Seattle, WA; [5]Ctr. for Sensorimotor Neural Engin. (CSNE), Seattle, WA

Abstract: Users of current brain-computer interface (BCI) technology must rely on visual feedback of cursor or robotic arm movement. The inherently long delays of visual processing likely contribute to relatively slow and unnatural control of BCIs. Despite increasing numbers of electrode sites and ever growing complexity of control algorithms, BCI technology has yet to achieve rapid and dexterous control comparable to an intact human arm and hand. We believe the lack of tactile perception and proprioceptive input imposes a fundamental limit on speed and accuracy of BCI controlled prostheses or reanimated limbs. By restoring this comparatively low-latency pathway directly to the brain via intracortical microstimulation (ICMS), BCI performance may be improved. To enable artificial sensory feedback, we are exploring sensitivity to several stimulation strategies in order to identify a high resolution space in which to provide feedback. We have developed a novel center-out task for rodents, including automated increments in difficulty to achieve autonomous training. We then use ICMS to present patterns of artificial stimulation directly to the sensorimotor cortex to enable animals to complete the task. Prior to stimulation, neural populations surrounding stimulation electrodes are categorized as responsive to sensory inputs, correlated with features of the behavioral task or as unrelated sites. We can then present stimulation both to correlated populations and unrelated sites and compare learning performance in each case. Stimulation encodes joystick position relative to a target as a proportional feedback signal, rather than simply a cue for a particular target. After determining the just noticeable difference of various stimulation patterns, we present graded stimulation via one or multiple cortical electrodes. By comparing the ability of animals to solve a forelimb spatial exploration task, we can measure comprehension of the encoded stimulation pattern at physiologically correlated and uncorrelated locations in the cortex. Our goal is to

determine an optimal strategy for providing a high resolution sensory feedback directly to the cortex. Using such ICMS strategies, we hope to restore low-latency sensory feedback and improve BCI control. This may enable participants with spinal cord injury and stroke to improve control of their prosthetic or reanimated limb when using a BCI, and also advance our understanding of natural sensory coding in the brain.

Disclosures: D.A. Bjanes: None. **A.L. Fairhall:** None. **C.T. Moritz:** None.

Poster

058. Neuroprosthetics: Processing Techniques

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Program#/Poster#: 58.03/GG8

Topic: E.05. Brain-Machine Interface

Support: NSF EEC-1028725

NINDS 5R01NS065186

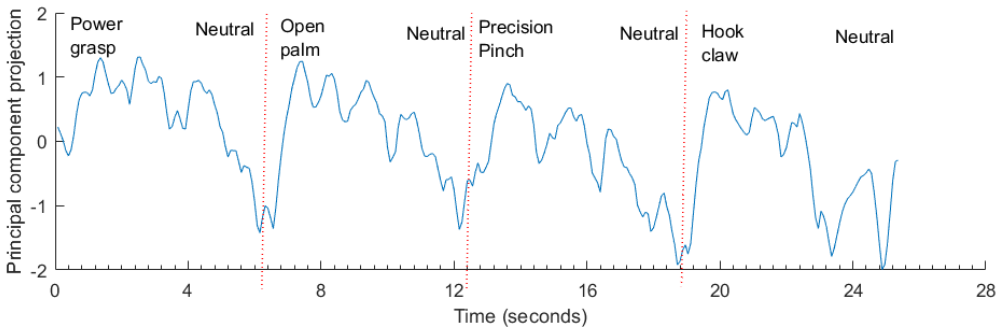
Washington Research Foundation

Title: Identification of stage transitions of imagined hand movements with electrocorticography

Authors: *J. WU[1,2], L. BASHFORD[6,7], J. A. CRONIN[2,1], D. J. CALDWELL[2,1], N. R. WILSON[1,2], D. SARMA[1,1], B. W. BRUNTON[3], R. P. N. RAO[4,1], J. G. OJEMANN[5,1]; [1]Ctr. for Sensorimotor Neural Engin., [2]Bioengineering, [3]Biol., [4]Computer Sci. and Engin., [5]Neurosurg., Univ. of Washington, Seattle, WA; [6]Neurobio., Imperial Col. London, London, United Kingdom; [7]Bernstein Ctr., Univ. of Freiburg, Freiburg, Germany

Abstract: Electrocorticography (ECoG) is a promising technique for direct brain control of dexterous upper-body robotic prostheses. However, the underlying stages and stage transitions in ECoG signals during imagined movement remain unclear. Improved understanding of the dynamics of imagined motor movement, and their similarity to movement planning behavior, is required for more rapid development of brain-computer interfaces (BCIs) for the volitional control of assistive prosthetics. We investigated several spatial-time-frequency models of ECoG signals during imagined and overt hand grasping behavior recorded in epileptic patients from clinical platinum subdural ECoG grids (10mm spacing). We matched dimensionally-reduced ECoG "movement staging" features, derived from principal component projections of wavelet transformed recordings during overt hand movement, with corresponding ECoG features in imaginary movement using dynamic time warp (DTW). Our decoding was tested in three patients implanted with 8×8 lateral temporal-parietal clinical grids during a dynamic movement

task with imagined hand movements mimicking a software robotic hand. Decoding with two principal movement axes (power grasp-open palm and pinch-hook) in this closed-loop imagined movement BCI achieved identification of the type of continuous movement with regularized discriminant analysis at an accuracy of 45%, with an above-chance performance in predicting stage transitions within a movement type. These results suggest the presence of stage-linked ECoG features of imaginary movement, and spatiotemporally localized stage transitions during imagined hand movements.



Disclosures: J. Wu: None. **L. Bashford:** None. **J.A. Cronin:** None. **D.J. Caldwell:** None. **N.R. Wilson:** None. **D. Sarma:** None. **B.W. Brunton:** None. **R.P.N. Rao:** None. **J.G. Ojemann:** None.

Poster

058. Neuroprosthetics: Processing Techniques

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Topic: E.05. Brain-Machine Interface

Support: Center for Sensorimotor Neural Engineering (CSNE), a National Science Foundation Engineering Research Center (EEC-1028725).

Washington Research Foundation, University of Washington Institute for Neuroengineering (UWIN)

Title: Surface and penetrating glassy carbon integrated microelectrode array for recording low and high frequency neural signals

Authors: N. GOSHI[1,2], M. VOMERO[1,2], T. J. RICHNER[3,2,4], E. MAGGIOLINI[9], E. ZUCCHINI[10], E. CASTAGNOLA[9], D. BJANES[5,2,4], I. DRYG[6,2], W. SHAIN[7,2], S. I. PERLMUTTER[8,2], D. RICCI[9], L. FADIGA[9,10], *C. T. MORITZ[3,2,4,8], S. KASSEGNE[1,2];

[1]Mechanical Engin., San Diego State Univ., San Diego, CA; [2]Ctr. for Sensorimotor Neural Engin. (CSNE), Seattle, WA; [3]Dept Rehabil. Med., [4]UW Inst. for Neuroengineering (UWIN), [5]Electrical Engin., [6]Bioengineering, [7]Neurolog. Surgery, [8]Physiol. & Biophysics, Univ. Washington, Seattle, WA; [9]Ctr. for Translational Neurophysiol. of Speech and Communication (CTNSC), Inst. Italiano di Tecnologia, Ferrara, Italy; [10]Section of Human Physiol., Univ. of Ferrara, Ferrara, Italy

Abstract: A major research challenge in the area of brain computer interface is the creation of a recording platform that combines high signal quality and spatial resolution with minimal tissue damage and chronic stability. Historically, implantable recording devices have been divided into two main categories, penetrating and surface electrode arrays. Penetrating devices typically have a greater signal quality and spatial resolution as compared to surface electrodes, while surface electrodes are minimally invasive and produce a less severe immune response. Previously recording of single action potentials have been limited to the more invasive penetrating electrodes, however new technologies have recently been reported that can record neuronal spikes from a subdural μECoG array. With these new technologies, surface μECoG arrays have greatly improved recording capabilities and spatial resolution, while maintaining their minimally invasive state and superior chronic stability. We have quantified the in-vitro biocompatibility of the devices and the in-vivo tissue response to glassy-carbon surface μECoG electrodes during chronic implantation, both during passive recording and active stimulation. Tissue response was minimal under recording electrodes, likely due to the inert nature of the carbon electrode and location on the brain surface, with no severe immune response observed. Additionally, we show that thin-film μECoG arrays implanted subdurally in rat models are stable over chronic implantation timescales. Further, we introduce the concept of a combined surface and penetrating device - integrated within a single flexible substrate - to validate the ability to record single unit action potential using a non-penetrating electrode and to compare the quality of the signals at different depths above and within the cortex. By fabricating a device with both surface and penetrating electrodes that cover the same cortical regions, we are better able to characterize the signals recorded simultaneous at different depths. Additionally, by combining the signals recorded by the surface and penetrating sites, we may be able to produce a 3D recording map of the area of interest. To realize this potential, we fabricated a flexible microelectrode array, composed of both cortical and penetrating glassy carbon electrodes. We are currently optimizing the method of insertion of the flexible penetrating probe and validating the device with *in vitro* electrochemical tests and *in vivo* characterization. If successful, the ability to record stable single unit action potentials from the brain surface will enable long-term clinical applications of brain computer interfaces.

Disclosures: **N. Goshi:** None. **M. Vomero:** None. **T.J. Richner:** None. **E. Maggiolini:** None. **E. Zucchini:** None. **E. Castagnola:** None. **D. Bjanes:** None. **I. Dryg:** None. **W. Shain:** None. **S.I. Perlmutter:** None. **D. Ricci:** None. **L. Fadiga:** None. **C.T. Moritz:** None. **S. Kassegne:** None.

Poster

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Topic: E.05. Brain-Machine Interface

Support: NIH Grant NS065186

NIH Grant 2K12HD001097-16

NIH Grant 5U10NS086525-03

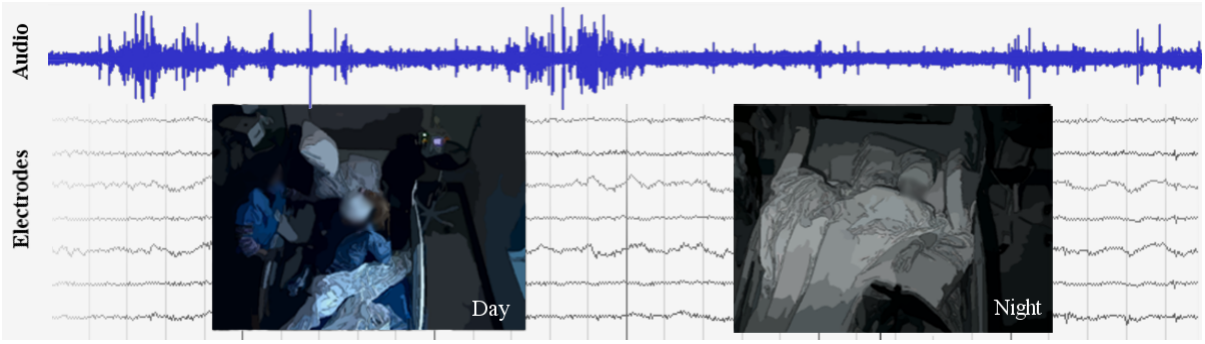
NSF EEC-1028725

Washington Research Foundation

Title: Unsupervised decoding of long-term, naturalistic human neural recordings with automated video and audio

Authors: *X. WANG[1], A. FARHADI[1], J. G. OJEMANN[2], B. W. BRUNTON[3], R. P. N. RAO[1]; [1]Computer Sci. and Engin., [2]Neurolog. Surgery, [3]Biol., Univ. of Washington, Seattle, WA

Abstract: There have been many successes in using experimental ECoG (Electrocorticography) and EEG (Electroencephalography) studies to decode brain activity; however, natural data has been rarely analyzed. Signals collected from humans "in the wild" are affected by a noisy environment and modulated by a complex range of behaviors. The goal of home Brain-Computer Interface (BCI) use and long-term neural monitoring will need to be robust to these factors. As well, with the great advancements in machine learning and computer vision in recent years, recordings of multiple modalities can provide information about a patient's behavior without the need of strict experimental protocols. In this project, we collected thousands of hours of simultaneous video (including a depth channel), audio and neurophysiological data from patients undergoing clinical long term monitoring for more than 10 ECoG patients. Previously, we were able to automatically cluster behaviorally relevant categories as well as functionally map the brain from the ECoG signals without any supervision nor manual labelling with the aid of computer vision techniques on video. In our current work, preliminary results show that the latest state-of-the art techniques based on deep learning can detect human poses accurately from the video. Our results show the brain mapping and decoding results from the ECoG signals using the automated pose detection from video. Our techniques overall show the suitability of natural data combined with fully automated video and audio analyses in brain activity decoding and prediction.



Disclosures: **X. Wang:** None. **A. Farhadi:** None. **J.G. Ojemann:** None. **B.W. Brunton:** None. **R.P.N. Rao:** None.

Poster

058. Neuroprosthetics: Processing Techniques

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Support: NSF EEC-1028725

National Institute of Neurological Disorders and Stroke 5R01NS065186

Washington Research Foundation Fund for Innovation in Neuroengineering

Achievement Rewards for College Scientists

Title: Error-related potentials for co-adaptive cortical brain-computer interfaces.

Authors: ***N. R. WILSON**[1], D. SARMA[1], J. D. WANDER[1], J. G. OJEMANN[2], R. P. N. RAO[3]; [1]Bioengineering, [2]Neurosurg., [3]Computer Sci. & Engin., Univ. of Washington, Seattle, WA

Abstract: When one's intended action does not match with what has occurred, an error-related potential (ErrP) may occur in various frequency bands in the encephalogram. These potentials may be used to promote unsupervised reinforcement learning in brain-computer interfaces (BCIs), promoting co-adaptation between the user and the decoder. Previous work has shown ErrPs occurring in the frontal and motor cortices with overt movement, in both the high gamma (70-100 Hz) and delta (0-4 Hz) frequency bands. However, ErrPs across different areas in BCI control tasks have yet to be thoroughly examined using electrocorticography (ECoG). We investigated changes in cortical signals during a one-dimensional center-out BCI task, recorded

with clinical subdural cortical electrode grids in epilepsy patients. We presented five subjects with a BCI task in which they controlled the velocity of a cursor to hit a target located above or below the center starting point. The subjects controlled the cursor by modulating their high gamma (70-100 Hz) activity in the electrode most strongly associated with hand or tongue motor imagery. We extracted high gamma and delta (0-4Hz) band amplitude for all trials, and epoched instances where the BCI incorrectly decoded subject intention. For each frequency band and for all 64 channels, we then compared the amplitudes of the ErrP epochs to the correctly decoded epochs. Clinical grid placement varied per subject, so we organized individual channels by location, in order to investigate regional variation in ErrP. We then performed cross-subject analysis to investigate commonality in ErrPs across subjects. Preliminary results suggest that potentials associated with decoder error are different and distinguishable from those associated with correct performance in cortical BCI tasks, and may share common features across subjects (Figure 1).

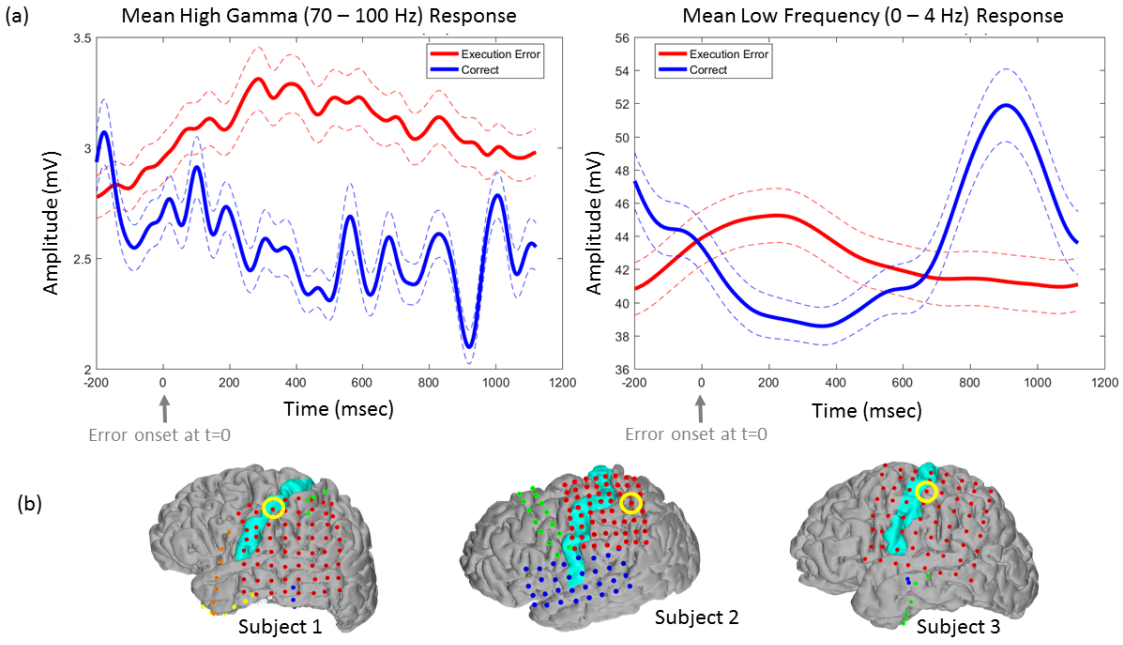

Figure 1. Error-related potentials in M1. (a) The mean responses across the entire primary motor cortex (M1) for both correct and decoder error epochs. (b) Three subjects had left hemisphere M1 coverage. The pre-central gyrus is highlighted in blue and the electrode used for BCI control is circled in yellow.

Disclosures: N.R. Wilson: None. **D. Sarma:** None. **J.D. Wander:** None. **J.G. Ojemann:** None. **R.P.N. Rao:** None.

Poster

058. Neuroprosthetics: Processing Techniques

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NIH NS065186

NIH NS079200

NIH U10NS086525

NSF EEC-1028725

NSF DGE-1256082

NSF IIS-1514790

Title: Somatosensory feedback via direct cortical electrical stimulation in humans

Authors: *J. A. CRONIN[1,5], J. WU[1,5], D. J. CALDWELL[1,5], K. L. COLLINS[2,5], D. SARMA[1,5], R. P. N. RAO[3,5], J. G. OJEMANN[2,5], J. D. OLSON[4,5];
[1]Bioengineering, [2]Neurolog. Surgery, [3]Computer Sci. and Engin., [4]Rehabil. Med., Univ. of Washington, Seattle, WA; [5]Ctr. for Sensorimotor Neural Engin., Seattle, WA

Abstract: Objective: Somatosensory feedback is essential for efficient and precise motor output. Cortical stimulation through electrocorticographic (ECoG) electrodes is a potential method for providing such feedback. Previous research has demonstrated that subjects can distinguish between ECoG stimulation of different frequencies or amplitudes; however, little is understood about how human subjects perceive these novel sensations.
Methods: Subjects were hospitalized for clinical monitoring of epilepsy with implanted ECoG grids. We used Tucker-Davis Technologies hardware to deliver constant-current stimulation using trains of 200 μs biphasic square pulses. We determined the perceptual threshold for stimulation by incrementally increasing the current amplitude, and examined response times and stimulation waveform parameters. For task-specific sensory feedback, subjects wore a 22 degree-of-freedom dataglove to measure their hand position. Subjects opened and closed their hand while they received state feedback via ECoG stimulation. Three states were encoded: 1) hand too open, no stimulation; 2) within the target position, low intensity stimulation; and, 3) too closed, higher intensity stimulation.
Results: Subjects perceived the stimulation on their hands as an abstract sensation, sometimes

described as vibration and/or pressure. Perceptual thresholds were in the range of 1.5 to 2.25 mA. One subject was able to achieve accuracies and R^2 values considerably greater than chance level, with performance dropping during a catch trial (Figure 1).

Conclusions: These results suggest that subjects can react to cortical sensory stimulation and use it as feedback to modulate motor behavior.

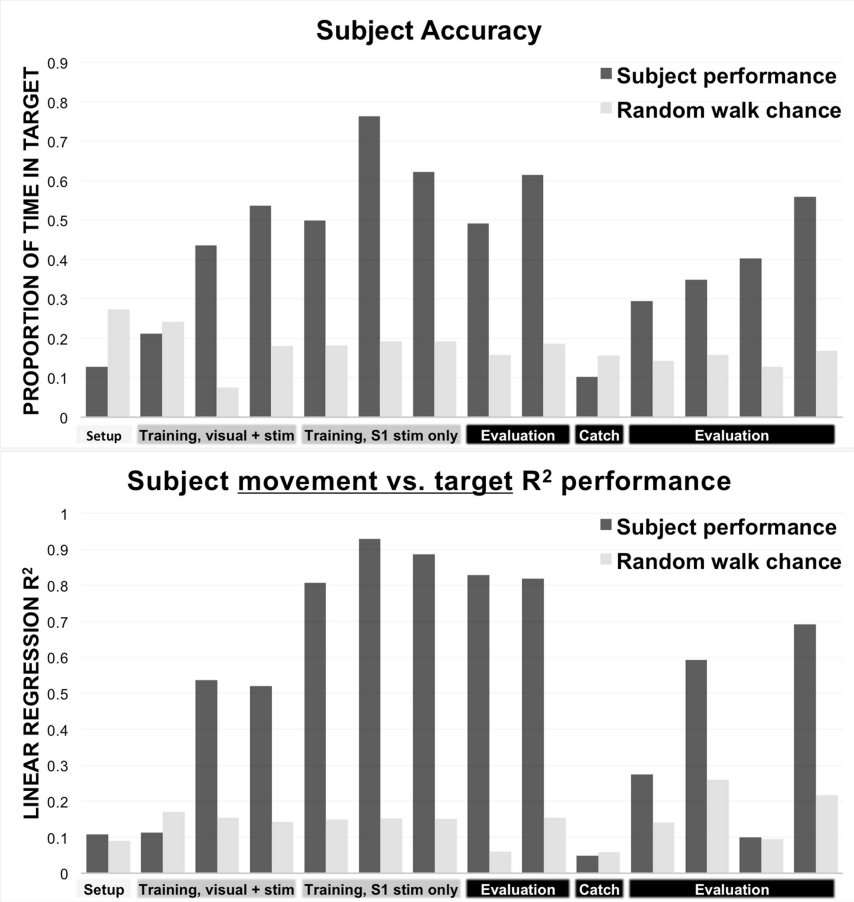

Figure 1. Subject performance in sensory feedback task. Accuracy (top panel) was calculated as *(samples inside target range)/(total samples)*. R^2 values (bottom panel) compare the subject's hand path to the ideal target path. Chance levels were determined by averaging performance on 1,000 simulated random walks. Catch trial used the same stimulation amplitude regardless of the state.

Disclosures: **J.A. Cronin:** None. **J. Wu:** None. **D.J. Caldwell:** None. **K.L. Collins:** None. **D. Sarma:** None. **R.P.N. Rao:** None. **J.G. Ojemann:** None. **J.D. Olson:** None.

Poster

058. Neuroprosthetics: Processing Techniques

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Program#/Poster#: 58.08/GG13

Topic: E.05. Brain-Machine Interface

Support: DOD CDMRP SCIRP SC120209

Center for Sensorimotor Neural Engineering (CSNE), a National Science Foundation Engineering Research Center (EEC-1028725).

Title: Intraspinal activation of respiratory muscles depends on phase of respiratory cycle

Authors: *M. D. SUNSHINE[1,4,5], C. N. GANJI[1,4], P. J. REIER[6], D. D. FULLER[5,7], C. T. MORITZ[1,4,2,3];
[1]Rehabil. Med., [2]Physiol. & Biophysics, [3]Univ. of Washington Inst. for Neural Engin. (UWIN), Univ. of Washington, Seattle, WA; [4]Ctr. for Sensorimotor Neural Engin. (CSNE), Seattle, WA; [5]Physical Therapy and McKnight Brain Inst., [6]Neurosci., [7]Ctr. for Resp. Res. and Rehabil., Univ. of Florida, Gainesville, FL

Abstract: While the anatomy of respiratory circuits within the spinal cord is well described, little is known about the ability to activate these circuits using intraspinal microstimulation (ISMS). In this study we quantified how ISMS activates the three primary respiratory muscles in spinally intact rodents. Female Long Evans rats were anesthetized with ketamine/xylazine and the spinal cord was exposed unilaterally from the second cervical (C2) through the first thoracic (T1) segment. Bipolar electromyography (EMG) electrodes were placed ipsilateral to the stimulated spinal cord in the diaphragm (DIA), intercostals (ICs), and sternocleidomastoid (SCM) muscles. An epoxy-coated tungsten electrode (impedance = 300-500 kOhm) was advanced ventrally along 20 tracks each beginning at the dorsal surface and ending at a final depth ranging from 1800-2400 μm, the 20 tracks were arranged in 2 columns 0.5mm and 1mm from midline. Single biphasic stimuli were delivered at amplitudes from 10-90 μA at each location within the spinal cord. To quantify the interaction of ongoing respiratory rhythms with ISMS, stimulation was delivered during each phase of respiration based on integrated, rectified diaphragm EMG. A total of 10 stimulation pulses at each amplitude were delivered in each phase, with the order of delivery randomized. ISMS evoked respiratory muscle activity at many spinal sites that were outside of the locations of the motor neuron pools for the respective muscles based on retrograde tracing with wheat germ agglutinin (WGA). Intraspinal stimulation delivered during expiration produced lower amplitude muscle contractions compared to stimulation during inspiration. Furthermore, the location of muscle excitation during out of phase stimulation was more centralized to the motor pools. Taken together, these results suggest that ISMS activates a network of spinal interneurons related to respiration, and collaborates with

phase-dependent respiratory networks. The ability of ISMS to interact with residual spinal networks controlling respiratory muscle activity will be critical for improving ventilatory function following spinal cord injury.

Disclosures: M.D. Sunshine: None. **C.N. Ganji:** None. **P.J. Reier:** None. **D.D. Fuller:** None. **C.T. Moritz:** None.

Poster

058. Neuroprosthetics: Processing Techniques

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Topic: E.05. Brain-Machine Interface

Support: NIH Grant RO1 NS12542

NSF ERC Grant for CSNE EEC-1028725

Title: A wireless bidirectional brain machine interface

Authors: *Y. OZTURK[1], Y. SU[1], K. S. MOON[2], S. PERLMUTTER[3], S. ZANOS[3], E. FETZ[3]; [1]Electrical and Computer Engin., [2]Mechanical Engin., San Diego State Univ., San Diego, CA; [3]Univ. of Washington, Seattle, WA

Abstract: Brain machine interfaces (BMI) provide a communication link between the brain and physical devices by offering an alternative path, bypassing physiological pathways that are no longer intact. An implantable BMI provides better signal quality for a broad range of neural activities and allows delivery of activity-dependent electrical stimulation. Wireless bidirectional interfacing with implanted electrodes is an important step towards increasing the clinical potential of BMIs.
We designed a small form factor, wireless bidirectional BMI (BBMI) system. The core component of the open-architecture BBMI system is a communication system on a chip (SoC) with an embedded processor responsible for wireless data transmission, data acquisition, and controlling delivery of stimulation. The sense electronic module provides 32 input channels with a gain of 45.67dB. The stimulation module provides 4 output channels supporting unipolar 20V stimulus with
programmable frequency and pulse duration. A novel ultrasonic wireless power delivery mechanism developed for efficient delivery of power into a deeply implanted system. The current consumption of the system is in the range of 18.47mA to 19.96mA when the device was in normal operation status. Bench tests were conducted by replaying a set of pre-recorded

intracortical signals recorded from an animal model using a research-grade recording system. The stimulation module was
validated by using a scope to monitor the output stimulus amplitude and frequency. In vivo tests of the system were performed on an awake monkey, with an intracortical array implanted in the primary motor cortex. Local field potentials (LFP) were recorded wirelessly at a sampling rate of 1 KHz. Single-pulse stimuli (biphasic, 0.2ms each phase, 40 uA) were delivered wirelessly through one of the intracortical electrodes at a constant rate of 0.6Hz. Stimulus-triggered signal averaging revealed a stimulus-elicited increase of power in high frequency LFP components at neighboring cortical sites at Gamma (30-60Hz) and high-gamma (60-100 Hz) ranges. The changes in the LFP spectrum might reflect a change in the behavioral state following by the stimulation such as arousal. A wireless BBMI module is being integrated to Neurochip-3 to acquire and deliver EMG signals as an implantable sensor and a spinal stimulation module for delivering stimulation to spinal
cord under the control of Neurochip-3.

Disclosures: **Y. Ozturk:** None. **Y. Su:** None. **K.S. Moon:** None. **S. Perlmutter:** None. **S. Zanos:** None. **E. Fetz:** None.

Poster

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NIH NS065186

NIH NS079200

Title: Exploration of the phase and dose dependence of cortico-cortical evoked potentials during beta-oscillation triggered direct electrical stimulation in humans

Authors: ***D. J. CALDWELL**[1], J. D. OLSON[2], J. D. WANDER[1], S. ZANOS[3], D. SARMA[1], D. SU[4], J. A. CRONIN[1], K. COLLINS[5], J. WU[1], L. JOHNSON[5], K. WEAVER[6], K. CASIMO[7], E. FETZ[3], R. P. N. RAO[8], J. G. OJEMANN[5];

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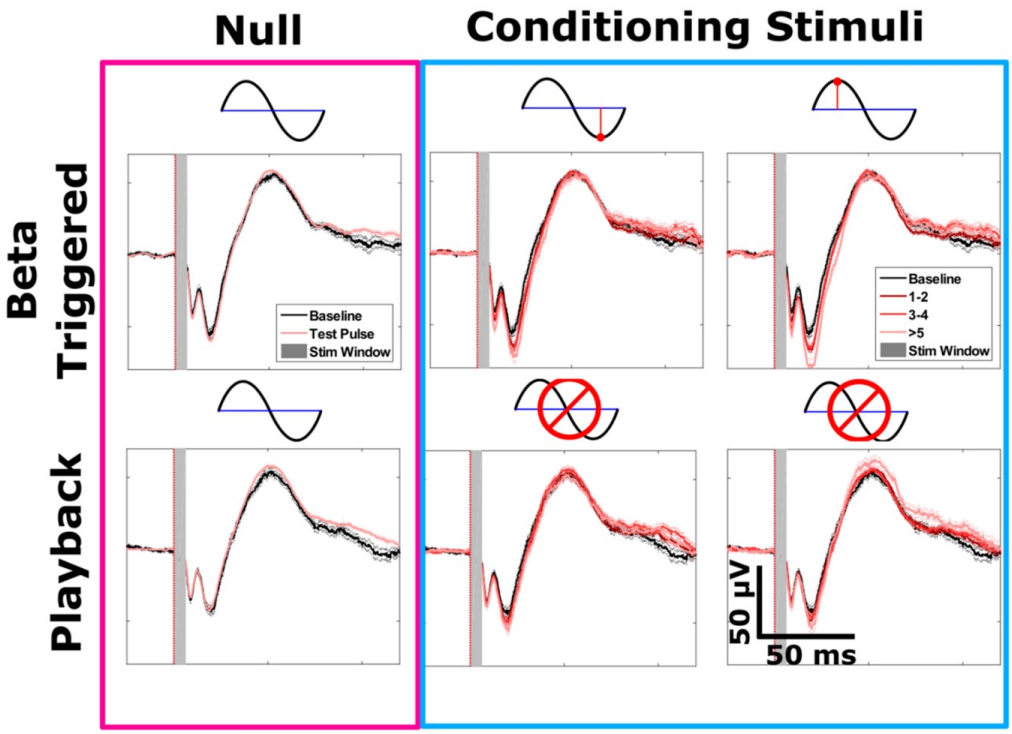
Abstract: Objective: Neuromodulation through activity-dependent cortical electrical stimulation may improve neurorehabilitation outcomes by enhancing synaptic plasticity. Cortico-cortical evoked potentials (CCEPs) are a way to assess stimulation-induced changes in plasticity. Inspired by results from nonhuman primate studies, we studied beta-oscillation triggered cortical stimulation in humans.
Methods: Humans with epilepsy were implanted with electrocorticographic (ECoG) grids for clinical monitoring. The band-passed signal (12-20 Hz) at an electrode in somatosensory cortex triggered stimulation across a separate electrode pair. During periods of heightened oscillations, bipolar, biphasic conditioning pulses were delivered at 0°, 90°, 180°, 270° phases. Test pulses outside of conditioning were used to analyze conditioning effects on CCEPs using z-scored CCEP magnitudes. Resting state analyses were performed on 8 minutes of resting state data before and after stimulation.
Results: ANOVA revealed a dose-dependent enhancement of CCEPs. Within subject analysis revealed no clear phase dependence of stimulation and no consistent change in resting state rhythms as assessed by phase locking value in beta. Increased numbers of conditioning pulses delivered during beta-bursts resulted in greater enhancement of CCEPs relative to baseline. In general, the largest CCEPs and greatest enhancement were seen in the beta-recording channel, though effects were also seen on adjacent channels.
Conclusions: Beta-activity triggered cortical electrical stimulation in human subjects demonstrated enhancement of cortico-cortical evoked potentials, which are a marker of short-term plasticity.
Figure representing dose-dependent enhancement of CCEP magnitude. This example is at an electrode adjacent to the beta triggering electrode. No clear phase dependence is seen. Null indicates no conditioning stimuli delivered. The numbers in the legend indicate the number of conditioning stimuli delivered. Playback represents stimuli delivered irrespective of beta.



Disclosures: **D.J. Caldwell:** None. **J.D. Olson:** None. **J.D. Wander:** None. **S. Zanos:** None. **D. Sarma:** None. **D. Su:** None. **J.A. Cronin:** None. **K. Collins:** None. **J. Wu:** None. **L. Johnson:** None. **K. Weaver:** None. **K. Casimo:** None. **E. Fetz:** None. **R.P.N. Rao:** None. **J.G. Ojemann:** None.

Poster

058. Neuroprosthetics: Processing Techniques

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 58.11/HH2

Topic: E.05. Brain-Machine Interface

Support: GSK Bioelectronics Innovation Challenge

Center for Sensorimotor Neural Engineering (CSNE), a National Science Foundation Engineering Research Center (EEC-1028725)

Washington Research Foundation

Title: Closed-loop neural interfacing strategies for the bladder

Authors: *T. J. RICHNER[1,2,3,6], B. J. MAHONEY[4,6], S. D. BOYER[2,6], V. RANGANATHAN[4,6], M. D. SUNSHINE[2,6], G. MOORE[5,6], R. SOLINSKY[2], G. D. HORWITZ[1,3], P. O. ANIKEEVA[7,6], J. R. SMITH[4,5,3,6], J. W. FAWCETT[8], C. T. MORITZ[2,1,3,6]; [1]Physiol. and Biophysics, [2]Rehabil. Med., [3]UW Inst. for Neuroengineering (UWIN), [4]Electrical Engin., [5]Computer Sci. and Engin., Univ. of Washington, Seattle, WA; [6]Ctr. for Sensorimotor Neural Engin. (CNSE), Seattle, WA; [7]Materials Sci. and Engin., MIT, Cambridge, MA; [8]Dept. of Clin. Neurosciences, Cambridge Univ., Cambridge, United Kingdom

Abstract: Neurogenic bladder, both overactive and underactive, has many causes including spinal cord injury, multiple sclerosis, stroke, spina bifida, and Parkinson's disease. Common to these causes is the loss of closed-loop control over the autonomic nervous system by the CNS. Closed-loop control is a feature of homeostasis and requires repetitive measurement, comparison to a set point, and fast actuation. The slow kinetics and lack of measurement by pharmaceuticals means that drugs are generally open-loop. Moreover, systemic drugs often have side effects caused, in part, by a lack of specificity. Encouragingly, the innervation of the bladder often remains intact despite injury or disease. In principle, a system that could record, stimulate, and block the neural activity of an innervated organ could reinstate closed-loop control and thereby restore homeostatic function.

We are developing a closed-loop bladder neuroprosthesis in the rat animal model. Our system features a neural interface and wireless record/stimulate control device. The neuroanatomy of the bladder offers five peripheral targets for interfacing (proximal to distal): the L6-S1 nerve trunk, the preganglionic pelvic nerve, the major pelvic ganglion (MPG), the postganglionic pelvic nerve, and the nerve endings on the bladder muscle. The proximal structures are large enough to cuff or insert probes, while the most distal structures are far too small. We are therefore investigating electrical interfaces in the proximal structures and optogenetics in the distal structures. Proximal structures are potentially less specific when stimulated, so we measured bladder pressure, colon pressure, and pelvic floor EMG while electrically stimulating each structure with a concentric electrical probe. We are also testing viral optogenetic delivery in the bladder muscle and directly to the MPG. We expect that a combination of electrical and optogenetic methods will be required to sufficiently control bladder pressure while avoiding off target effects in the nearby organs. In parallel, we built an implantable wireless device capable of recording electroneurography signals, applying current pulses, and driving LEDs. Our initial tests found that we could power the in vivo device inductively (300 mW for 60 seconds) without significantly heating the surrounding tissue. This charging period was able to power the device for several minutes, depending on LED parameters. Discovery both the principles of selective activation of visceral nerves, as well as implanted device development will lead to applications for bioelectronics medicines in the bladder and other organ systems.

Disclosures: **T.J. Richner:** None. **B.J. Mahoney:** None. **S.D. Boyer:** None. **V. Ranganathan:** None. **M.D. Sunshine:** None. **G. Moore:** None. **R. Solinsky:** None. **G.D. Horwitz:** None. **P.O. Anikeeva:** None. **J.R. Smith:** None. **J.W. Fawcett:** None. **C.T. Moritz:** None.

Poster

058. Neuroprosthetics: Processing Techniques

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 58.12/HH3

Topic: E.05. Brain-Machine Interface

Support: NIH NS079200

NIH NS065186

NIH U10NS086525

Title: Cortical network changes in individuals learning a bimanual task in coordination with an electrocorticographic brain-computer interface.

Authors: *D. SARMA[1], J. WU[2], J. G. OJEMANN[3], R. P. N. RAO[4];
[2]Bioengineering, [3]Neurolog. Surgery, [4]Computer Sci. and Engin., [1]Univ. of Washington, Seattle, WA

Abstract: Introduction: There are large populations of patients that retain some residual natural motor function and who, even with degradation, could benefit from modulation through BCIs. An important question in evaluating the applicability of BCIs to real world bimanual natural movement is: can subjects use BCIs simultaneously coordinated with overt motor activity? Understanding the learning process and how the neural signal adapts during coordination, could be significant in development of BCIs.
Methods: 7 subjects with intractable epilepsy were implanted with electrocorticographic (ECoG) grids for clinical monitoring prior to surgery. g.USBamp biosignal amplifiers (g.Tec) were used to acquire neural signals from the ECoG electrodes. Subjects were trained to interact with a 2-D center-out cursor task in which y-velocity was driven by the modulation of high-gamma (70-200Hz band) activity in a single electrode over right motor cortex related to left-hand motor imagery and x-position was controlled by keyboard presses from the right hand. Eight targets, equidistant from center were radially distributed and randomly presented for 6 seconds at a time. Targets along xy-diagonals required coordinated control of right (overt) and left (imagined) hand behaviors.
Results: In preliminary analysis, one subject, with lifelong hemiparesis, achieved accurate and precise control despite confounding neural activation from ipsilateral motor activity (Figure 1). As user-control improves, high-gamma activation patterns consolidate over time as per target y-axis modulation requirements (y+/y-), countering the significant HG activations related to the right hand motor activity (x+/x-). HG activation across cortical areas also changes in relation to strength of modulation in the control channel.
Conclusion: Subjects' ability to modulate and adapt their HG signal despite confounding

ipsilateral interference underscores the utility of BCI-training even with damaged cortical areas. These results provide steps towards adapting BCIs for dexterous coordinated bimanual control.

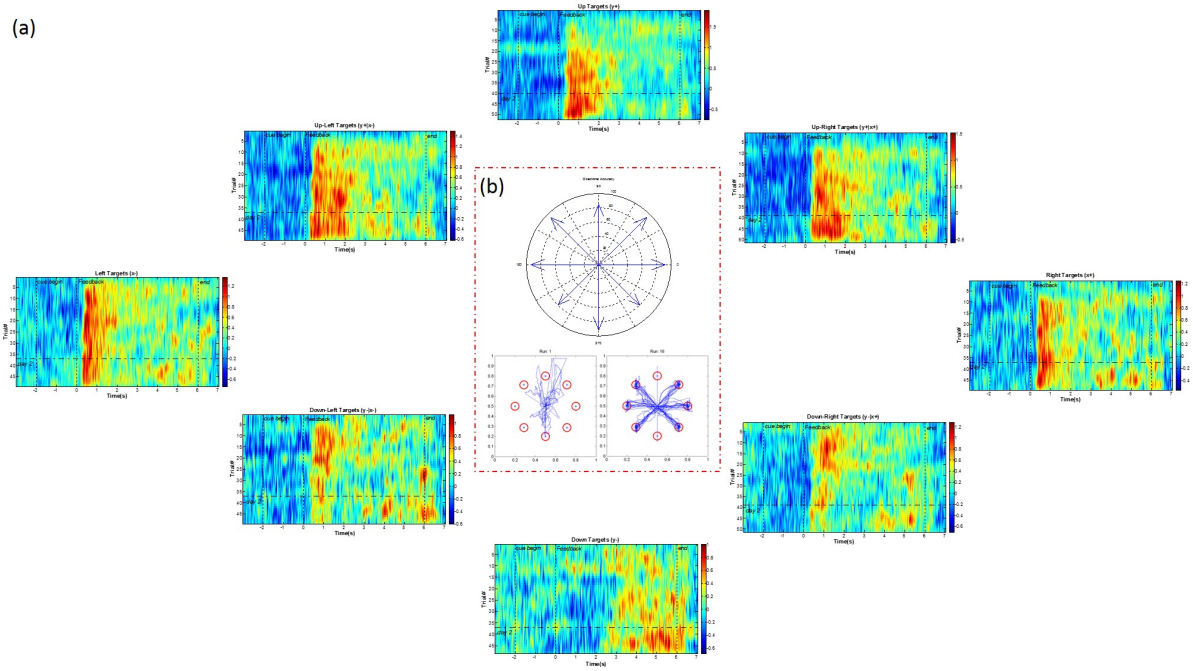


Figure 1 - Trial by Trial HG activation at BCI Control Channel. Recordings were common-average re-referenced over the whole grid. Channel data was band pass filtered for the high-gamma (HG) range (70-200 Hz) using a fourth-order Butterworth filter. A time variant estimate of the HG-band power was calculated from the square of the magnitude of the Hilbert Transform. Data was then log normalized and further z-normalized with respect to the rest (behaviorally quiescent) periods. The HG activation for each trial, concatenated top to bottom, is plotted as an image for each target (in relative spatial orientation). (a) Subject initially had extremely poor performance with left-hand motor imagery. As subject improves, HG activation is seen to increase over the trials, but only in relation to the feedback cue, indicating purposeful modulation. HG suppression or reduction is seen to increase over the trials as control improves for downwards imagery control. For the x+/x- only targets along the horizontal, the right hand key presses result in a HG activation starting from the feedback cue, continuing as right-hand behavior is re-triggered to fight the gravity element along the x-axis. For the up diagonal targets, as control improves, HG activation increases, though activation due to key presses persists. This ipsilateral HG Activation is similar over the trials for the down diagonal targets as well. As control improves, HG activation decreases, though activation due to key presses persists. However, this decrease mirrors the HG decrease for the Down targets and is inverse of the activation pattern for Up Diagonals. (b) Center-inset: the average Directional Accuracy is shown for all targets, plotted radially. In addition, traces of the real cursor behavior are plotted from the first and last runs the subject performed, showing the difference in trajectories between final and initial control.

Disclosures: D. Sarma: None. **J. Wu:** None. **J.G. Ojemann:** None. **R.P.N. Rao:** None.

Poster

058. Neuroprosthetics: Processing Techniques

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 58.13/HH4

Topic: E.05. Brain-Machine Interface

Support: ERC-2012-AdG 320708-iCONNECT

Title: Controlling false positives on a BCI implant for communication

Authors: *M. P. BRANCO[1], Z. V. FREUDENBURG[1], E. G. M. PELS[1], E. J. AARNOUTSE[1], S. LEINDERS[1], M. A. VAN DEN BOOM[1], T. DENISON[2], M. J. VANSTEENSEL[1], N. F. RAMSEY[1];
[1]Brain Ctr. Rudolf Magnus, Univ. Med. Ctr. Utrecht, Utrecht, Netherlands; [2]Medtronic Neuromodulation, Minneapolis, MN

Abstract: Electrocorticography-based Brain-Computer Interfaces (BCI) are an emergent technology that enables paralyzed patients to restore or replace motor function. Up until now, most BCI systems report classification accuracies ranging from 75 to 90%. However, when aiming for spelling with an implant, 90% accuracy is not good enough. Recently, a fully implantable BCI communication system (Utrecht NeuroProsthesis, UNP) was implemented, which translates neuronal activity elicited upon hand attempted movements into a binary control signal for selection of characters in spelling software.
The UNP system was implanted in a locked-in patient who suffers from late stage Amyotrophic Lateral Sclerosis, with a four-electrode strip covering hand sensorimotor cortex. The best bipolar pair was chosen based on the best R^2 response to a motor localizer task, where the patient alternated between trials of attempted movement and rest.
A standard Cursor Pong task (in BCI2000) was used to estimate the optimal signal processing parameters for a one-dimensional continuous control signal. Across several months the average Pong performance using high-frequency broadband power (65-95 Hz) was 90.73±6.42 % (N=70 runs), which is significantly above chance (50%, $p<0.01$). However, the same set of parameters used for the Pong task showed an unexpected large number of false positives (FP) during a spelling task where a power value exceeding a set threshold was translated to a binary active state, which was converted to an item selection (click). There were on average 2.26 FP/min (N=20 7-letter words), yielding a FP rate of approximately 30% and a true positive (TP) rate of 88%. To reduce the FPs, while preserving the TPs, a number of strategies were applied: 1) the signal threshold for determining binary active state was adjusted; 2) a smoothing filter was added to reduce the rate of spurious active states; 3) an additional narrowband power channel (beta, 20±2.5 Hz) was added to the control signal with an unitary negative weight; 4) and, finally, the number of consecutive active states required to generate a click was increased.
Results indicate that changes in the threshold reduce both TPs and FPs. By smoothing the data TPs were preserved while the rate of FPs declined slightly. The addition of the beta signal showed a clear improvement from previous strategies. Finally, increasing number of active states required for a click improved performance, but did so at the expense of increased patient effort. A compromise was reached where effort was acceptable while performance was improved, resulting in a score of 1.14 FP/min (N=13, 5-letter words, resulting FP-rate of 16%), while keeping TP rate at 88%.

Disclosures: **M.P. Branco:** None. **Z.V. Freudenburg:** None. **E.G.M. Pels:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Funded by the Dutch Technology foundation STW with co-funding from Medtronic Europe. **E.J. Aarnoutse:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Funded by the Dutch Technology foundation STW with co-funding from Medtronic Europe. **S. Leinders:** None. **M.A. van den Boom:** None. **T. Denison:** A. Employment/Salary (full or part-time): Medtronic Neuromodulation USA. **M.J. Vansteensel:** None. **N.F. Ramsey:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Funded by the Dutch Technology foundation STW with co-funding from Medtronic Europe.

Poster

058. Neuroprosthetics: Processing Techniques

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 58.14/DP04 (Dynamic Poster)

Topic: E.05. Brain-Machine Interface

Support: EU grant ERC-Advanced 320708

Netherlands Government grant STW 12803

Netherlands Government grant STW UGT7685

Netherlands Government grant HCMI 056-10

Netherlands Government

Title: Autonomous communication at home by a person suffering from Locked In Syndrome achieved with a completely implanted permanent Brain-Computer Interface system

Authors: ***N. F. RAMSEY**[1], M. J. VANSTEENSEL[2], E. PELS[2], S. LEINDERS[2], M. P. BRANCO[2], M. A. VAN DEN BOOM[2], T. DENISON[3], E. J. AARNOUTSE[2];
[2]Neurol. and Neurosurg., [1]Brain Ctr. Rudolf Magnus, Univ. of Utrecht, Utrecht, Netherlands; [3]Neuromodulation, Medtronic, Minneapolis, MN

Abstract: People with Locked In Syndrome are not able to communicate at their free will. They can interact by means of very basic channels such as eye blinks to indicate yes or no to closed questions and as such depend heavily on their caregivers. We here report on the first Brain-Computer Interface system that is fully implanted (sensors, amplifiers, signal processing and transdermal wireless transmission) (Investigational Activa PC+S and Nexus System by Medtronic) and enables the user to generate binary signals with which dedicated ‘switch’

software such as a speller can be used autonomously without expert assistance.
A 58 year old women in late stage of Amyotrophic Lateral Sclerosis underwent surgery for placement of several 4-electrode strips under the dura. The exact location of electrodes was based on the combination of a 3T fMRI scan for attempted right-hand movement with the typical location of the hand knob on motor cortex. Strips were positioned through accurately planned burrholes, under the dura. Strips were connected to the device which allowed for bipolar signal acquisition within strips.
After surgery there were no visible signs of the implant, meeting the wish of the patient. During 2 meetings per week (6 months per may 2016), the team visited the patient at her home for testing the system with 4 types of tasks, each designed to address specific features of the bipolar brain signal generated by attempting to make one or more hand movements (fingertapping). Tasks were: the widely used Pong task (general BCI control), a cueing task with short and long 'active states' where the patient was required to regulate the duration of activation (timing properties), an item selection task with scanning software (reliability of decoding), and spelling to test the BCI system for real-life use.
The patient regarded participation as highly motivating due to continuous improvement of performance, in part due to her training but mostly due to development, implementation and optimization of multiple filters for transformation of raw brain signals to a control signal. With deteriorating residual communication capability (minimal speech, eye tracking) she expects to rely on the system for communication in the near future and has started to use the speller autonomously at home without the presence of the research team.
These results indicate feasibility of offering Locked In patients a new means of communication. Mass production of the device, currently a commercially manufactured investigational device, is expected to make the system affordable for a wide range of candidates, and surgery is expected to be straightforward and efficient (4 hours in OR, 3 days of admission to the ward)

Disclosures: **N.F. Ramsey:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Medtronic. **M.J. Vansteensel:** None. **E. pels:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Medtronic. **S. leinders:** None. **M.P. branco:** None. **M.A. van den boom:** None. **T. denison:** A. Employment/Salary (full or part-time): Medtronic Inc. **E.J. aarnoutse:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Medtronic.

Poster

058. Neuroprosthetics: Processing Techniques

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 58.15/HH5

Topic: E.05. Brain-Machine Interface

Support: Dutch Technology Foundation STW, number 12803

Title: Demonstration of minimal invasive surgery for an invisible, permanent Brain-Computer Interface

Authors: *E. J. AARNOUTSE[1], M. J. VAN STEENSEL[1], E. G. M. PELS[1], T. DENISON[2], B. H. VERWEIJ[1], P. GOSSELAAR[1], P. C. VAN RIJEN[1], N. F. RAMSEY[1];
[1]Brain Ctr. Rudolf Magnus, Utrecht, Netherlands; [2]Medtronic Neuromodulation, Minneapolis, MN

Abstract: Increasingly, researchers and users appreciate that an implanted Brain-Computer Interface (BCI) for communication has merits over a non-invasive BCI, such as EEG or NIRS, even when the control signal would be equally reliable. Users of a BCI for communication, e.g. Locked-in patients, benefit from a permanent solution to use at home, with minimal requirements of expertise and time of caretakers. At the same time users feel very strong about esthetics, so a BCI device ideally is invisible (Nijboer et al, 2014). An implanted BCI fits both requirements, but the prerequisite of acceptance of implanting a BCI under the skull is a minimal invasive approach. We have demonstrated that it is feasible to implant a BCI which is permanent, invisible and easy to use.
A 58-year-old female late stage ALS patient, on invasive mechanical ventilation, participated in our study. Functional MRI was used to localize the exact location of the regions with highest activity in a motor task (fingertapping) and working memory task (mental calculation). The peak t-values within the predefined areas of interest were identified ("hotspots") and electrode strip (four electrodes) positions were chosen to cover the hotspots maximally. During electrode implant surgery a question mark incision was made to lift part of the scalp from the skull. Five 1 cm burr holes were made guided by the intraoperative neuronavigation system using the 3D cortical surface renderings with the hotspots and planned locations of the burr holes superimposed. Electrode strips were guided subdurally between two burr holes and fixated. We also implanted two spare electrode strips near the hotspots. Subsequently, leads were tunneled to the thorax and extended with temporary leads that externalized through the abdominal skin. After electrode implant surgery, the subject repeated the localizer tasks to select the strips with highest correlation of the bipolar ECoG signal in the gamma frequency range with the task. In a second surgery we attached two electrode strips to an Activa PC+S neurostimulator (Medtronic) with sensing capabilities, implanted subcutaneously in the thorax.
CT images aligned with the fMRI t-value map show positioning of electrodes within 2 mm over the hotspots. During electrode selection ECoG measured between bipolar electrode pairs was highly correlated with the task (r2=0.93). Correlation analysis between t-values and the gamma signal from bipolar electrodes measured with the Activa PC+S showed a high correlation (r2=0.85).
In conclusion, this is a first demonstration that minimal invasive surgery can be used to implant an invisible, wireless, permanent BCI to be used at home by a user for communication.

Disclosures: **E.J. Aarnoutse:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Funded by the Dutch Technology foundation STW with co-funding from Medtronic Europe. **M.J. Van Steensel:** None. **E.G.M. Pels:** C. Other Research Support

(receipt of drugs, supplies, equipment or other in-kind support); Funded by the Dutch Technology foundation STW with co-funding from Medtronic Europe. **T. Denison:** A. Employment/Salary (full or part-time): Medtronic Neuromodulation. **B.H. Verweij:** None. **P. Gosselaar:** None. **P.C. Van Rijen:** None. **N.F. Ramsey:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Funded by the Dutch Technology foundation STW with co-funding from Medtronic Europe.

Poster

058. Neuroprosthetics: Processing Techniques

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 58.16/HH6

Topic: E.05. Brain-Machine Interface

Support: ERC-2012-AdG 320708-iCONNECT

Title: ECoG control signal optimization for use of a communication BCI implant in a person with Locked-In Syndrome

Authors: *Z. V. FREUDENBURG[1], M. P. BRANCO[2], E. J. AARNOUTSE[3], S. LEINDERS[2], M. A. VAN DEN BOOM[2], T. DENISON[4], M. J. VANSTEENSEL[2], N. F. RAMSEY[5]; [1]UMC Utrecht-Rudolf Magnus Inst., Utrecht, Netherlands; [2]Brain Ctr. Rudolf Magnus, Univ. Med. Ctr. Utrecht, Utrecht, Netherlands; [3]Univ. Med. Center, Brain Ctr. Rudolf Magnus, Utrecht, Netherlands; [4]Medtronic Neuromodulation, Minneapolis, MN; [5]Univ. Med. Ctr. Utrecht, Brain Ctr. Rudolf Magnus, Utrecht, Netherlands

Abstract: For continuous real-life use of a BCI implant the standards of performance are much higher than those needed for proof on concept in a controlled lab setting. Optimal neural control signals in real-life use can differ significantly from those established for short experiments in the lab given the requirement for reliable and robust decoding for daily use. In this context it is important to characterize the timing and duration of single intentional brain events used to drive the BCI, and find the optimal signal features.
The Utrecht Neural Prosthesis (UNP) is a fully implanted communication BCI based on bi-polar ECoG signals recorded from the primary motor cortex (Investigational Activa PC+S and Nexus System, Medtronic). A subject in late-stage ALS using the UNP generated a large number of cued short intentional neural events with 3-5s intervals by attempting single hand movements in a task repeated over many sessions across days and weeks at the home.
In this context we investigate the characteristics on the impulse response of several often used electrophysiological bandlimited control signal features (mu (6-12Hz), beta1 (13-16Hz), beta3, (21-30Hz) and high frequency broadband HFB (37-90Hz) neural activity [1]) to single generated

neural events. We then evaluated reliability of the responses for these deliberate events for each bandwidth separately and for combinations thereof.
As expected, the lower frequencies displayed a strong and highly significant drop in power following the cue, whereas the HFB significantly increased in power. The lower frequencies also displayed a distinct rebound response with power significantly exceeding baseline levels. Timing of the bandwidth responses differed between bands, with mu, beta 1 and beta 3 starting to decline immediately after the cue, versus HFB increasing at approximately 0.8 s after the cue. All signal features returned to baseline at approximately 2.6-2.8 s after the cue. The low frequency rebound peaked at about 4 s after the cue. A combination of the features, notably beta3 and HFB, increased significance of the response and improved the reliability of detecting the response against baseline. The rebound contributed to a rapid termination of the combined response, thereby improving control in BCI application such as a spelling device
Our results demonstrate the advantage of combining the beta3 band, associated with task related attention, with the HFB, associated with increased neural activity, to improve the detectability of the neural impulse response. Combined features proved to contribute to a more reliable BCI control signal

Disclosures: **Z.V. Freudenburg:** None. **M.P. Branco:** None. **E.J. Aarnoutse:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Funded by the Dutch Technology foundation STW with co-funding from Medtronic Europe. **S. Leinders:** None. **M.A. van den Boom:** None. **T. Denison:** A. Employment/Salary (full or part-time): Medtronic Neuromodulation USA. **M.J. Vansteensel:** None. **N.F. Ramsey:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Funded by the Dutch Technology foundation STW with co-funding from Medtronic Europe.

Poster

058. Neuroprosthetics: Processing Techniques

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 58.17/HH7

Topic: E.05. Brain-Machine Interface

Title: A flexible modular neural recording and stimulation system for both surface and penetrating electrodes

Authors: *R. LILJEMALM, T. STIEGLITZ;
Univ. of Freiburg, Freiburg, Germany

Abstract: As the technology for miniaturization of bioelectronics is making progress, the interest for high density electrode recordings in the neural systems increase. A modular system

that can easily be expanded to the desired number of electrodes would be beneficial with that in mind. A high density electrode implant would also increase the possibility to choose specific electrodes, e.g. in the proximity of the desired neural target, or active sites instead of silent. Furthermore, a system that uses surface electrodes, but at the same time allows the use of penetrating probes would make it highly adaptable to the need of the researcher, for studying cortical activity in the brain. In our group we have developed several structures based on the polymer polyimide, which is a flexible, stable and biocompatible polymer, therefore excellent for neural probes, especially for chronic applications with high demands on reliability. We present a modular polyimide based implant for cortical surface recordings of neural activity. Furthermore, the implant design offers the possibility to also use penetrating probes for in-tissue recordings.

Disclosures: R. Liljemalm: None. **T. Stieglitz:** None.

Poster

058. Neuroprosthetics: Processing Techniques

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 58.18/HH8

Topic: E.05. Brain-Machine Interface

Support: Wellcome Trust Grant RES01657592

Title: Multi-channel neural logger for recording neural activity in free behavior non-human primates.

Authors: *F. DE CARVALHO, W. XU, A. JACKSON;
Newcastle Univ., Newcastle Upon Tyne, United Kingdom

Abstract: Advances in chronic electrode arrays allow action potentials to be recorded from multiple neurons in freely behaving animals. However, long-term continuous recording in non-human primates remains a challenge since wireless systems typically have a limited communication range and battery lifetime. Therefore, to obtain continuous recordings from large numbers of neurons during free behaviour and sleep we have developed a custom battery-powered neural data logger. The device uses an off-the-shelf microcontroller (STM32F415) and two INTAN amplifiers to record 8 channels at 20 kHz (for spikes) and 32 channels at 1000 Hz (for local field potentials) with 16 bit resolution. Data is stored to 64 GB flash memory (µSD card) and subsequently transferred to a computer for off-line analysis. Two 3.6V, 1800mAh Li-Ion batteries (Ultralife UBP001) allow continuous recording for up to 28hrs. Neural activity was recorded over a 24-hour period from the primary motor cortex of freely behaving macaque monkeys. During the night, the local field potential revealed cyclic transitions between slow-

wave sleep (high delta power) and REM sleep (low delta power). The firing rates of most neurons were elevated during REM periods. During slow-wave sleep, the neural spiking was strongly phase-locked to low-frequency local field potentials. We have successfully recorded local field potentials and stable spike activity from multiple neurons continuously over 24 hour periods. Initial analyses reveal the known architecture of brain activity during sleep. This technology will enable studies of network dynamics across multiple behavioural states and the mechanisms of memory consolidation.

Disclosures: F. De Carvalho: None. **W. Xu:** None. **A. Jackson:** None.

Poster

058. Neuroprosthetics: Processing Techniques

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 58.19/HH9

Topic: E.05. Brain-Machine Interface

Support: STW grant 12803

Title: Implanted brain-computer interface signal stability over time

Authors: *E. G. M. PELS[1], E. J. AARNOUTSE[1], S. LEINDERS[1], Z. V. FREUDENBURG[1], M. P. BRANCO[1], M. A. VAN DEN BOOM[1], T. DENISON[2], M. J. VANSTEENSEL[1], N. F. RAMSEY[1];
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Abstract: In recent years implanted Brain-Computer Interfaces (BCI) gained more interest. Relying on implanted electrodes these systems carry the advantage of brain signals gathered at the source. Successes in this field have been reported in controlling robotic arms, intended for severe paralysis or arm amputation. For people who have lost most or all ability to communicate, a BCI implant for communication would meet the most important of their needs. The Utrecht NeuroProsthesis (UNP) is designed to restore communication by means of item selection in spelling software, and is completely implanted. Here we report on the stability of signals obtained from the first person implanted with the UNP.
The UNP consists of two four-electrode ECoG-strips connected to an implanted amplifier/transmitter (AT-device; Investigational Activa PC+S and Nexus System, Medtronic). The strip reported here is placed over the left hand sensorimotor cortex. The AT-device amplifies the signal and sends it wirelessly to a computer outside the body. Adapted BCI2000 software is used to analyze and process the signal further.

For this study, a partially locked-in patient (58-year-old female) who suffers from late stage Amyotrophic Lateral Sclerosis (ALS) was implanted with the device. The best performing bipolar electrode pair within the strip was selected based on the correlation of the signal (high frequency band power, 65-95 Hz) with task conditions (expressed as R^2) in a localizer task. For this, the patient alternated attempted movements with rest periods.
At home, weekly repetitions of the localizer tasks, measured in time-domain, enabled us to track R^2 over time, as an indication of signal stability. Additionally, the patient learned to control her brain signal using a one-dimensional continuous cursor control task ('Pong'; measured in the energy saving power-domain), where she had to hit a target at either the top or the bottom of a screen.
The results of the localizer task show a slight increase in R^2 over time (0.88 to 0.93 in 6 months for the bipolar used), and stable baseline power in the gamma band (0.486 ± 0.063 a.u.) indicating that the implanted electrodes are durable and information transfer is preserved. The results from the Pong, however, show a fluctuation in the baseline gamma-band power of 5.7%. The cause of this variation is uncertain but possibilities include temperature, level of fatigue and circadian rhythm. Calibration during each session, nonetheless, solved this issue and the average performance over 6 months with sensorimotor control was $90.73 \pm 6.42\%$, which is significantly above chance (50%, $p<0.001$).

Disclosures: **E.G.M. Pels:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Funded by the Dutch Technology foundation STW with co-funding from Medtronic Europe. **E.J. Aarnoutse:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Funded by the Dutch Technology foundation STW with co-funding from Medtronic Europe. **S. Leinders:** None. **Z.V. Freudenburg:** None. **M.P. Branco:** None. **M.A. van den Boom:** None. **T. Denison:** A. Employment/Salary (full or part-time): Medtronic. **M.J. Vansteensel:** None. **N.F. Ramsey:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Funded by the Dutch Technology foundation STW with co-funding from Medtronic Europe.

Poster

058. Neuroprosthetics: Processing Techniques

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 58.20/HH10

Topic: E.05. Brain-Machine Interface

Title: OMNI: A distributed and modular device for wireless neural recording and closed-loop neuromodulation

Authors: *G. ALEXANDROV[1], S. R. SANTACRUZ[2,1], A. MOIN[1], A. J. ZHOU[1], F. BURGHARDT[1], B. C. JOHNSON[3], E. ALON[1], J. RABAEY[1], J. M. CARMENA[2,1], R. MULLER[1,3];
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Abstract: Technology for chronic neural recordings in humans is limited and tends to be constrained by elements such as low channel counts, minimal flexibility in signal acquisition specifications, and wired connections. Recordings in nonhuman primate (NHP) subjects also typically rely on wired connections, restricting the ability to record during natural behavior. To resolve these impediments, we have developed OMNI, a wireless and autonomous neurotechnology for continuous high-throughput streaming of neural data. The OMNI device features reconfigurable stimulation units that can be dynamically assigned to any electrode, as well as components to support closed-loop neuromodulation and behavioral state classification, eclipsing current commercial implantable technology.
The OMNI device has been deployed in one NHP subject for continuous cortical and subcortical recording during free, natural behavior, as well as closed-loop microstimulation. Neural data was synchronized to behavioral events (wake/sleep transitions) using the accelerometer and gyroscope incorporated in the technology. The OMNI device was utilized in this pilot study to determine sleep states, stimulate target sites with implanted microelectrodes at certain detected time points, and simultaneously record local field potential (LFP) activity in corticostriatal circuitry. LFP signal fidelity was preserved in the streamed data compared to data acquired in a standard cabled electrophysiology equipment. Features of the LFP signal were used to determine the closed-loop stimulation protocol for administration of constant-current biphasic pulses. The realization of this pilot NHP study attests to the comprehensive functionality of the OMNI device.

Disclosures: G. Alexandrov: None. **S.R. Santacruz:** None. **A. Moin:** None. **A.J. Zhou:** None. **F. Burghardt:** None. **B.C. Johnson:** None. **E. Alon:** None. **J. Rabaey:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cortera Neurotechnologies. **J.M. Carmena:** None. **R. Muller:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cortera Neurotechnologies.

Poster

058. Neuroprosthetics: Processing Techniques

Location: Halls B-H

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Program#/Poster#: 58.21/HH11

Topic: E.05. Brain-Machine Interface

Support: NIH Grant DA026452

Title: A real-time sense-and-stimulate intracranial system detects and slows impending movements

Authors: *B. D. MOORE, IV[1], A. R. ARON[2], N. TANDON[1];
[1]Neurosurg., Univ. of Texas McGovern Med. Sch., Houston, TX; [2]Psychology, UCSD, San Diego, CA

Abstract: A major goal of recent work in human neural prosthetics is to deliver stimulation to neural circuits in a manner time locked to ongoing activity. It is well known that voluntary movements are preceded by motor planning related activity in the supplementary motor area (SMA). This lag between neural activity and observable behavior provides a test bed for the development of a decoding system that can then be used to control an external device. Here we tested a method that aimed to detect impending activity in the SMA and then deliver direct cortical stimulation to influence behavior on the same trial.
We focused on electrodes placed in the SMA in a cohort of 4 patients undergoing invasive surgery for treatment of refractory epilepsy. First, the patients performed a task that involved a series of bilateral finger movements. On each trial a cue instructed them to move their fingers in a coordinated sequence. Electrocorticographic (ECoG) signals recorded from electrodes in the SMA were analyzed for pre-movement gamma frequency power increases. We then configured a closed loop control system to detect and quantify gamma power changes in real time. When gamma power exceeded a pre-defined threshold, a transient electrical stimulus was delivered to SMA electrodes. Control and stimulation trials were randomly interleaved such that both patient and experimenter were double-blinded to trial type. The dependent measure was the reaction time measured when the contralateral hand first pressed a button. In a separate experiment carried out in each patient, stimulation was delivered chronometrically, based only on the anticipated time of movement initiation and independent of the detection of pre-movement activity.
Subsequent analysis of the ECoG signal showed that in three of the four patients, the system successfully detected pre-movement gamma activity in the SMA. In these three patients, stimulation based on the detection of this activity resulted in significant slowing of behavior in stimulated versus control conditions (mean for three subjects: 100 ± 19 ms, all < 0.05). Similarly, chronometric stimulation also resulted in significantly delayed behavioral responses (mean for three subjects: 289 ± 25 ms, all < 0.001). The timing of chronometric stimulation was much closer to movement execution, possibly accounting for the larger effect. At the current time, not much is known about when and how to perform real time modulation of endogenous brain activity to alter behavior. Our results show that, within a trial, impending movement can be detected and used to trigger direct current stimulation of SMA to influence ongoing motor behavior safely and in a manner imperceptible to the subject.

Disclosures: B.D. Moore: None. **A.R. Aron:** None. **N. Tandon:** None.

Poster

058. Neuroprosthetics: Processing Techniques

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 58.22/HH12

Topic: E.05. Brain-Machine Interface

Support: SPAWAR HR0011-15-C-0036

Title: Wireless implantable multichannel myoelectric system with infrared data transmission

Authors: *D. R. MERRILL, S. HIATT, B. CROFTS, C. SMITH, K. S. GUILLORY, D. MCDONNALL;
Ripple, Salt Lake City, UT

Abstract: Modern hand and arm prostheses provide multiple degrees of freedom (DoF) of motion, yet their use is severely limited by the biological control interface. Current prostheses are controlled by EMG signals recorded from surface electrodes on the residual limb. These recordings are a composite of signals from several muscles and typically do not provide enough independent sources for simultaneous multi-DoF control. Additionally, the surface recording sites are unreliable. These impediments limit transradial prostheses to two DoF with sequential control. Ripple has developed an implantable system which simultaneously records 32 channels of myoelectric data from multiple residual muscles, and transmits these data to an external transceiver placed in the prosthetic socket. Our objective is to provide simultaneous multi-degree of freedom prosthesis control, ultimately providing an intuitive control experience. This approach supports a high number of independent control signals and provides access to EMG from deep muscles that cannot be accessed with surface electrodes. The system comprises a hermetic implanted module from which nine EMG leads emerge. Eight of the leads contain four electrode sites each for 32 total recording channels. A ninth lead provides the reference electrode. The implant receives power inductively from an external transceiver and sends digitized EMG data to the external transceiver via infrared light. By using a single subcutaneous module for telemetry from which several leads emerge, power coupling efficiency remains high. We have demonstrated high data rate transmission using infrared light in chronically implanted canine. Devices were implanted in deltoideous and the long and lateral heads of triceps. Recorded EMG demonstrate very low noise and clearly indicate antagonistic activity of the gait muscles. We have completed initial safety and performance testing. These efforts demonstrate the ability to amplify and transmit muscle signals and confirm safety and performance requirements. This approach has the potential to provide simultaneous multi-degree of freedom prosthesis control, especially if used with advanced hand and arm prostheses, targeted muscle reinnervation patients, and recently developed pattern recognition algorithms.

Disclosures: **D.R. Merrill:** A. Employment/Salary (full or part-time): Ripple LLC. **S. Hiatt:** A. Employment/Salary (full or part-time): Ripple LLC. **B. Crofts:** A. Employment/Salary (full or part-time): Ripple LLC. **C. Smith:** A. Employment/Salary (full or part-time): Ripple LLC. **K.S. Guillory:** A. Employment/Salary (full or part-time): Ripple LLC. **D. McDonnall:** A. Employment/Salary (full or part-time): Ripple LLC.

Poster

058. Neuroprosthetics: Processing Techniques

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 58.23/HH13

Topic: E.05. Brain-Machine Interface

Support: Neilsen Senior Research Grant #340943

Title: Development of cortically-controlled muscle stimulation to restore treadmill locomotion and overground navigation in spinal cord injured rats

Authors: *A. A. KINKHABWALA, M. K. JANTZ, J. A. GALLEGO, T. A. VERNON, M. C. TRESCH, L. E. MILLER;
Physiol., Northwestern Univ., Chicago, IL

Abstract: We have shown previously that FES (functional electrical stimulation) driven by neural activity in the motor cortex can be used to reestablish the ability to grasp and move objects. That work, in monkeys, used only temporary paralysis induced by peripheral nerve block. In order to move this technology closer to clinical application we have begun developing similar methods to restore locomotion to rats with spinal cord injury (SCI).
We are developing techniques to predict muscle activity during locomotion from cortical activity and to restore functional hindlimb movements with FES. We are using a Plexon MAP system to record neural activity from 32 channel microwire arrays implanted 1.4-1.6 mm below the cortical surface in the hindlimb motor cortex, and examining the modulation of neurons beginning several days after surgery. Neural activity is recorded while rats run on a treadmill (Columbus Instruments) or along a custom built hallway. Hindlimb kinematics are measured with a Vicon system along with cortical activity with the rats running either on a level or inclined treadmill, or moving freely along the hallway. Obstacles added to either of these arenas require the rats to plan and execute more complex, cortically-dependent movements. We extract functional parameters from the recorded kinematics to characterize the locomotion and then relate these parameters to the simultaneously recorded cortical activity. Preliminary results show a good modulation of single and multiunit cortical activity to the kinematics of the ongoing locomotor cycle. Our goal is to characterize the information content of the cortical activity under different

locomotor conditions and evaluate its adequacy to serve as an FES control signal.
In parallel, we are using a real-time controllable 16 channel stimulator from Ripple to deliver FES to multiple hindlimb muscles. We monitor the kinematics of evoked movements to evaluate the efficacy of the FES. We have begun by using EMG signals recorded during locomotion from intact rats to specify the FES patterns. In initial experiments, we have been able to produce basic flexion/extension cycles similar to those observed during locomotion. Our goal is to optimize muscle stimulation in order to produce appropriate locomotion movements and ground reaction forces while minimizing fatigue.
Ultimately, we will use the estimates of motor intent obtained from cortical recordings to specify the FES control signals in animals with spinal cord injury, enabling restoration of voluntary locomotion and potentially facilitating functional rehabilitation.

Disclosures: A.A. Kinkhabwala: None. **M.K. Jantz:** None. **J.A. Gallego:** None. **T.A. Vernon:** None. **M.C. Tresch:** None. **L.E. Miller:** None.

Poster

058. Neuroprosthetics: Processing Techniques

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 58.24/HH14

Topic: E.05. Brain-Machine Interface

Support: DARPA HAPTIX Contract HR0011512791

Title: Body-wearable platform for un-tethered, closed-loop stimulation and recording for BCI and neuroprosthetic research with hundreds of electrodes

Authors: *S. HIATT, T. POULSON, E. BARCIKOWSKI, R. ROUNDY, S. BARRUS, C. SMITH, A. WILDER, K. S. GUILLORY, D. MERRILL, D. MCDONNALL;
Ripple, Salt Lake City, UT

Abstract: Human neuroprosthetic research has demonstrated potential major improvement in the quality of life for patients with severe neural impairments. This work has primarily been confined to short laboratory-based sessions due to the size and lack of integration of the instruments required and the computational burden of these applications. Advances in this field require new systems that enable experimental designs focused on real-life unscripted patient activities.
Ripple has been developing the core technologies necessary to facilitate un-tethered ambulatory studies with hundreds of bio-potential recording channels combined with researcher-specified on-board processing algorithms and control of hundreds of independent stimulation channels.

We have developed a wearable neural interface system from these core technology components that meets the size, weight, and performance required for on-subject portability. The device has an internal battery to facilitate short-duration un-tethered subject movement, and with options to attach additional batteries to facilitate longer term portable use. An internal hard drive is included for logging of full bandwidth data and algorithm performance metrics. Wireless interfaces (WiFi and Bluetooth) provide experimenters real-time access to data and control of tunable processing parameters.
This work is being completed in compliance with IEC 60601-1 and portions of IEC60601-2 that are applicable to the instrument, with FDA QSR Title 21, Part 820, ISO 13485, and ISO 14971 and we anticipate a version of the device to be cleared for human use in the US and Europe.

Disclosures: **S. Hiatt:** A. Employment/Salary (full or part-time): Ripple LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ripple LLC. **T. Poulson:** A. Employment/Salary (full or part-time): Ripple LLC. **E. Barcikowski:** A. Employment/Salary (full or part-time): Ripple LLC. **R. Roundy:** A. Employment/Salary (full or part-time): Ripple LLC. **S. Barrus:** A. Employment/Salary (full or part-time): Ripple LLC. **C. Smith:** A. Employment/Salary (full or part-time): Ripple LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ripple LLC. **A. Wilder:** A. Employment/Salary (full or part-time): Ripple LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ripple LLC. **K.S. Guillory:** A. Employment/Salary (full or part-time): Ripple LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ripple LLC. **D. Merrill:** A. Employment/Salary (full or part-time): Ripple LLC. **D. McDonnall:** A. Employment/Salary (full or part-time): Ripple LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ripple LLC.

Poster

058. Neuroprosthetics: Processing Techniques

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 58.25/HH15

Topic: E.05. Brain-Machine Interface

Support: W911NF-15-2-0054

Title: A neuromodulation integrated circuit for high-channel count, bidirectional, and minimally invasive neural interface.

Authors: *S. JUNG[1], T. L. HANSON[2], E. ALON[1], J. RABAEY[1];
[1]Electrical Engin. and Computer Sci., Univ. of California, Berkeley, Berkeley, CA; [2]Univ. of California, San Francisco, San Francisco, CA

Abstract: With a desire to better understand the brain, there have been continuous efforts to develop neural interfaces that support 1) fine (microsecond, micrometer) and broad (year, centimeter) coverage, 2) simultaneous bidirectional communication (recording and stimulation), and 3) minimally invasive hardware. We present a building block toward such a system: a low-power, low-area, high-channel count neuromodulation integrated circuit (IC) for both recording and stimulation. These ICs are controlled via a field programmable gate array that communicates with them via an efficient time-multiplexed serial protocol. The FPGA in turn is linked to a PC for analysis and closed-loop experiments via full-duplex gigabit Ethernet. This tightly integrated system realizes the microelectronics part of a thousand-channel bidirectional neural interface. As a key module in the designed interface system, the IC has state-of-the-art features: energy-efficient programmable 64 recording channels (<3uW/channel); programmable stimulators (variable shape, amplitude, pulse width, and pulse rate); and programmable compression filters for action potential extraction. These tight integration works make the IC an ultra-low power (200uW on average) low-area (4.78mm^2) signal processing hardware. By wire-bonding this IC on a small printed circuit board along with directly bonded fine-pitch electrodes, we are on track to realize a fully miniaturized implant device. All the integrated elements are anticipated to yield improved safety, longevity, robustness, and lower infection risk.

Disclosures: S. Jung: None. **T.L. Hanson:** None. **E. Alon:** None. **J. Rabaey:** None.

Poster

058. Neuroprosthetics: Processing Techniques

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 58.26/HH16

Topic: E.05. Brain-Machine Interface

Support: Craig H. Neilsen foundation

NSF GRFP

Title: Design and testing of a low power neural interface module for the networked neuroprosthesis system

Authors: *A. J. BULLARD[1], Z. T. IRWIN[1], K. E. SCHROEDER[1], B. SMITH[3], A. CAMPEAN[3], P. G. PATIL[2], H. P. PECKHAM[3,4], K. L. KILGORE[3,4,5], C. A. CHESTEK[1];

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Abstract: Brain Machine Interfaces (BMIs) have demonstrated great potential for generating prosthetic control signals. However, current BMIs still require transcutaneous leads connecting indwelling electrodes to recording devices outside of the body. Several fully implantable, wireless systems have been designed to improve clinical viability, but are limited by high signal bandwidth requirements. The Networked Neuroprosthesis (NNP) developed at Case Western Reserve University is a system of implantable modules that can perform combinations of neural stimulation and recording for controlling grasp and other motor functions. The existing capabilities are not sufficient for intracortical recording; however, the architecture was designed to accept new modules with added functionality. As an extension to the NNP, we have designed a custom module to record neural data from a 96-channel Utah array and generate command signals for grasping using low power circuitry and spiking band techniques as seen in Stark and Abeles (2007). A six layer printed circuit board was designed to be compatible with the existing NNP hardware and software. The module is designed to fit the dimensions of a 1x4 cm flexible PCB. The electronics consist of off the shelf components: primarily, three 32-channel Intan bioamplifiers and a 32-bit Atmel microcontroller (MCU). The bioamplifiers can record from 96 channels in total with 16-bit ADC resolution. The MCU serves as the data processor and central controller, configuring amplifier settings and communicating with existing NNP modules. Testing was performed by exercising the amplifiers, using simulated neural data from a Blackrock Neural Signal Simulator. The required amplifier power was reduced by extracting the signal power in the 300-1kHz frequency band and using a sampling frequency of 2kSps for each channel. Amplifying and digitizing 96 active channels used only 14.8mW. Decoding performance was investigated using neural data previously recorded during motor and sensory experiments and resampled at 2kSps to validate the spiking band technique used to lower power requirements in our device architecture. Using linear discriminant analysis, we performed a 1of 3 classification to predict either finger flexion (97.6% correct) or finger stimulation (97.9% correct). This suggests that extracting signal power within this band is effective for decoding while saving significant power. The device is currently undergoing preliminary bench top testing to optimize settings and reduce power consumption. This low power neural recording module along with the existing NNP represents a path towards a fully implantable cortical BMI system.

Disclosures: **A.J. Bullard:** None. **Z.T. Irwin:** None. **K.E. Schroeder:** None. **B. Smith:** None. **A. Campean:** None. **P.G. Patil:** None. **H.P. Peckham:** None. **K.L. Kilgore:** None. **C.A. Chestek:** None.

Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 59.01/HH17

Topic: E.09. Spinal Cord Injury and Plasticity

Support: CIHR

NSERC

CFI

HBI

FRQS

Title: Time-dependent bilateral cortical plasticity parallels locomotor recovery after unilateral spinal cord injury in rats

Authors: *A. R. BROWN[1,2], M. MARTINEZ[1,2];
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Abstract: After an incomplete spinal cord injury (iSCI), supraspinal structures, which initiate movement, and spinal circuits, which generate them, remain partially connected through residual descending pathways. In this scenario we and others have shown that, even after extensive iSCI that disrupts all sensorimotor tracts on one side (hemisection at thoracic level), animals recover locomotion spontaneously. The contributing neural structures and neuroplastic mechanisms supporting locomotor recovery are poorly understood. By using a combination of intracortical microstimulation (ICMS) and behavioural techniques, we evaluated the cortical mechanisms of hindlimb locomotor recovery for 12 weeks after a spinal hemisection at T8 in rats. In intact rats, the location and size of the hindlimb representation within the motor cortex (hindlimb motor map) was consistent between animals, and hindlimb motor maps contained representations of contralateral hindlimb joints exclusively. After iSCI, rats exhibited hindlimb locomotor deficits on the side of the lesion with a plateau in recovery developing by 5 weeks. During the early recovery stage (first 3 weeks), the representation of the affected hindlimb disappeared within the contralatesional (de-efferented) cortex and emerged within the ipsilesional motor cortex. Specifically, ICMS applied on the ipsilesional motor cortex evoked movements of both hindlimbs, indicating that, after iSCI, the ipsilesional motor cortex functionally reorganizes to encode bilateral hindlimb movements. By the fourth week of recovery, the ipsilesional motor cortex lost its ability to produce bilateral movements and regained its normal control over the contralateral (non-affected) hindlimb. During the late recovery stage (12 weeks), the

representation of the affected hindlimb re-appeared within the contralesional cortex. These data suggest that the recovery involves the ipsilesional motor cortex during the early recovery stage and the contralateral motor cortex during the late recovery stage. These dynamic plastic changes in motor cortical areas may represent an adaptive strategy for functional compensation after SCI that would ultimately help identifying therapeutic strategies to harness cortical activity and catalyze functional recovery following iSCI.

Disclosures: A.R. Brown: None. **M. Martinez:** None.

Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 59.02/II1

Topic: E.09. Spinal Cord Injury and Plasticity

Support: Grant-in-Aid for Scientific Research (C) from the Japanese Ministry of Education 26350564

Title: Short-term effect of repetitive electrical nerve stimulation on spinal reciprocal inhibition depends on the phase of passive stepping.

Authors: *H. OBATA[1], T. OGAWA[2], T. KITAMURA[3], N. KAWASHIMA[4], K. NAKAZAWA[2];
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Abstract: Introduction: Combination of electrical nerve stimulation (ES) and passive or active intermittent movements (i.e., pedaling and stepping) has been suggested to induce stronger short-term effect in the spinal circuits compared to either intervention alone. The purpose of the present study was to determine whether the effects of electrical stimulation and passive stepping are dependent on the phase of the stepping. Methods: In 10 healthy subjects, two interventions were assessed at intervals of over 1 week between interventions. ES to the common peroneal nerve (CPN) at the motor threshold intensity was applied during 1) swing phase (SW intervention); and 2) stance phase (ST intervention) of passive ground stepping for 30 min. We measured the H-reflex in the soleus muscle and spinal reciprocal inhibition from the tibialis anterior muscle before (baseline), as well as 5, 15 and 30 min after each intervention. Spinal reciprocal inhibition was assessed by conditioning the soleus H-reflex with ES to the CPN. Results: The amount of spinal reciprocal inhibition was significantly increased 5 and 15 min

after the SW intervention as compared to the baseline values ($P < 0.05$). The ST intervention did not have an effect. Neither intervention affected the H-reflex amplitude in the soleus muscle. Conclusion: These results show a phase-dependent effect of ES of the CPN on spinal reciprocal inhibition during passive stepping. Significance: The results suggest that phase-dependent timing of ES during intermittent passive movement is important for inducing changes in the spinal circuits.

Disclosures: H. Obata: None. **T. Ogawa:** None. **T. Kitamura:** None. **N. Kawashima:** None. **K. Nakazawa:** None.

Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

Location: Halls B-H

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: The general insurance association of Japan - Grant

Title: Suicide gene therapy - induced cell death using HSV-1tk for tackling tumorigenesis following human induced pluripotent stem cell-derived neural stem/progenitor cell transplantation for spinal cord injury

Authors: *K. KOJIMA[1,2], H. MIYOSHI[2], S. ITO[2], T. OKUBO[2], M. OZAKI[2], K. SUGAI[2], S. KAWABATA[2], Y. NISHIYAMA[2], G. ITAKURA[2], N. NAGOSHI[2], A. IWANAMI[2], M. MATSUMOTO[2], M. NAKAMURA[2], H. OKANO[2];
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Abstract: Background: We have previously reported functional recovery in rodent spinal cord injury (SCI) models following human induced pluripotent stem cell-derived neural stem/progenitor cell (hiPSC-NS/PC) transplantation. In certain cell lines, however, there have been reports of tumorigenicity, which has raised the issue of "safety". Thymidine kinase/ganciclovir -mediated (TK/GCV) suicide is a system whereby the herpes simplex virus thymidine kinase (HSV-TK) converts ganciclovir (GCV) into a toxic product, which allows selective elimination of TK+ cells. The aim of this study is to evaluate the use of suicide gene therapy as a failsafe mechanism for tumorigenic changes following hiPSC-NS/PC transplantation for SCI. Methods: Efficacy of TK/GCV *in vitro* - A lentiviral vector was employed to introduce the HSK-TK gene into hiPSC-NS/PCs derived from a cell line that is known to have a high risk of tumorigenicity (Nori et al, 2015). Differentiation was induced by culturing these hiPSC-NS/PCs without FGF and LIF. Five days after the induction, GCV was administered to trigger

the apoptosis. Efficacy of TK/GCV *in vivo* - hiPSC-NS/PCs with the HSV-TK gene was transplanted into the injured spinal cord of NOD/SCID mice nine days after the injury. Eight weeks following the transplantation, GCV was intra-peritoneally administered for three weeks (GCV+ group). A control group was produced at the same time(GCV- group). Bio-imaging was used to monitor and compare cell proliferation through photon counts. Results: *In vitro* - When the cells were cultured with GCV, the nestin positive cells had noticeably decreased (GCV(+)18% vs GCV(-) 68%). With Tuj-1 positive cells, however, the drop was less pronounced (GCV(+) 58% vs GCV(-) 80%). *In vivo* - The photon count gradually increased in all mice transplanted with the hiPSC-NS/PCs. GCV+ group showed a decrease in the photon count ($-5.7x10^6$) whereas in the GCV- group, the photon count continued to increase ($+1.8x10^7$). Conclusion: TK/GCV-mediated suicide is cell cycle dependent, which explains why the undifferentiated nestin positive cells were affected more than the differentiated Tuj-1 positive cells in *vivo*. We believe that this approach will, therefore, allow us to conserve the functional recovery gained from the transplantation whilst treating the graft derived tumor at the same time.

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Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 59.04/II3

Topic: E.09. Spinal Cord Injury and Plasticity

Support: NJCSCR Grant CSCR15IRG010

Title: Impact of complete spinal cord injury on skin pressure ulcer healing in a mouse model

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Abstract: Spinal cord injury (SCI) leads to loss of sensory and motor functions below the level of the lesion besides muscle atrophy, pressure ulcers (PUs), infections, and respiratory problems. PUs are a serious, costly, and life-long complication experienced by SCI individuals. SCI persons are at high risk for developing PUs because of lack of sensation, immobility, moisture, and multiple other risk factors. The prevalence of PUs in SCI ranges from 14-32%, and

recurrence rates have been reported to range from 31-79%. Based upon the clinical data, there are likely several physiological processes in wound healing affected after SCI. However, there have been no reports studying these factors in animal models of SCI. Therefore, there is a critical need for a PU animal model to investigate the effects of SCI on the dynamics of wound development and healing. We developed a mouse model of PU with or without SCI (complete transection at T10). All experiments were conducted using 10 week-old BALB/c male mice. A PU model was developed using 2 magnets applied on either side of a skin fold on the back of the mouse for 12 h. PUs were induced in both normal and SCI mice, and then mice were housed individually. First, the dynamics of development of the PUs and its healing processes were assessed via gross morphology. It was found that after a single 12 h magnet application, a PU developed within a few days. The wound tissue samples were prepared for histological evaluation at early time points. The wounded areas demonstrated tissue edema at day 0, immediately after a single 12 h magnet application, and disappearance of the epidermis by day 3, which persisted until day 7. Although the wounds in SCI mice exhibited more inflammation as compared to normal mice, the PU grades were very similar to that of regular mice. PUs in normal mice showed complete closure by 21 days as compared to 35 days in SCI mice. Thus, there was a significant delay in wound closing process in SCI mice ($p<0.001$). We also compared the healing time of 1 cm x 1 cm excisional wounds (in which case the full-thickness of the skin is excised on the back of the mice) between normal and SCI mice. Again, the healing time was significantly slower in SCI mice ($p<0.001$). The BMS score of all mice post-injury ($n=8$) was 0 at day 2 and 0.375 ± 0.18 at week 5, thus confirming the severity of the SCI. With these results, we show that skin healing is much slower after SCI in this mouse model, which is consistent with clinical observations in SCI patients. This animal model can be used to assess, in a systematic fashion, the impact of SCI on the dynamics of various cellular processes in wound healing, as well as a system to test compounds that may improve wound healing in SCI patients.

Disclosures: **S. Kumar:** None. **M.L. Yarmush:** None. **F. Berthiaume:** None.

Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

Location: Halls B-H

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Program#/Poster#: 59.05/II4

Topic: E.09. Spinal Cord Injury and Plasticity

Support: Strategic Research Program for Brain Sciences by MEXT of Japan

Grant-in-Aid for Scientific Research on Innovative Areas (“Adaptive Circuit Shift”) by MEXT of Japan

Title: Contribution of propriospinal neurons to recovery of hand dexterity after corticospinal tract lesions in monkeys

Authors: *T. TOHYAMA[1,4], M. KINOSHITA[5], K. KOBAYASHI[2,6], K. ISA[3], D. WATANABE[7], K. KOBAYASHI[8], M. LIU[4], T. ISA[3,9];
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Abstract: The direct cortico-motoneuronal connection is believed to be essential for the control of dexterous hand movements, such as precision grip in primates. It was reported, however, that even after lesion of the corticospinal tract (CST) at the C4/C5 segment, precision grip largely recovered within 1 to 3 months, suggesting that the recovery depends on transmission through intercalated neurons rostral to the lesion, such as the propriospinal neurons (PNs) in the midcervical segments. To obtain direct evidence for the contribution of PNs to recovery after CST lesion, we applied a pathway-selective and reversible blocking method using double viral vectors to the PNs in 6 monkeys after CST lesions at C4/C5. Two kinds of viral vectors were injected into the cervical spinal cord of each monkey. The first was a highly efficient retrograde gene transfer vector, HiRet or its derivatives (FuG-E, NeuRet) carrying the sequences for tetracycline-inducible, green fluorescent protein-tagged enhanced tetanus neurotoxin light chain. This vector was injected into the ventral horn of the C6-Th1 segments, where motoneurons innervating distal forelimb muscles are located. The second was the adeno-associated viral (AAV) vector carrying the sequence for the highly efficient Tet-on sequence, rtTAV16. This vector was injected into the intermediate zone of the caudal C2 to caudal C4 segment, where the cell bodies of PNs are located, thus enabling tetracycline derivatives, doxycycline (Dox)-inducible blockade of PNs. CST lesions were then made between the injection sites of the two viral vectors, mostly in C4/C5. In 4 monkeys that showed nearly full or partial recovery, transient administering Dox for 1-3 weeks after recovery caused partial impairment of recovered-precision grip. In the other 2 monkeys, continuous administration of Dox outlasted the entire period of postoperative observation (3-4.5 months). In these monkeys, precision grip recovery was not achieved. At the end of the behavioral observation, by recording presumably PN-mediated field potentials in the deep radial motor nuclei evoked by stimulation of the contralateral pyramid under anesthesia, PN-transmission was significantly blocked in the affected side compared with the intact side in 4 of 5 monkeys examined. Anti-GFP immunohistochemistry could show that axons of double-infected PNs directly connected with motoneurons of forelimb muscles. These results provide evidence for causal contribution of the PNs to recovery of hand dexterity after CST lesions; PN transmission is necessary for promoting the initial stage recovery; however, their contribution is only partial once the recovery is achieved.

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Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 59.06/II5

Topic: C.09. Brain Injury and Trauma

Title: Lateral olfactory tract usher substance (LOTUS) enhanced axonal regeneration and functional recovery after spinal cord injury in adult mice

Authors: ***S. ITO**[1], A. IWANAMI[1], S. SHIBATA[1], M. SHINOZAKI[1], N. NAGOSHI[1], S. KAWABATA[1], M. MATSUMOTO[1], K. TAKEI[2], M. NAKAMURA[1], H. OKANO[1]; [1]Keio Univ., Tokyo, Japan; [2]Mol. Med. Biosci. Lab., Yokohama City Univ. Grad. Sch. of Med. Life Sci., Yokohama, Kanagawa, Japan

Abstract: [Introduction]Lateral olfactory tract usher substance (LOTUS) is a membrane protein that functions as an axonal guidance molecule, and combines with the Nogo receptor 1 (NgR1) to antagonize several axonal growth inhibitors. It has been reported that in LOTUS knockout (LOTUS-KO) mice, motor function recovery after spinal cord injury (SCI) significantly deteriorated as compared with wild-type mice. The purpose of this study is to evaluate the effects of LOTUS on axonal regeneration and motor function recovery after SCI using LOTUS-overexpressing mice.[Method]Contusive SCI was induced at the Th10 level in LOTUS-overexpressing mice (LOTUS group) and wild-type mice (control group) as reported previously, and hindlimb motor function was evaluated until six weeks after SCI using BMS score, DigiGate system and rotarod test. 6 weeks after SCI, biotinylated dextran amine (BDA) was injected into the primary motor cortex of the mice in both groups to trace the corticospinal tract (CST). Two weeks after injection, electrophysiological analysis using spinal cord-evoked potential was performed as reported previously. After the mice were sacrificed, histological analyses were performed.[Result]In the LOTUS group, the BMS score showed significantly greater functional recovery compared with that in the control group at four weeks after SCI and thereafter (LOTUS group; 3.31±1.22 vs. control group; 2.35±0.38, $p<0.05$). DigiGate analysis revealed significantly longer stride length in the LOTUS group (3.67±0.53 vs. 2.97±0.23, $p<0.01$), and rotarod test showed significantly longer total run time in the LOTUS group (53.63±35.29 vs. 22.38±16.76, $p<0.05$) six weeks after SCI. Electrophysiological analysis showed significantly shorter latency in the LOTUS group eight weeks after SCI (3.91±0.68 vs. 4.95±0.79, $p<0.05$). Histological analyses revealed that the CST axons labeled with BDA significantly increased at the rostral

sites, but not at the caudal sites to the lesion epicenter in the LOTUS group compared to the control group. In contrast, the 5-HT positive serotonergic fibers significantly increased at the lesion epicenter and the caudal sites in the LOTUS group compared to the control group.[Conclusions]LOTUS overexpression promoted axonal regeneration after SCI, thereby contributing to motor functional recovery. In the future, we will evaluate the effects of exogenous LOTUS on the injured spinal cord in wild-type mice.

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Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

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Topic: C.09. Brain Injury and Trauma

Support: Olympia-Morata-Program of the Heidelberg University to BS

Interdisciplinary Neurobehavioral Core (INBC) in Heidelberg

Title: Behavioral and histological effects of epothilone D after moderate spinal cord contusion injury in Fischer 344 rats

Authors: *B. SANDNER[1], M. MELANIE[2], R. PUTTAGUNTA[2], J. RUSCHEL[3], F. BRADKE[3], N. WEIDNER[2], A. BLESCH[2,4];
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Abstract: Fibroglial scarring is a major obstacle for axonal regeneration after spinal cord injury. Previous studies have shown that local administration of the microtubule-stabilizing drug taxol or epothilone B (EpoB) reduce fibrotic scar formation after a moderate (150kDyne) contusion injury. Systemic administration of EpoB also promoted axon regeneration and functional recovery. In this study, we investigated the effects of epothilone D (EpoD), a less toxic analog of EpoB with a greater therapeutic index on fibrotic scaring and functional recovery after SCI. Adult Fischer 344 rats underwent a midthoracic moderate contusion injury (150kDyn), followed by intraperitoneal injection of EpoD (1.5mg/kg*)* at day 1 and 15 post- injury. Animals receiving vehicle injection served as controls. Eight weeks post-injury, there was a strong trend towards

improvement in sensorimotor recovery (reduced number of footfalls when crossing a regular horizontal ladder) in EpoD-treated animals compared to vehicle controls. Based on the variability in spinal cord displacement and the importance of displacement on functional parameters, we are currently analyzing groups subdivided into displacements of more or less than 1000 µm prior to any functional testing. Furthermore, hindlimb motor function in the BBB open field locomotor rating scale and gait analysis (Catwalk) will be analyzed. Adverse effects such as weight loss were not observed. Morphologically, parameters such as spared white matter (histological analysis of Eriocrome Cyanine staining), fibrotic scar tissue (laminin positive area) and number of serotonergic (5-HT positive) fibers will be investigated. Results of this study will give an independent insight, whether EpoD treatment may promote sensorimotor recovery following SCI and whether it may represent a candidate towards clinical translation.

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Poster

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: Thomas Hartman Center for Parkinson's Research at Stony Brook University

The SUNY Brain Network of Excellence

Title: Using serial cystometry to evaluate effectiveness of treadmill training on recovery of lower urinary tract function in a rat contusion model of SCI

Authors: *F. QURESHI[1], P. KUNG[2], H. CHO[3], N. P. PHAGU[4], S. A. SISTO[1], W. F. COLLINS[4];
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Abstract: Lower urinary tract (LUT) dysfunction is common in spinal cord injury (SCI). The aim of the study was to evaluate the effectiveness of treadmill training using serial measurements of LUT function over15 weeks pre- and post-SCI. Five female Sprague-Dawley rats (250-300g) underwent moderate spinal cord contusion (210 Kd) at T8. Treadmill training was started 2 weeks post-SCI and continued for 8 weeks. Pre-SCI and pre-, mid-, and post-treadmill training, LUT function was assessed using serial transurethral cystometry (STUC) with external urethral

sphincter (EUS) EMG recording. For each STUC session, the rat was anesthetized (Ketamine/Xylazine; 90/10 mg/kg, i.p.), and a sterile catheter (PE50) was inserted transurethrally into the bladder and connected in series to a pressure transducer to record bladder pressure and to a 60cc syringe attached to a syringe pump. EUS EMG recordings were obtained by inserting two sterile fine wire electrodes percutaneously into or near the EUS muscle. Repetitive reflex micturition events were elicited by continuous infusion of saline into the bladder. Bladder Pressure and EUS EMG data were acquired for ~2 hours after which the catheter and electrodes were removed and the rat was allowed to recover from anesthesia. For each micturition event, bladder threshold pressure (TP), peak pressure (PP) and contraction duration (CD) were measured as well EUS burst duration and frequency. All rats pre-SCI exhibited normal patterns of threshold-driven voiding bladder contractions with coordinated bladder and EUS activity and no large amplitude non-voiding contractions. Before training, SCI rats exhibited rhythmic non-voiding contractions (NVCs) with no clear TP and elevated PP. Rhythmic NVCs continued during bladder filling until leakage occurred (n=4). One rat showed a repeating pattern of 2-4 NVCs followed by a voiding contraction. Treadmill training reduced PP in every rat and improved LUT function in 4 rats characterized by reduced incidence of NVCs, increased frequency of voiding contractions, and increased EUS bursting activity during voiding. Return to normal micturition patterns occurred in 2 rats. In conclusion, STUC is an effective method for repeated evaluation of therapeutic interventions over time in individual rats following SCI.

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Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

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Program#/Poster#: 59.09/II8

Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH grant R01 HL96750

Title: Post-synaptic effects of BDNF/TrkB signaling on functional recovery after cervical spinal cord injury

Authors: *H. M. GRANSEE[1], C. B. MANTILLA[1,2], P. SEKAR[1], W.-Z. ZHAN[1], G. C. SIECK[1,2];
[1]Physiol. & Biomed. Engin., [2]Anesthesiol., Mayo Clin., Rochester, MN

Abstract: Unilateral spinal cord hemisection at C_2 (SH) results in loss of descending premotor drive to phrenic motoneurons and paralysis of the ipsilateral diaphragm muscle (DIAm). Over time there is spontaneous neuroplasticity and gradual recovery of rhythmic DIAm activity after SH. Although both pre- and post-synaptic mechanisms likely contribute to the strengthening of spared contralateral synaptic inputs to phrenic motoneurons over time, their relative contribution to recovery post-SH is not clear. Following spinal cord injury, brain-derived neurotrophic factor (BDNF) acting through tropomyosin related kinase receptor subtype B (TrkB) regulates both pre- and post-synaptic plasticity and BDNF/TrkB signaling plays an important role in recovery. Previous studies found that increasing BDNF near phrenic motoneurons by intraspinal transplantation of BDNF-expressing mesenchymal stem cells (BDNF-MSCs) and upregulating TrkB in phrenic motoneurons by intrapleural AAV7-mediated transduction increase ipsilateral DIAm EMG activity after SH, although the extent of recovery is incomplete. Whereas BDNF availability may exert pre- and post-synaptic effects, TrkB expression in motoneurons likely exerts only post-synaptic effects. We hypothesized that post-synaptic effects at motoneurons play a predominant role in recovery post-SH and that by combining both AAV-TrkB and BDNF-MSCs treatment the synaptic mechanism could be elucidated. Adult Sprague-Dawley rats were intrapleurally injected with AAV7-TrkB or AAV7-GFP at 3 weeks prior to SH, and BDNF-expressing MSCs or wild type-MSCs were injected at C_2 at the time of SH surgery. DIAm EMG electrodes were implanted bilaterally to record activity across motor behaviors. Functional recovery during eupnea at 14D was evident in 100% of the BDNF-MSC/AAV-TrkB treated rats compared to 78% of control MSC/AAV-GFP treated rats. At 14D after SH, treatment with BDNF-MSC/AAV-TrkB or AAV-TrkB alone increased DIAm root mean square (RMS) EMG amplitude during eupnea, whereas control treatment had lower RMS EMG amplitude compared to pre-SH. Combined treatment with AAV-TrkB and BDNF-MSC had no additional benefit on extent of DIAm functional recovery during eupnea compared to AAV-TrkB treatment alone. Enhancing TrkB in phrenic motoneurons was sufficient to promote recovery of DIAm activity after SH, suggesting that post-synaptic mechanisms evoked by BDNF/TrkB signaling are sufficient to enhance eupneic recovery after SH.

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Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: CIHR MOP44358

Title: Locomotor recovery after spinal contusion at T10 and following a complete spinalisation at T13 in the cat

Authors: H. DELIVET-MONGRAIN, M. DEA, L. AHMED, J.-P. GOSSARD, *S. ROSSIGNOL;
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Abstract: Following a unilateral spinal hemisection at T10-11, cats recover quadrupedal locomotion and, after a second and complete lesion at T13, they can walk with the hindlimbs within 24hrs which is much faster than the 2-3 weeks needed after a single complete spinalisation. This suggests that the spinal cord (SC) below the hemisection undergo plastic changes leading to a much faster recovery of hindlimb spinal locomotion. To move closer to the clinical situation, we developed a model of bilateral spinal contusion at T10-11. Nine cats were implanted chronically with electromyographic (EMG) electrodes. The SC was contused using an Infinite Horizon impactor with a force of 800kdynes (8N) for 30s through a 5mm diameter flat circular tip. Thereafter, cats were trained 5 times a week on the treadmill and EMG recordings synchronized to video were made weekly. After 5wks, 2 cats were prepared for an acute experiment to record fictive locomotion (see abstract Gossard et al, SFN) while 3 other cats were perfused for histology. Four other cats were contused and trained for 5wks, then surgically spinalised and kept for 2 weeks for locomotor evaluation before the acute experiment. We found that the contusion produced very large lesions affecting all quadrants bilaterally. Only very few "normal" fibers remained in the ventral quadrants, with no residual grey matter at the epicenter of the lesion containing 5-7mm cavities. For most cats, hindlimb paralysis was complete during the first week following contusion, but quadrupedal locomotion gradually recovered thereafter. At the end of the fifth week, most of the cats could walk unaided on all four limbs and some could even step over obstacles on the treadmill. Moreover, 3 of the 4 spinalised cats walked with the hindlimbs within 24 hours of the spinalisation. We conclude that, after a very large bilateral spinal contusion leaving only sparse fibers in the white matter, neuroplastic changes occurring below the contusion must be part of locomotor recovery since spinal locomotion reappears with 24-48 hours, as after hemisection. Whereas in the hemisected model it could be hypothesized that the spinal changes below the hemisection were driven mainly from the important contingent of remaining descending fibers in the other half of the cord, the present results showing only sparse remaining fibers after severe contusion suggests that it is probably the increase of afferent inputs provided by locomotor training that may largely be implicated in inducing such major sub-lesional functional changes This conclusion is very important when considering potential therapeutic targets for improving locomotor recovery in humans with SCI.

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Poster

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH grant R01 HL96750

Title: Reorganization of respiratory motor control following cervical spinal cord injury

Authors: *C. B. MANTILLA[1,2], H. M. GRANSEE[2], W.-Z. ZHAN[2], G. C. SIECK[1,2];
[1]Anesthesiol., [2]Physiol. & Biomed. Engin., Mayo Clin., Rochester, MN

Abstract: Animal models of incomplete spinal cord injury to the upper cervical spinal cord such as unilateral C_2 hemisection (SH) are widely used to examine the organization of motor systems underlying respiration. Premotor drive to phrenic motoneurons innervating the main inspiratory muscle in mammals, the diaphragm muscle, is primarily ipsilateral, although latent contralateral pathways are thought to underlie spontaneous recovery over time post-SH. The diaphragm muscle is active across a range of motor behaviors from ventilatory behaviors to higher force behaviors necessary for airway patency. Previous studies show that ipsilateral diaphragm EMG activity can be enhanced by interventions that increase premotor drive to phrenic motoneurons. Premotor drive to phrenic motoneurons measured by the root mean square (RMS) EMG activity at 75 ms is increased in response to airway occlusion, but not during sighs, although these behaviors require generating similar levels of force (~70% of maximal force in rats). We hypothesized that presence of sighs ipsilateral to SH in the absence of eupneic activity would reflect bilateral premotor drive to phrenic motoneurons during sighs compared to eupnea. Adult male Sprague-Dawley rats underwent bilateral diaphragm EMG electrode placement for chronic recordings followed by SH. Absence of ipsilateral diaphragm EMG activity was verified at the time of SH surgery and at 3 days post-SH. In lightly anesthetized, spontaneously breathing rats, EMG recordings were conducted during eupnea. Sighs occurred periodically every few minutes, and were determined as being present by contralateral diaphragm RMS EMG amplitude at least twice that of eupneic breaths. Sighs were evident in all animals post-SH, despite absence of eupneic activity, and the RMS EMG amplitude was 63 +/- 18% of pre-injury sigh levels. Consistent with the lack of increased premotor drive during sighs, RMS EMG amplitude at 75 ms was not significantly different compared to pre-injury eupnea or sigh. The results of the present study support the notion of primarily unilateral premotor drive to phrenic motoneurons for eupnea and bilateral projections for sighs. Reorganization of premotor drive to motoneurons following incomplete spinal cord injury is likely an important mechanism for recovery that can be directly addressed in therapeutic intervention.

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Poster

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: NYS DOH SCIRB C30606GG

NYS DOH SCIRB C30835GG

Title: Combined chronic theta burst stimulation of motor cortex and trans-spinal direct current stimulation in rats promote coticospinal tract outgrowth caudal to a cervical spinal contusion and motor recovery

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Abstract: An important strategy for promoting motor function after SCI is to augment sprouting of the spared axons of motor pathways. We showed in a rat pyramidal tract lesion model that motor cortex (M1) electrical stimulation augments sprouting of spared corticospinal tract (CST) axons and promotes motor recovery (Carmel et al, 2010). In the present study we translated M1 stimulation to a moderate cervical contusion injury. We modified our published M1 stimulation protocol and used an intermittent theta burst electrical stimulation (iTBS) pattern. We combined iTBS with spinal cord cathodal DC stimulation to co-activate spinal circuits during M1 stimulation, based on our recent pyramidal tract lesion study (Song et al 2016). We examined CST axonal labeling caudal to the injury and several behavioral outcomes in 2 animal groups: injury only (n=14); injury + combined iTBS and cervical spinal DC stimulation (n=13). Bilateral contusions (Infinite Horizons; 200kdyn) were made at the C4 level. Stimulation began 7 days after injury, and continued for 10 days for 30 minutes daily. iTBS was delivered through epidural electrodes bilaterally over forelimb M1 and cervical cathodal DC stimulation, through cutaneous electrodes. Analyses were done blind. There were no differences in lesion volume between the two groups. Nor were there differences in the number of spared CST axons in the white matter for the two groups. We measured total CST axon length and axon varicosity number within the C6 gray matter. As expected, for both groups gray matter axon length increased as the number of spared CST axons in the white matter increased. However, this relationship was greater for the stimulated group, where there was approximately a 2-fold increase in axon length per spared white matter CST axon. A similar increase in CST axon varicosities (ie., presynaptic sites) was observed for the stimulated compared with non-stimulated group. Von Frey hair testing revealed

no change in either group, relative to uninjured controls. For the IBB test, there was no difference in initial post-injury scores for the two groups and only the stimulated group showed a significant improvement compared with the non-stimulated group (non-stimulated: 7.4%; paired t-test; p=0.31; stimulated: 17.7%; p=0.009). Analyses are in progress to determine treatment effect on locomotion (horizontal ladder walking and treadmill). Our findings show that combined M1 and spinal cord cathodal DC stimulation promoted spared CST axonal outgrowth and significantly improved forepaw manipulation skills after a moderate cervical contusion injury.

Disclosures: M. Shinozaki: None. **N. Zareen:** None. **D. Ryan:** None. **H. Alexander:** None. **S. Naeem:** None. **J.H. Martin:** None.

Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 59.13/II12

Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH R01 NS085167 02

Title: Enhancing plasticity and recovery following spinal cord injury

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Abstract: Cervical spinal cord injury (cSCI) leads to long lasting impairments in forelimb function. The location and extent of cSCI determines the subsequent impairment. It has long been assumed that the degree of functional recovery is similarly determined by the lesion. There is growing evidence that this assumption is incorrect. We tested the hypothesis that functional recovery following chronic cSCI is primarily limited by ineffective neural plasticity. Rats were first trained to proficiency on the isometric pull task to assess volitional forelimb strength and given a C5/C6 hemicontusion. Repeatedly pairing vagus nerve stimulation (VNS) with forelimb movement beginning six weeks after cSCI promoted 75% more recovery of forelimb function compared to intense rehabilitation alone. In spite of significant forelimb strength enhancement,

we observed no differences in awake behaving forelimb muscular dynamics or forelimb pain between groups. We next used intracortical mappings of motor cortex and retrograde transsynaptic tracing from the forelimb musculature to examine neural plasticity. The addition of VNS as an adjuvant to rehabilitation substantially improved the anatomical and physiological connectivity of forelimb grasping musculature with motor cortex, without altering the extent of spinal damage. These findings suggest that neural plasticity, and not lesion extent, primarily limits recovery from cSCI. However, enhanced recovery from hemicontusion does not suggest that VNS paired with rehabilitation will improve recovery from cSCI in humans, because most patients have bilateral cervical spinal damage. To address this limitation, we repeated the behavioral assessments above in rats with midline cervical spinal contusions. Repeatedly pairing VNS with forelimb movement beginning eight weeks after bilateral cSCI again enhanced recovery of forelimb function compared to intense rehabilitation alone. These findings provide new hope for patients and suggests that plasticity-based therapies may prove to be clinically useful.

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Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: U01EB007615

Title: Recovery of voluntary control following spinal injury in rodents

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Abstract: Recent studies in paraplegic humans have demonstrated a remarkable recovery of lower limb voluntary control when provided epidural and transcutaneous stimulation over the lumbosacral enlargement. Since the foundational research for these studies was conducted after complete spinal transection in animals, where no recovery was observed, the mechanism for this functional recovery in humans is unclear. To explore how epidural stimulation allows communication to occur across the spinal injury site, we have developed a novel behavioral task

in the rodent. Eight Sprawg-Dawley rats were trained to move their right hindlimb when prompted by an auditory cue. This was accomplished by initially pairing the cue with a vibration of a small motor attached to the animal's leg. The vibration intensity was chosen to evoke leg movement, and then slowly decreased until the animal responded to the auditory cue alone (which took one week). After training, each animal underwent an implantation surgery and (a month later) a spinalization. During the implantation, epidural stimulating electrodes were inserted over L2 and S1 spinal levels, and EMG recording electrodes were embedded in the hindlimb muscles (R-ST, R-VL, R-TA, R-Sol, L-TA). The spinal surgery consisted of a double hemi-section injury at the right T7 and left T10 spinal levels. This injury results in lower limb paralysis by eliminating all long tract descending connections, but preserves a continuous tract of neural tissue. Following injury, the animals received sessions of sub-threshold epidural stimulation and basic treadmill step training. After one month, the spinalized animals were able to perform the behavioral task, but only when provided epidural stimulation. These animals showed a significant increase in the reaction time to the auditory cue, as well as an increase in the duration the muscle response, suggesting reorganization of the underlying neural circuit. These results demonstrate a simplified behavioral task that mimics the results found in the human. This task is unique since the EMG activity is time-locked to the auditory cue, but can have a variable latency and amplitude. This provides a metric to judge optimal stimulation patterns and pharmacological interventions, as well as other injury models. This can also be used to study how the cortex reorganizes to enable recovery of voluntary control after the loss of all direct supraspinal connections due to spinal injury.

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Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

Location: Halls B-H

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation 2015

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Title: The effect of CaV1.3 channel blocker on long-lasting root reflex

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Abstract: After spinal cord injury (SCI), prolonged, involuntary muscle contractions, or spasms, can develop in muscles innervated below the level of injury. One source of these spasms is the increased excitability post-injury caused by an increase of persistent inward currents (PICs) in motoneurons. PICs are produced, in part, through the activation of the L-type calcium channel, CaV1.3. Recently, a new drug, SKP004C08 (SKP), has been developed to specifically block CaV1.3. In this study, we evaluated this new drug for its potential therapeutic effects on spasms. We used the *in vitro* sacral cord preparation to measure long-lasting reflexes (LLRs) in the sacral roots of mice with a chronic thoracic transection SCI. The ventral roots at sacral segment 2 - 3 (S2 - 3) were placed on metal electrodes to record LLRs evoked by stimulating the corresponding dorsal roots. The LLRs were evoked by single or a five pulse train stimulation of the dorsal root after blocking inhibitory synapsis with 5 μM strychnine, an antagonist of the glycine receptor, and 10 μM bicuculline, an antagonist of the GABAA receptor. Prior to application of SKP, the results showed that the spinal motor system in the SCI mouse was hyperactive as it frequently expressed a synchronized bursting firing among the ventral roots and showed less depression in repetitive firings in response to a short train stimulation. After SKP administration, however, the LLRs were significantly and dose-dependently reduced 10 - 90% of control levels as the concentration of SKP ranged from 20 - 200 μM. Interestingly, the LLRs were more resistant to SKP in the chronic SCI mouse than the control, acutely injured SCI mouse, suggesting the involvement of other channel types in the LLRs of the SCI mouse. These data suggest a potential therapeutic effect of SKP on spasms post-SCI and indicate a complex mechanisms underlying the LLR.

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Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

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Program#/Poster#: 59.16/II15

Topic: E.09. Spinal Cord Injury and Plasticity

Support: ICORD Seed Grant

Title: Can arm cycle training effect postural control and voluntary trunk muscle activation in people with spinal cord injury?

Authors: *J. BROUSSEAU[1,2], R. MALIK[1,2], A. E. CHISHOLM[1,2], A. LYNN[2], A. WILLIAMS[1,2], T. LAM[1,2];
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Abstract: Objectives: For people with spinal cord injury (SCI), regular exercise is crucial for improving functional mobility, as well as reducing the risk of secondary health concerns such as cardiovascular disease and obesity. Seated arm cycling has been shown as an effective form of aerobic exercise for people with SCI, which also offers the potential to engage the trunk muscles. Recent studies have demonstrated motor preservation of trunk musculature below the level of injury for people with complete cervical and high thoracic SCI. Since there is potential to improve trunk muscle activation, it is important to develop interventions to target these muscles. Training trunk muscles may enhance seated balance control which can directly impact independence in activities of daily living and functional mobility. The aim of this study is to evaluate the effect of an 8-week arm cycle class on seated balance control and the voluntary activation of trunk muscles for those living with SCI.
Methods: Modeled after conventional spin classes, individuals with SCI attend a 1 hour arm cycle class every week for 8 weeks. Classes vary in difficulty by changing resistance levels, cycling cadence, and sitting posture (analogous to standing pedaling bouts in conventional spin classes) to challenge the cardiovascular system and encourage core stability. We evaluated seated balance control and voluntary trunk muscle activation patterns pre and post 8 weeks. Seated balance control was assessed by having participants sit on a forceplate while calculating their center of pressure (COP) during quiet unsupported sitting with eyes open (EO) and eyes closed (EC). We also tested the limits of stability (LOS) by asking participants to lean as far as they could in the 8 cardinal directions, and calculating the total distance the COP moved. EMG patterns were recorded from the rectus abdominus, external oblique and erector spinae (trunk flexion and rotation, lateral flexion, hollowing). We also recorded heart rate (HR) during each class to verify the aerobic intensity of the activity.
Results: Individuals with SCI showed limited trunk muscle activation and impaired static (EO and EC) and dynamic (LOS) seated balance control prior to joining the arm cycle class. Following 8 weeks of arm cycling individuals with SCI tended to show improvements in trunk muscle function and core stability.
Conclusion: Preliminary findings indicate that an 8-week arm cycle class could potentially improve postural control and elicit training effects in trunk musculature. Moreover arm cycle classes may provide aerobic benefits from an increase in HR reducing the risk of secondary health complications commonly found in those with SCI.

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Poster

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Program#/Poster#: 59.17/II16

Topic: E.09. Spinal Cord Injury and Plasticity

Support: Swiss National Science Foundation

Christopher and Dana Reeve Foundation

European Research Council (ERC) advanced grant (Nogorise)

Title: Time course of sprouting of hindlimb corticospinal fibers after thoracic spinal cord injury in rats

Authors: *N. RUSSI, A. K. ENGMANN, M. P. SCHNEIDER, A.-S. HOFER, S. IMOBERSTEG, L. TRUSCELLO, K. FRICKE, R. SCHNEIDER, M. E. SCHWAB;
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Abstract: In humans as well as in experimental animal models of CNS injury certain degrees of functional recovery are observed depending on the severity of the injury. Rodent studies investigating anatomical changes showed neurite outgrowth of both spared and axotomized fiber tracts, also referred to as sprouting, in different spinal cord and brain regions and different CNS lesion paradigms. Following a thoracic spinal cord injury in adult rats, axotomized fibers of the hindlimb corticospinal tract (CST), which is crucial for fine motor skills, were found to sprout into the cervical spinal cord at different rostro-caudal levels. Retrograde and anterograde tracings showed new projections of motor cortical hindlimb fibers to the cervical spinal cord. Microstimulations of the former hindlimb sensorimotor area were found to elicit forelimb, trunk, shoulder and head movements. However, the precise time course of cortical reorganisation as a consequence of cervical CST sprouting and its effects on physiological forelimb motor functions remain to be elucidated. A combination of three retrograde neuroanatomical tracers injected at different time points into three different locations in the spinal cord was used to study sprouting, target specificity of the new collaterals and cortical reorganisation following a thoracic bilateral transection of the dorsal spinal cord including the CST in adult rats. Preliminary results suggest major rearrangements of the new forelimb connections from the former hindlimb cortex over 1 to 12 weeks after the thoracic CST lesion. Animal behaviour on the irregular horizontal ladder and in the Montoya staircase test was assessed to study possible functional changes of skilled forelimb motor functions. Rats achieved a plateau of high performance with their (intact) forelimbs which was not disturbed in a major way by the new hindlimb-originating fibers. More challenging skilled reaching tests are currently being applied.

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Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

Location: Halls B-H

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: Friends of Nancy Lieberman Spinal Cord Research Fund

NYS DOH C030843GG

Title: Pharmacological targeting of ion channels leads to rapid and sustained behavioral improvements following spinal cord injury

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Abstract: In central nervous system tissue, the injury response follows a stereotypical pathway, the ultimate result of which is glial cell, neuronal and axonal loss. In spinal cord injury (SCI), acute cell death is responsible for the motor deficit, but it is the slower degeneration of axons that survived the initial insult that prevents future recovery. Current approaches aim to either stop the injury progression, or attempt to replace lost neurons or oligodendrocytes by cell transplants. We however, have hypothesized that restoring function to surviving neurons will be sufficient to cause behavioral improvements and we have approached this idea in two ways. Previous work from our lab has shown that the transplantation of astroglial support cells, which will improve the environment for surviving cells, is sufficient to improve motor function following SCI (PLoS One. 6, e17328). Our recent work with acute spinal cord injury, however, looks to identify a pharmacological agent that would directly act on neurons to improve function and increase survival. To achieve this, we targeted ion channels that are critical for action potential propagation as a way of increasing neuronal activity.
We identified an FDA approved, ion channel-modulating drug that we find leads to rapid behavioral improvements when given 24 hours after spinal cord injury. The speed of recovery suggests reactivation of the surviving neurons, and remarkably the behavioral improvements are sustained even when the drug is no long administered. Furthermore, we find the drug is able to reduce cell death and consequent lesion volume as early as three days of treatment. Ongoing work investigates the mechanism of action for the rapid and persistent improvements we see.

Disclosures: **H. Natola:** None. **M. Noble:** None. **C. Proschel:** None.

Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: Veterans Administration

NIH R-01 NS042291

Title: *In vivo* direct neuronal reprogramming of fibroblasts grafted to fill sites of spinal cord injury

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Abstract: Previously we have demonstrated that neural stem cells (NSCs) derived from induced pluripotent stem cell (iPSC) and embryonic stem cell (ESC) sources extend large numbers of axons long distances when grafted to sites of spinal cord injury (SCI). We and others have reported that these grafts can support recovery of motor function, even in severe SCI models. However, major barriers to the clinical use of these grafts remain: (1) ESC-derived grafts are not autologous, necessitating immune suppression, and (2) without extensive long-term safety characterization of individual clones, iPSC-derived NSC grafts are occasionally teratogenic after implantation into sites of SCI.

Direct reprogramming strategies to convert fibroblasts to induced neurons (iNs) via the expression of lineage-instructive transcription factors can produce autologous neurons without progressing through an iPSC intermediate stage, and may represent a safer cell source for the treatment of SCI. Autologous fibroblasts are readily available clinically, robustly survive grafting into sites of SCI, and can be very rapidly reprogrammed to neurons. As such, in addition to an improved safety profile, we hypothesize directly-reprogrammable autologus sources of new neurons can allow more rapid intervention during the subacute phase of SCI compared to iPSC derivatives, and may accordingly encourage greater connectivity with actively regenerating host systems, leading to superior motor function recovery.

We have preliminary data demonstrating in vivo generation of rat iNs within acute SCI lesion sites. Transgenic GFP^+ rat fibroblasts were infected in vitro with doxycycline-inducible lentiviral constructs expressing neuronal lineage transcription factors, and then grafted to fill GFP^- immunocompetent rat dorsal column lesion injury sites. After administration of doxycycline for

a period of 3 weeks, graft-derived GFP^+ iNs extended long processes away from the injury site, and expressed neuronal markers Tuj1, NeuN, and Map2. Next we propose to assess synaptic connectivity with these grafts, and to perform behavioral tests of motor recovery.

Disclosures: A. Adler: None. **M. Tuszynski:** None.

Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: National Science Foundation Grant # HRD-1363399

Title: Investigating the long term changes in the spinal cord due to neuromuscular electrical stimulation through a dynamic learning mechanism

Authors: I. PEREZ[1], *D. S. WON[2], L. TONG[1], P. ARGUELLO[1];
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Abstract: Recent advancement of computational models of central pattern generator (CPG) spinal circuitry has led to accurate simulation of many characteristics of locomotion. Some of the behaviors that have been achieved include controlling the phases of locomotion (extension or flexion) and coordination of left and right limbs. Achievement of these simulated behaviors is made possible by a 2-level organizational principle of the CPG model: the rhythm generation level generates the intrinsic rhythmic behavior of the spinal cord, and a pattern formation layer is responsible to project the rhythmic behavior as patterns to the motoneurons.
One key characteristic of the spinal circuitry that is of great importance for understanding the process of post-injury rehabilitation but missing from current CPG models is the long term effects of afferent nerve stimulation on synaptic efficacy. Incorporating a learning mechanism for the spinal circuitry model will provide insight to changes of the spinal cord for rehabilitation purposes. The work presented here is framed within the context of developing a neuromuscular electrical stimulation therapy in a rodent model of spinal cord injury.
In order to incorporate the long term effects of neuromuscular electrical stimulation, the spinal circuitry model will incorporate a set of dynamic weights that are dependent on intracellular calcium concentration of the post-synaptic neuron. This type of learning mechanism is based on the modulation of NMDA and AMPA receptors that are responsible for the long term synaptic changes. The influx of calcium ions is known to be a second messenger in the post synaptic neuron for synaptic efficacy. With the addition of the learning mechanism in the spinal circuitry

model, changes of the synaptic efficacy due to simulated neuromuscular electrical stimulation are analyzed. In this work, we demonstrate the ability to control the operating point of the dynamic weights, signifying the efficacy of synapses between the afferent Ia fibers and the motoneurons in the CPG, by applying afferent stimulation to the CPG network. These capabilities have implications for studying the capability of using peripheral nerve stimulation to systematically modify and rehabilitate spinal cord locomotor function.
The funding for this program is provided by the National Science Foundation under Grant # HRD-1363399.
Keywords: Central Pattern Generators, Neuromuscular electrical stimulation, NMDA and AMPA receptors, synaptic plasticity

Disclosures: I. Perez: None. **D.S. Won:** None. **L. Tong:** None. **P. Arguello:** None.

Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: FINEP 01.12.0514.00

AASDAP

AACD

Itau Bank

Title: Virtual anatomical biofeedback for motor rehabilitation in spinal cord injury patients

Authors: *G. BAO[1], S. SHOKUR[1], A. C. DONATI[1,2], Y. BYUN[3], D. CAMPOS[1], D. FISCHER[1,2], M. NICOLELIS[4,5,3];
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Abstract: Biofeedback (BF) has been long used in rehabilitation for treatment of motor deficiency in neurological disorders. Studies have shown that BF is beneficial for ligament reconstruction, chronic pain treatment, cerebral palsy, balance training etc. In these treatments,

patients are reported to engage more intensively when BF is used. Moreover, it offers the opportunity to improve accuracy during functional tasks.
We have proposed a novel BF tool, using a realistic anatomical muscle model linked to online EMG, for improving motor control in spinal cord injury patients. Two key lower limb muscles engaged in locomotion, namely gluteus maximus (GMx) and rectus femoris proximal (RFP), used respectively for hip flexion and extension, were trained in our setup.
Patients were vertically supported in a orthostatic position, while they observed an anatomical avatar through a head mounted display. They were asked to alternatively flex and extend their hips. Seven SCI patients (one ASIA A, one ASIA B, five ASIA C) were trained for three sessions with our apparatus. Alternatively, in a control experiment no visual feedback was given to the patients.
Five patients reached significant levels of contraction for both left and right RFP and GMx muscles. In two patients (both ASIA C), stronger activations in the order of 5-10% ($p<0.01$, Ttest) were systematically observed when the anatomical avatar was shown compared to the control case for both RFP and GMx.

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Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

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Program#/Poster#: 59.22/JJ4

Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH

NYS DOH

Title: Spinal to sciatic direct current stimulation caused long term reduction in spasticity in mice with spinal cord injury

Authors: W. MEKHAEL[1], M. HASSAN[2], S. SAMANDDAR[1], S. BEGUM[2], P. TORUNO[2], M. AHMED[2], M. SALEH[2], A. GORIN[2], M. MAISANO[2], M. AYAD[2], M. ANDRAWIS[2], *Z. AHMED[3];
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Abstract: Muscle tone abnormalities cause functional limitations and can severely override the weak ability to produce movements after spinal cord, or brain injuries. Muscle tone could be viewed as a background noise that when increased, could corrupt movement accuracy. In our previous study, we found that passing direct current from the lumbar spinal cord to the sciatic nerve reduces muscle tone in spastic spinal cord injured mice. In the present study, we tested the long-term effects of stimulation and the effects of repeated sessions of stimulation on muscle tone and on skilled and unskilled locomotion in spastic mice with spinal cord injury. To test spasticity, we constructed a computer-controlled motorized system that stretches, at different levels of speeds, the triceps surae muscle in awake restrained mice. Animals were stimulated transcutaneousaly for seven consecutive days (20 min/day) using a stimulation system manufactured in our lab. Animals were followed for four weeks after stimulation ended. Various indices of spasticity were significantly reduced during and for four weeks after stimulation. Skilled locomotion was tested using a computer-controlled ladder wheel that is similar to the horizontal ladder test. Spinal-to-sciatic stimulated animals showed significant improvement in skilled locomotion compared to sham, and sciatic-to-spinal stimulated animals. Animals also showed significant improvements in treadmill walking tested by the DigiGait system. We also studied the underlying electrophysiological and molecular mechanisms of spinal-to-sciatic stimulation. Treated animals showed significant increase in the rate-dependent depression (RDD) of Hoffman reflex. Spinal to sciatic stimulation affected the expression levels of VEGF, trkB, KCC2, and pKCC2. Also, we tested the safety of the stimulation using Nissil staining, NF200, TUNL assay, and microglia activation. None of these assays showed differences compared to control animals. We concluded that spinal to sciatic direct current stimulation is an effective therapeutic technique to ameliorate spasticity developed after spinal cord injury.

Disclosures: **W. Mekhael:** None. **M. Hassan:** None. **S. Samanddar:** None. **S. Begum:** None. **P. Toruno:** None. **M. Ahmed:** None. **M. Saleh:** None. **A. Gorin:** None. **M. Maisano:** None. **M. Ayad:** None. **M. Andrawis:** None. **Z. Ahmed:** None.

Poster

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: NSF Grant HRD-1463889

Title: Training induced muscular adaptations associated with improved cardiometabolic profiles in individuals with spinal cord injury (SCI)

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Abstract: Debilitating spinal cord injury (SCI) results in marked reductions in functional neuromuscular activity below the area of lesion. Activity based therapies that target spinal locomotor networks have been associated with improvements in walking ability with varying levels of success after traumatic SCI. A typical regimen of locomotor training exposes the paralyzed individual with prolonged, full-body, load-bearing exercise of moderate intensity. Thus, in addition to promoting recovery of neuromuscular function, this type of training is also associated with providing benefits to health, including improved cardiometabolic function. The purpose of this pilot study was to investigate whether improved function of skeletal muscle mitochondria could explain the interaction between how locomotor training promotes dual benefits to the neuromuscular and cardiometabolic systems. We hypothesize that activity-based training that elicits pronounced electromyographic activity is associated with improvement in mitochondrial oxidative capacity (MOC) and overall cardiometabolic health. **Methods**: Able-bodied and SCI participants performed the MOC protocol. Rate constants indicative of skeletal muscle MOC were determined using near infrared spectroscopy and a repeated arterial occlusion protocol. In addition, muscle activity will be assessed using surface electromyography during stepping with BWST. Finally, blood samples will determine whether changes in performance or resting measures are associated with overall improvement in cardiometabolic health. **Results:** In order to complete the MOC protocol, arterial blood flow to the muscle was occluded using a rapid-inflation cuff system after electrical muscle stimulation was used to exhaust local oxygenation. Slopes of recovery were used to calculate the time constant to indicate the speed of recovery. The time constants correspond to the mitochondrial activity in the muscles of interest. We found that these time constant values were reproducible within subjects and can be used to differentiate variability in fitness across participants. **Conclusion:** Preliminary data are promising in determining methods to assess the role of training on mitochondrial capacity that takes place in parallel with recovery of neuromuscular function and overall cardiometabolic health.

Disclosures: S. Guzman: None. **J. Ramirez:** None. **S. Keslacy:** None. **K. Yamazaki:** None. **C.J. Dy:** None.

Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

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Title: Acute intermittent hypoxia alters plasticity-related and hypoxia-related protein expression in a rat model of cervical spinal injury

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Abstract: One promising approach to treating spinal cord injury (SCI) is through augmentation of spontaneous plasticity in uninjured neural pathways. Acute intermittent hypoxia (AIH) is a therapeutic intervention which has been shown to facilitate plasticity in respiratory and non-respiratory pathways after SCI. AIH, breathing alternating levels of normal (21%) and low (11%) oxygen for brief periods, increases phrenic nerve output (phrenic long term facilitation, pLTF) and tidal volume in rats with SCI. This is accompanied by changes in hypoxia- and plasticity-related proteins in the spinal cord. We have previously shown that AIH, when combined with specific task training on a ladder task, improves ladder performance in rats with cervical SCI. The purpose of this study is to determine whether the same hypoxia- and plasticity-related proteins associated with AIH-induced respiratory plasticity are also altered by AIH treatments in SCI rats that have undergone task training. Adult male Lewis rats received a dorso-lateral spinal cord lesion at the C2 vertebral level. Four wks after SCI, rats received up to 7 d of daily AIH treatment, consisting of 10 episodes of 5 min 11% O_2 alternating with 5 min 21% O_2. Control rats received continuous exposure to 21% O_2 for the same duration. AIH treatment was followed by daily ladder task training. Rats were sacrificed after training on treatment day 1 or day 7. Spinal cord segments C6-7 and L4-5 were dissected and processed for immunohistochemistry for plasticity- and hypoxia-related proteins. Expression of brain derived neurotrophic factor (BDNF), its high affinity receptor (trkB), and the phosphorylated form of trkB (ptrkB) was significantly increased in motoneurons of SCI rats treated with 7 d AIH compared to normoxia- treated control SCI rats. Expression of vascular endothelial growth factor (VEGF) was also significantly increased after 7 d AIH compared to normoxia-treated control rats, while hypoxia inducible factor 1α (HIF-1α) protein levels were significantly increased relative to control rats after either 1 or 7 d of AIH treatment. These results are consistent with previous work and suggest that similar mechanisms might underlie both respiratory and non-respiratory plasticity associated with AIH.

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Poster

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH R01 HD084645

NIH R01 HD082109

Title: Dose estimate of low force exercise needed to alter gene expression in paralyzed human skeletal muscle

Authors: *M. A. PETRIE[1], C. L. MCHENRY[1], E. FAIDLEY[1], M. SUNEJA[2,3], R. K. SHIELDS[1];
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Abstract: The loss of physical activity after a spinal cord injury results in metabolic dysfunction. Rehabilitation often overlooks the importance of physical activity in paralyzed limbs for systemic metabolic health. There is a need for safe and feasible exercise interventions to increase physical activity levels for people with chronic paralysis. Low-force exercise using neuromuscular electrical stimulation has been shown to acutely increase the expression of key transcription factors that regulate fiber type; however, the number of sessions needed each week to induce change in paralyzed muscle is unknown. **Purpose:** The goal of this work was to develop an estimate for the dose of low-force exercise needed to train human paralyzed muscle. **Methods:** Seven people with a complete spinal cord injury (ASIA-A) of at least 1-year duration participated in this study. Subjects unilaterally trained the quadriceps muscle with a low-force exercise for at least 12 weeks while keeping the other limb untrained. Subjects trained with a range of sessions per week from 1 to 6. Each subject received two bouts of 60 contractions per day. The stimulation train consisted of 10 stimulus pulses delivered at a 5Hz stimulation frequency and a between train work rest ratio of 1 on to 2 off. Before and after training, subjects performed a muscle performance assessment to evaluate muscle fatigue. After training, subjects underwent a percutaneous muscle biopsy of the trained and untrained limb to determine a gene expression signature. **Results:** At the conclusion of the training, we had a range of training doses from 1.8 to 6.3 low force exercise sessions performed weekly. We found a positive relationship between the changes in fatigue index and the number of training sessions per week (R^2=0.81, $p<0.006$). We found a positive relationship between training dose and changes in gene expression. For instance, we found a positive relationship between the changes in fatigue index after training and the expression of some key metabolic genes like mitochondrial pyruvate carrier 1 (MPC1, R^2=0.63), mitochondrial pyruvate carrier 2 (MPC2, R^2=0.50), and myosin heavy chain

2 (MYH2, R^2=0.89). **Conclusions:** Taken together, this study suggests low-force training exercise should target at least 5 days of training per week using at least 6,000 stimulus pulses per session. Using this dose of exercise will help future studies maximize potential effects low force training on chronically paralyzed human skeletal muscle.

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Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH R01 HD084645

NIH R01 HD082109

Title: Low force electrically induced exercise regulates distinct metabolic transcription factors in people with spinal cord injury (SCI)

Authors: *R. K. SHIELDS[1], M. A. PETRIE[1], A. SHARMA[2], M. SUNEJA[3,4];
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Abstract: Low force and long duration exercise is associated with improved metabolic homeostasis in humans without paralysis. One method to induce long duration activity in people with paralysis is to activate the skeletal muscle at a low frequency to minimize force but recruit the entire muscle. **Purpose:** We compared the effect of two low frequency protocols (1 Hz and 3 Hz) on metabolic gene regulation in humans with chronic paralysis. We also assessed the fatigue induced and each subject's perception of the two protocols to determine the feasibility for a long term training study. We hypothesized that the 3 Hz protocol would be most effective at metabolic gene regulation and perceived as most feasible. **Methods:** Eight subjects with a spinal cord injury (ASIA-A) of at least 2 year duration participated in this study. Subjects completed 2 sessions of a unilateral muscle exercise using muscle stimulation at a 1 Hz or 3 Hz frequency. The opposite limb remained untrained to serve as a within subject control. Three hours after the completion of the long duration and low force exercise (1 or 3 Hz), the trained and untrained vastus lateralis muscle was biopsied. We extracted RNA from trained and untrained muscle samples and analyzed the exon expression using Affymetrix HTA 2.0 microarray gene chips. We

also tested the fatigability of the muscle and assessed the subjects' perception of each protocol for feasibility. **Results:** Over 30,000 genes were analyzed and determined to have patent hybridization signal. Of those 71 and 179 genes were increased by at least 50% three hours after a single session of 1 Hz and 3 Hz muscle stimulation, respectively. 10 of the top 20 most highly induced genes ($p < 0.05$) were common for each protocol (1 and 3 Hz) including PGC-1α, NR4A3, ABRA, FOSB, EGR1, and XIRP1. Only two of the most repressed genes ($p < 0.05$) were common among both protocols; MIR4524A and GHR. The 3 Hz protocol showed greater regulation of several major metabolic transcription genes as compared to the 1 Hz protocol; consistent with our findings of greater fatigue induced with the 3 Hz stimulation. Subjects reported the 3 Hz protocol was more feasible for routine and long term utilization. **Conclusions:** We demonstrate that 3 Hz stimulation yields greater gene regulation in human paralyzed muscle as compared to 1 Hz stimulation. The 3 Hz protocol regulated a family of genes that were also distinct from the 1 Hz protocol; most notably genes associated with insulin receptor regulation. The 3 Hz protocol was reported to be "more acceptable" than the 1 Hz protocol and induced greater fatigue. We conclude that the 3 Hz protocol is the optimal training protocol for long duration exercise in people with chronic spinal cord injury.

Disclosures: R.K. Shields: None. **M.A. Petrie:** None. **A. Sharma:** None. **M. Suneja:** None.

Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: Michael Smith Foundation for Health Research

Canadian Institutes of Health Research

Title: The performance and retention of a skilled walking task among among people with an incomplete spinal cord injury

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Abstract: Background: Many people with a motor-incomplete spinal cord injury (m-iSCI) can recover some basic walking ability; however they experience difficulty when performing more skilled walking tasks (e.g. stairs and obstacles). There is potential for task specific locomotor

training based on principles of motor learning to improve the motor control of walking in people with m-iSCI. The purpose of this study is to determine how well people with m-iSCI can learn a new skilled walking task, and retain their performance after a week.
Methods: Participants with m-iSCI (American Spinal Cord Injury Assessment; ASIA D) and age-matched controls performed a skilled walking task focused on foot height during the swing phase. They were presented with a virtual target that they were instructed to match during the swing phase along with real-time visual feedback of their foot height for 600 steps. The target height changed for each step based on a percentage of their maximum step height. We conducted a baseline and post-training test of 20 steps without visual feedback. A 20 step retention trial without visual feedback was repeated 24 hours, 48 hours and 1 week later. Following the retention trial at 1 week, participants performed 300 steps with visual feedback to re-learn the task. Foot trajectory error was measured as the vertical distance between the target and actual foot height. We also measured lower limb muscle activity and sagittal joint angles.
Results: SCI and control groups reduced their average foot trajectory error to 16.7 mm and 10.2 mm, respectively, in the last 20 steps of training. Performance was maintained during the post-test without visual feedback at 17.8 mm and 12.8 mm, respectively. However, average performance on retention trials increased to baseline levels for both groups (SCI: 86.2 mm, control: 43.1 mm). During the initial training session, it took SCI participants 39-87 steps and controls 30-53 steps to learn the task (i.e. 5 consecutive steps within 2 standard deviations of the final performance). Participants were able to re-learn the task faster at 1 week when visual feedback was given (SCI: 9-20 steps, control: 16-30 steps).
Conclusions: Preliminary findings indicate that individuals with m-iSCI were able to learn a skilled walking task at a similar level to abled-bodied adults. Although performance is not maintained after 24 hours, people are able to re-learn the task at a faster rate. Further data collection is warranted to understand the capacity and limitations of individuals with m-iSCI to acquire and learn new locomotor skills.

Disclosures: **A.E. Chisholm:** None. **A.M.M. Williams:** None. **G. Eginyan:** None. **T. Lam:** None.

Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

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Program#/Poster#: 59.28/JJ10

Topic: E.09. Spinal Cord Injury and Plasticity

Support: NIH R01 HD062507

Title: H-reflex depression in spinal cord injury: Impact of long term vibration training

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Abstract: Spinal cord injury (SCI) precipitates a range of secondary health conditions including lower limb fractures and involuntary spasms. We are currently studying whether the reintroduction of mechanical loads via vibration is an effective anti-osteoporosis countermeasure in SCI. Anecdotal reports from participants in this study indicated that after vibration training, lower limb spasms subsided for several hours. **Purpose:** We investigated spinal reflex excitability in the trained and untrained limbs of these participants in order to determine whether vibration training initiated changes in soleus H-reflexes. Altered H-reflex depression may indicate the presence of spinal reorganization. We hypothesized that vibration training would trigger partial restoration of H-reflex paired-pulse depression, which is typically lost after SCI. **Methods:** Five men with chronic complete (AIS-A) SCI received vibration training (30 Hz, 0.6 *g*) to one lower limb while seated in a wheelchair. The contra-lateral limb served as a within-subject control. Subjects received an average of 47 vibration sessions over an average of 29 weeks. At the end of their participation, we elicited soleus H-reflexes via paired-pulse stimulation (inter-pulse interval = 500 ms) to the tibial nerve in both the trained and untrained limbs. We examined the depression of the 2^{nd} H-reflex (H2) in relation to the unconditioned H-reflex (H1), which provides an index of post-activation depression within the spinal reflex arc. We examined H-reflex depression at rest (Pre), during a brief session of 30 Hz vibration (Vibration), and 5 minutes after vibration (Post). **Results:** In the Pre and Post epochs, 4 of 5 participants showed a between-limb difference in H2 depression in the hypothesized direction (Trained > Untrained; average difference = 29.19%) but it was not statistically significant ($p = 0.125$). During Vibration, all 5 participants showed a between-limb difference in the hypothesized direction (average difference = 34.98%; $p = 0.06$). **Conclusion:** These findings support that vibration training modulates post-activation depression in humans with chronic paralysis. Future studies will determine whether vibration training will reduce the incidence or severity of lower extremity spasms in individuals with SCI.

Disclosures: S. Dudley-Javoroski: None. **C. Yen:** None. **C.L. McHenry:** None. **M.A. Petrie:** None. **R.K. Shields:** None.

Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

Location: Halls B-H

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Program#/Poster#: 59.29/JJ11

Topic: E.09. Spinal Cord Injury and Plasticity

Title: Trunk muscle activation patterns during walking with robotic exoskeletons in high thoracic motor-complete sci

Authors: R. ALAMRO, A. E. CHISHOLM, ***T. LAM**;
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Abstract: Background: The International Standards for Neurological Classification of Spinal Cord Injury relies on sensory tests only to evaluate motor function in the thoracic segments of the spinal cord, which could result in uncertain assumptions about the motor function of these segments. Indeed, recent studies using targeted approaches to assess the trunk have revealed sparing of trunk muscle function in individuals with SCI classified with thoracic or cervical motor-complete injuries. Therefore, finding training techniques to recruit this preserved muscle function in the trunk could enhance their activation and potentially lead to better improvements in postural control and function. Robotic exoskeletons, such as the Lokomat and Ekso, are used in gait rehabilitation for people with SCI, but it remains unknown the extent to which they engage those trunk muscles that are normally activated during walking. The Lokomat and Ekso use different methods to provide gait training. The Lokomat provides gait training on a treadmill with the trunk passively supported by an overhead harness that provides weight support. However, the user's body is rigidly held within the Lokomat, which could imply lesser degree of recruitment of postural muscles. In contrast, gait training in the Ekso is provided overground and requires continuous participation from the users to maintain their balance while shifting their weight from one limb to the other in order to activate the Ekso's legs to walk. This mechanism could lead to better postural muscle activation.
Objective: To characterize and compare the activation patterns of the axial muscles during walking with the Ekso and the Lokomat in people with high thoracic motor-complete SCI.
Methods: 1 individual with T3 chronic motor-complete SCI. The participant performed 3 speed matched walking conditions: Lokomat-assisted walking (Loko-TM), Ekso-assisted walking overground (Ekso-OG), and Ekso-assisted walking on a treadmill (Ekso-TM). Electromyography (EMG) signals were recorded bilaterally from rectus abdominis (RA), external oblique (EO), and erector spinae (ES) and normalized to a standardized voluntary contraction (SVC).
Results: For Loko-TM condition, right RA mean EMG amplitude was 61% of SVC, EO 24% and ES 15%. In the left side, RA was 37%, EO 21% and ES 16%. For Ekso-OG, right RA was 141.3% of SVC, EO 238.9%, and ES 48.4%. In the left side, RA was 84%, EO 74% and ES 36.3%. For Ekso-TM, right RA was 187%, EO 356% and ES 63%. In the left side RA was 132%, EO 167% and ES 52%.
Conclusion: Based on the results, Ekso-assisted walking was better in activating trunk muscles than the Lokomat-assisted walking.

Disclosures: R. Alamro: None. **A.E. Chisholm:** None. **T. Lam:** None.

Poster

059. Training, Rehabilitation, and Repair: Spinal Cord Injury Recovery

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Topic: E.09. Spinal Cord Injury and Plasticity

Support: CIHR Grant

Title: Improvements in lower limb proprioceptive acuity as a result of end-point based training

Authors: *T. QAISER[1,2], T. LAM[1,2];
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Abstract: Background: Human movement control relies critically on the ability to sense limb position and movement (proprioception). There is intriguing evidence from upper limb reaching studies that given appropriate feedback it is possible to improve proprioceptive sense through passive movement training. Furthermore, it has been suggested that individuals are more accurate at determining end-point limb location than the commonly assessed single joint angle position. Here we developed a robotic-based protocol for training proprioceptive sensation of the lower limb and to compare the effectiveness of end-point vs. joint-based proprioceptive training.
Methods: Baseline proprioceptive sense was assessed in able-bodied individuals with tests of knee joint position sense and end-point (heel) position sense using custom software controls of the Lokomat robotic exoskeleton. During knee joint position assessment, participants were asked to passively match their knee joint (with the help of a joystick) to a previously memorized position. The error was determined as the difference (in degrees) between the actual and memorized position. During end-point position tests, the participant's heel height was displaced upwards or downwards in a pseudo-random order from a previously memorized position. Participants were then asked to report whether the heel was higher or lower than before and the number of correct responses was scored. Subjects then underwent proprioceptive training, using the end-point position task described above, combined with visual feedback about their accuracy. After training, subjects repeated the knee joint and end-point position tests.
Results: Preliminary data show one participant improved knee joint position sense as a result of the end-point position training while remaining consistent in end-point position sense. Another participant started with high levels of initial proprioception and showed no improvements in either joint or end-point position sense.
Conclusion: This is the first study to test the idea whether lower limb proprioceptive sense could be improved with sensory training. Preliminary findings indicate that end-point based training might enhance proprioceptive acuity. Since these were individuals with intact proprioception, a ceiling effect may be possible. Implementation of lower position sense thresholds are needed to

further confirm the reliability of end-point based training intervention and to apply it to a population with sensory deficits such a spinal cord injury.

Disclosures: **T. Qaiser:** None. **T. Lam:** None.

Poster

060. HPG Axis I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

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Topic: F.03. Neuroendocrine Processes

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MBRS-RISE R25 GM061838

MBRS-RISE R25 GM066250

Title: Anabolic androgenic steroids modulate proteins related to glucose metabolism, stress response, GnRH and estrogen receptors in the GT1-7 hypothalamic cell line

Authors: *A. G. ALEMÁN-REYES[1], C. CALO-GUADALUPE[2], F. J. MARTÍNEZ-RIVERA[3], J. PÉREZ-LASPIUR[4], M. E. SANTIAGO-GASCOT[3], E. GARCÍA-SANTIAGO[2], I. OTERO-PAGÁN[5], Y. RODRÍGUEZ-PÉREZ[4], L. M. MELÉNDEZ[4,6], J. L. BARRETO-ESTRADA[3]; [1]Univ. of Puerto Rico, Río Piedras Campus, San Juan, Puerto Rico; [2]Dept. of Biotech., Univ. del Este, Carolina, 00983, Puerto Rico; [3]Dept. of Anat. and Neurobio., [4]Translational Proteomics Center-RCMI, [5]Dept. of Biostatistics and Epidemiology, Sch. of Publ. Hlth. Sci., [6]Dept. of Microbiology and Med. Zoology, Med. Sci. Campus, Univ. of Puerto Rico, San Juan, 00936, Puerto Rico

Abstract: The abuse of anabolic androgenic steroids (AAS) has been considered a major public health problem during decades. Supraphysiological doses of AAS may lead to a variety of neuroendocrine problems. Precisely, the hypothalamic-pituitary-gonadal (HPG) axis is one of the body systems mainly influenced by steroidal hormones. Fluctuations of the hormonal milieu result in alterations of reproductive function through changes in hypothalamic neurons expressing gonadotropin-releasing hormone (GnRH). Previous studies have shown that AAS

modulate the activity of these neurons through steroid-sensitive afferents. However, the potential molecular mechanisms induced by AAS in GnRH neurons remain undetermined. Here, we performed proteomic analyses of the murine hypothalamic GnRH (GT1-7) neuronal cell line after exposure to 17α-methyltestosterone (1μM). Two-dimensional difference in gel electrophoresis (2D-DIGE) and mass spectrometry analyses identified a total of 17 different proteins that were significantly affected by AAS. Proteins were associated to glucose metabolism (GAPDH), drug detoxification (GSTM1), cell cycle (ERH) and cellular transport (PEBP and ERP), among others. In addition, using Western blots, AAS exposure decreased the expression of GnRH and estrogen receptors (ER), without affecting androgen receptors (AR). Using Ingenuity Pathway Analyses (IPA) we studied protein predominant interactions with other proteins of signaling pathways. Particularly, signal transduction proteins pERK and p38MAPK were upregulated and downregulated, respectively, whereas AKT was unaffected. Together, our results suggest that androgenic compounds have the capacity to affect the neuroendocrine system by modulating key cellular processes for the control of reproductive function.

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Poster

060. HPG Axis I

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 60.02/JJ14

Topic: F.03. Neuroendocrine Processes

Support: ANR-14-CE12-0015-01

Title: AMH is mutated in patients with congenital hypogonadotropic hypogonadism and it regulates development and function of GnRH neurons

Authors: ***S. A. MALONE**[1], D. CASSATELLA[2], J. ACIERNO[2], C. XU[2], I. CIMINO[1], P. PIGNY[3], N. PITTELOUD[2], P. GIACOBINI[1];
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Abstract: Gonadotropin releasing hormone (GnRH) neurons, critical for reproduction, originate in the olfactory placode and enter the brain along vomeronasal and terminal axons during embryonic development. Alterations either in the development of this system or in the secretion of GnRH are associated with congenital hypogonadotropic hypogonadism (CHH) in humans, a condition characterized by failure of sexual competence. Kallmann syndrome (KS) associates congenital hypogonadism due to GnRH deficiency and anosmia, while a normal sense of smell is defined as normosmic CHH (nCHH). Here, we performed whole-exome sequencing in a cohort of 70 KS and 43 nCHH probands and identified several heterozygous missense mutations in the Anti-Mullerian Hormone *(AMH)* gene and in its receptors in nCHH individuals. Mutations in *AMH* resulted in impaired secretion of AMH by transfected COS-7 cells or reduced signalling activity of the secreted protein in the GN11 cell line derived from embryonic GnRH cells, which strongly suggests that these mutations have a pathogenic effect. We also show that AMH and its receptor (AMHR2) are expressed along the olfactory fibers and by GnRH neurons during GnRH migratory process. Pathohistological analysis of *Amhr2*$^{-/-}$ mice revealed defective embryonic migration of the neuroendocrine GnRH cells to the basal forebrain, leading to a significant reduction in the total number of GnRH neurons in the adult brains of these animals and reduced fertility. Our findings indicate that AMH signalling insufficiency contributes to the pathogenesis of nCHH and highlight a novel role for AMH in the correct development and function of GnRH neurons.

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Poster

060. HPG Axis I

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Topic: F.03. Neuroendocrine Processes

Support: ANR Research Grant, France

Title: Prenatal Anti-Mullerian Hormone (AMH) treatment leads to hypothalamic neuroendocrine deregulations and PCOS-like phenotype in mice

Authors: ***B. TATA**, P. GIACOBINI;
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Abstract: Reproduction in mammals is dependent on the function of specific hypothalamic neurons that secrete GnRH. In females, the pulse amplitude and frequencies of GnRH is essential

to shape the differential synthesis and secretion of two gonadotropic hormones from the anterior pituitary, luteinizing hormone (LH) and follicle stimulating hormone (FSH). Alterations in the development of this system or in the secretion of GnRH are associated with a number of reproductive disorders in humans. Among these disorders, Polycystic Ovary Syndrome (PCOS), the most common form of female infertility, affects up to 10% of women, and is characterized by increased ovarian androgen biosynthesis, oligo-anovulation, with high GnRH/LH pulse frequency. Interestingly, AMH levels are also elevated in PCOS patients and we recently showed an extra-gonadal role of AMH, whereby AMH acts directly on GnRH neurons, increasing their activity. Important, PCOS patients exhibit hormonal imbalances and endocrine alterations during gestation, which can contribute to an increased risk that their offspring will develop PCOS. We hypothesized that prenatal exposure to elevated AMH could contribute to the hormonal and gonadal alterations observed in PCOS through precocious activation of the GnRH system.
To test this, we subjected pregnant mice to daily injections of AMH during the late gestational periods and studied the reproductive parameters and the development of the hypothalamic neuronal network's in their offspring. Prenatal AMH-treated (PAMH) female offspring exhibited anovulation, hyperandrogenism and elevated LH (i.e, the major hallmarks of PCOS) as compared to control females. Prenatal AMH treatment combined with injections of a GnRH antagonist rescued the reproductive phenotype of PAMH mice. These experiments underscore that the disruptions observed in PAMH mice result from prenatal AMH-dependent precocious hyperactivation of the GnRH system. Indeed, PAMH offspring exhibited severe defects in hypothalamic neuronal networks that regulate GnRH function. Herein, AMH excess *in utero* disrupts hypothalamic neuronal systems, which could underlie the neuroendocrine anomalies in PCOS. In conclusion, we generated a novel animal model to study the neuroendocrine pathological development of PCOS.

Disclosures: **B. Tata:** None. **P. Giacobini:** None.

Poster

060. HPG Axis I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 60.04/JJ16

Topic: F.03. Neuroendocrine Processes

Support: R01 HD039916 to M.N.L. and R.L.G.

Title: Kappa opioid receptors are internalized in arcuate KNDy cells GnRH pulse termination in the ewe

Authors: *P. W. WEEMS[1], L. M. COOLEN[2], S. M. HILEMAN[4], S. HARDY[4], R. B. MCCOSH[4], R. L. GOODMAN[4], M. N. LEHMAN[3];
[1]Neurosci., [2]Physiol., [3]Neuobiology and Anatom. Sci., Univ. of Mississippi Med. Ctr., Jackson, MS; [4]Physiol., West Virginia Univ., Morgantown, WV

Abstract: The endogenous opioid peptide dynorphin (Dyn) is known to play an important role in mediating the negative feedback influence of progesterone on pulsatile GnRH secretion in sheep. Dyn action is mediated via its high affinity receptor, kappa opioid receptor (KOR). Recently we reported the presence KOR in kisspeptin/neurokinin B/dynorphin (KNDy) neurons of the sheep arcuate nucleus (ARC). The Dyn/ KOR interaction is believed to play a critical role within the KNDy model of GnRH pulse generation, acting as a "stop signal" terminating each pulse. KOR is an inhibitory G-protein coupled receptor and after repeated or sustained exposure to agonists, KORs are desensitized by receptor phosphorylation and endocytosis. The "KNDy hypothesis" of pulse generation predicts that KOR are bound and internalized by binding of Dyn, at the termination of each pulse. To test this hypothesis, GnRH pulses were artificially induced in unanesthetized, gonad-intact anestrus ewes via icv injection of 0.2 nmole neurokinin B (NKB) into the third ventricle. Portal blood samples were taken every two minutes prior to and following injection of NKB or vehicle to measure GnRH concentrations. Animals were sacrificed at times corresponding to either GnRH pulse onset or termination and brain tissue was collected for triple-label immunofluorescence analysis. 45μm hypothalamic sections containing the ARC were processed for immunofluorescent labeling of KOR in KNDy cells, using Dyn as a marker, and counterstained with Fluoro-Nissl to aid in the assessment of membrane-bound vs internalized KOR immunoreactivity via confocal microscopy. Numbers of KOR-immunoreactive endosome-like particles within the cytosol and not associated with the cell membrane were counted in the two 1 μm optical sections in the middle of each KNDy neuron (n = 20/animal). Animals that received NKB injection (n=4) showed significantly higher numbers of internalized particles compared to vehicle controls (n=2). Furthermore, there was a significant increase in the number of internalized particles during pulse termination (n=2) compared to onset (n=2) in NKB-injected ewes which was not seen in vehicle controls. Thus it appears that Dyn is released onto the KNDy network shortly after GnRH pulse onset activating and internalizing KOR on these cells resulting in pulse termination.

Disclosures: P.W. Weems: None. **L.M. Coolen:** None. **S.M. Hileman:** None. **S. Hardy:** None. **R.B. McCosh:** None. **R.L. Goodman:** None. **M.N. Lehman:** None.

Poster

060. HPG Axis I

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Program#/Poster#: 60.05/JJ17

Topic: F.03. Neuroendocrine Processes

Support: Hungarian Brain Project (KTIA_NAP_13-2014-0006)

Title: Effect of contraceptive residues at central and peripheral levels of the snail (Lymnaea stagnalis) reproductive system

Authors: *Z. ZRINYI, G. MAASZ, R. HORVATH, Z. PIRGER;
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Abstract: Estrogens and progestogens in combined application are the most frequently used oral contraceptives. As endocrine disruptors they can also be traced in the environment, including aquatic surrounding. Their presence in worldwide relevant concentration in several European natural waters has also been demonstrated by our research group. It is well-known that their target is the reproduction of the animals living in the aquatic environment. In spite of it, only few data are available on the effect of sex-steroid mixtures, especially in worldwide relevant concentration range. Therefore, we have investigated the effect of estrogens (17-β-estradiol, ethynil-estradiol) and progestogens (progesterone, drospirenone, levonorgestrel, gestaden) in environmentally relevant concentration (10 ng/L progestogens, 1 ng/L estrogens) on the reproductive system of the pond snail (*Lymnaea stagnalis*). As a first step, the distribution of gonadotropin-releasing hormone (GnRH)-like peptide containing neurons were visualized in the CNS by immunohistochemistry, using a rabbit polyclonal anti-GnRH antibody, both in control and treated snails. The various GnRH analogs were then identified in the immunopositive neurons by single cell capillary microsampling technique. Their quantitative levels were measured by liquid chromatography coupled electrospray ionization mass spectrometry in total ganglion homogenates. Finally, the sperm number was counted by flow cytometry after nuclear staining. GnRH-like peptides expressing neurons were found in the buccal, cerebral and visceral ganglia as well as varicose fibers of neuropil tissue also displayed immunoreactivity. Quantitative IHC revealed the significant reduction of fluorescence intensity of the labeled neurons after treatments with hormone residues. In the immunopositive buccal, cerebral and visceral neurons three forms of GnRH analog were identified. The amount of GnRH analogs was significantly reduced in the treated snails, compared to the control. Finally, the total sperm number per animal also significantly decreased from ~1.6 million to ~1 million following contraceptive administration, as a possible consequence of the lowered GnRH level in the central neurons responsible for male reproduction. In summary, it appears that the simultaneous presence of 10 ng/L progestogens and 1 ng/L estrogens in the aquatic environment can lead to the impairment of the control of male reproduction at the highest regulating level, i.e. the CNS. It is concluded that the release of endocrine disruptors into aquatic ecosystem represents a serious environmental risk, exerting harmful effects on non-target organisms.

Disclosures: **Z. Zrinyi:** None. **G. Maasz:** None. **R. Horvath:** None. **Z. Pirger:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Hungarian Brain Project (KTIA_NAP_13-2014-0006).

Poster

060. HPG Axis I

Location: Halls B-H

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Program#/Poster#: 60.06/KK1

Topic: F.03. Neuroendocrine Processes

Support: Grants-in-Aid for Scientific Research (C) 24590257

Title: Immunohistochemical detection of kisspeptin in the mouse pituitary

Authors: *Y. IKEDA, A. TAGAMI;
Aichi-Gakuin Univ. Sch. of Dent., Nagoya, Japan

Abstract: Kisspeptins encoded the Kiss1 gene are expressed in two hypothalamic regions, the preoptic area and the arcuate nucleus, and regulate the hypothalamic-pituitary-gonadal (HPG) axis. Hypothalamic kisspepsins bind to their receptor (Kiss1R) to release GnRH, and indirectly increase gonadotropin secretion in the pituitary. Thus, it has been considered that the primary site of the Kiss1/Kiss1R system in the regulation of the HPG axis is in the hypothalamus. Although both Kiss1 and kiss1R were found in the pituitary of rats and owes, it is controversial about the effects of pituitary kisspeptins on gonadotropin secretion. To understand the importance of pituitary kisspeptins, we evaluated the spatial and temporal distribution and the cellular localization of kisspeptins in the mouse pituitary, using immunohistochemistry. We performed single- and double-label immunohistochemistry using specific antibodies for kisspeptin and endocrine cell-lineage markers on sagittal and coronal sections of mouse pituitaries at various stages of embryonic and postnatal development and in adulthood. Kisspetin-immunopositive cells were detected in the anterior pituitary as early as embryonic day 13.5 through adulthood. Kisspetin was co-localized with Foxl2, the marker of aGSU cells, and partially with SF-1, the marker of gonadotropes. However, few co-localization of kisspeptin with Pit1, the marker of thyrotrope-/somatotrope-/lactotrope-lineage cells, was detected. We demonstrate that kisspeptins are expressed specifically by gonadotrope-lineage cells in the mouse pituitary, suggesting a possible involvement of kisspeptins in gonadotrope differentiation.

Disclosures: Y. Ikeda: None. **A. Tagami:** None.

Poster

060. HPG Axis I

Location: Halls B-H

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Program#/Poster#: 60.07/KK2

Topic: F.03. Neuroendocrine Processes

Support: CONACYT: Registro 003797, Registro de validez oficial: 09-00122

Title: Long term effects of prenatal stress on hypothalamic GnIH and GnRH expression

Authors: *A. G. GARCÍA-SOTO[1], L. GÓMEZ QUIROZ[2], W. PORTILLO MARTÍNEZ[4], L. JUÁREZ ROJAS[2], S. RETANA-MARQUEZ[3];
[1]Grad. Biol. of Animal Reproduction Universida, Ciudad de Mexico, Mexico; [3]Biol. of Reproduction, [2]Univ. Autonoma Metropolitana, Mexico, Mexico; [4]Insituto de Neurobiología, UNAM, Querétaro, Mexico

Abstract: Prenatal stress (PE) in mammals is involved in long term reproductive disorders. It causes alterations in adrenal and gonadal axes in adulthood. However, it is unknown whether neuroendocrine control of reproduction is disrupted by prenatal stress. Therefore, the aim of this study was to evaluate the content of gonadotropin-releasing hormone (GnRH) and gonadotropin-inhibiting hormone (GnIH) in the hypothalamus of adult male and female rats exposed to PE, as well as their sex hormone levels. Pregnant females were assigned to control group or stress by immersion in cold water. The pups were weaned on day 21, sexed and separated by gender. At the age of 3 months, the animals were euthanized and the hypothalamus was dissected. Blood samples were also obtained. Females were sacrificed at proestrus or diestrus stages of estrous cycle. Hypothalamic GnRH and GnIH content was evaluated by Western blotting. Sex hormones were quantified by High Performance Liquid Chromatography (HPLC). The results showed a decrease in GnRH content and an increase in GnIH content in prenatally stressed females at both stages (proestrus and diestrus). In males subjected to PE, an increase in GnIH and a decrease in GnRH were also observed. Estradiol levels were higher in prenatally stressed females at proestrus, and testosterone decreased in prenatally stressed males. These results indicate that PE is capable of disrupt the neuroendocrine control of reproduction both in females and males, causing endocrine disorders which may impair some reproductive aspects in both sexes.

Disclosures: A.G. García-Soto: None. **L. Gómez Quiroz:** None. **W. Portillo Martínez:** None. **L. Juárez Rojas:** None. **S. Retana-Marquez:** None.

Poster

060. HPG Axis I

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Program#/Poster#: 60.08/KK3

Topic: F.03. Neuroendocrine Processes

Support: DGAPA-PAPIIT grant IN-220014

CONACyT grant 29240

Title: The estradiol feedback in the regulation of spontaneous ovulation depends on the excised-ovary

Authors: *M. B. CRUZ[1], R. LIBRADO-OSORIO[2], A. ESPINOSA-VALDEZ[1], K. MACÍAS[1], I. ARRIETA-CRUZ[2], A. FLORES[1], R. DOMÍNGUEZ[1];
[1]Univ. Autonoma De México, Mexico DF, Mexico; [2]Natl. Inst. of Geriatrics, Mexico City, Mexico

Abstract: In rats, estradiol (E2) secreted in the day of diestrus-2 (D2) stimulates preovulatory LH secretion. We have previously shown that one hour after removing the left ovary (ULO-L) E2 serum levels are similar to the control group, while the removal of the right ovary (ULO-R) reduces it. In present study we evaluated if this asymmetry is maintained until 11:00 h or 17:00 h of the expected proestrus day (Pe). In addition, if the ovulation occurs at the predicted day of estrus (Ee) or at the next vaginal estrous (Ev). At 13:00h of D2 groups of rats was ULO or unilateral sham operated (USO), and sacrificed at Ee and Ev. Other groups were sacrificed at 11:00 or 17:00 h of Pe. In USO or ULO sacrificed at 11:00 h of Pe the number of immunoreactive (ir-) cells to estrogen receptors (ER) alpha and beta in preoptic-anterior hypothalamic (POA-AHA) region were evaluated. Groups of ULO rats were injected with LHRH at 14:00 h of Pe, or with estradiol benzoate (EB) at 14:00 h of D2. 4/19 ULO ovulated at the Ee while 19/20 USO rats did it ($p<001$). When the rats had its Ev (24 h after Ee), 6/8 of the ULO-R ovulated, while 0/8 of the ULO-L did it. In ULO-L rats, the E2 serum levels at 11:00 h of Pe were higher than in USO rats (61.3±8.8 vs. 14.4±4.0, $p<0.001$). Such effects did not occur in ULO-R (21.8±7.4 vs. 14.4±4.0). The concentration of FSH in both ULO groups were higher than in USO (ULO-L: 21.4±0.14; ULO-R >100 ng/mL vs. 10.07±0.7, $p<0.001$). In addition, at 17:00 h of Pe the LH serum levels were lower (ULO-L: 0.9±0.008, ULO-R: 1.4± 0.019 vs. control: 22.9± 7.0, $p<0.001$). At 11.00 of Pe, the number of ir-ERalpha cells in the POA-AHA of ULO rats was lower than in control rats (OVX-I: 983.7 ± 52.5 OVX-D: 926.2 ± 42.6 vs. control: 1226.4 ± 53.8, $p<0.05$). In rats with ULO-R the number of ir-REbeta cells was lower than control (ULO-R: 605.2±78 vs. control: 1070.4±78.5, $p<0.0001$). Rats with ULO-R or ULO-L ovulated after LHRH injection. The injection of EB to ULO-R induced ovulation in all treated rats (10/10 vs. 3/10, $p<0.0031$), but not in ULO-L rats (3/9 vs. 1/10, $p=0.3034$).The results

suggest that at D2, the ovulation regulatory mechanisms throughout the E2 feed-back depend on the in situ ovary, and that the right ovary secrets more E2 than the left one.

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Poster

060. HPG Axis I

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Program#/Poster#: 60.09/KK4

Topic: F.03. Neuroendocrine Processes

Support: NSF Career Award 1253126

Title: Impact of timing and duration of low-dose Bisphenol A exposure on extra-hypothalamic GnRH Neurons, social and locomotor behavior in adult Japanese medaka

Authors: *T. INAGAKI, J. A. O'LEARY, S. RAMAKRISHNAN;
Biol., Univ. of Puget Sound, Tacoma, WA

Abstract: Recent evidence indicates that chronic exposure to Bisphenol A (BPA), a xenoestrogen, in early life may disrupt development of normal brain function and behavior mediated by gonadotropin-releasing hormone (GnRH) pathways. In fish, previous work showed that chronic exposure to BPA (200 ng/ml) altered development of GnRH3 systems and locomotor behavior in medaka embryos and larvae. Yet impacts of timing and duration of BPA exposure and their consequences in adult brains and behavior are not well known. Here we examined the effects of different exposure to low-dose BPA on extra-hypothalamic GnRH3 systems, locomotor and social behavior in adult medaka fish (8-14 months) with GnRH3 neurons tagged with green fluorescent protein (GFP). Fertilized eggs were collected daily and exposed to BPA (200 ng/ml) with the following regimen- i) G0: no exposure (Group 0, control), ii) G1: life-long exposure (G1), iii) G2: during embryogenesis and early larval development (1-14 days post fertilization or dpf), iv) G3: only during neurogenesis (1-5 dpf) and v) G4: only during sex differentiation (5-14 dpf). Aside from G1, other subjects were raised to adulthood in regular fish water following initial exposure. Adult locomotor (distance covered, velocity) and social (mirror approach and mirror touching) behaviors were recorded for 3 minutes using Noldus Ethovision behavioral analysis system. Following behavior tests, the brains were dissected and sizes of extra-hypothalamic GnRH3-GFP neurons in the terminal nerve were measured. G1 fish showed significant hyperactivity compared to controls, for both the total distance covered ($p<0.05$) and the velocity of movement ($p<0.01$) compared to control fish. No differences in overall

locomotion were seen in other treatments. In using mirror approach to assess social behavior, G2 fish spent significantly more time near the mirror ($p<0.001$, 99.2% increase compared with control). However, fish in G1 ($p<0.01$), G2 ($p<0.001$) and G4 ($p<0.05$) spent significantly less time touching the mirror (67.6%, 79.8%, and 53.8%, respectively) in relation to unexposed controls. The soma sizes of GnRH3-GFP neurons were significantly smaller in G1 ($p<0.05$, 16.9% reduction compared with control) and G2 ($p<0.001$, 33.3% reduction compared with control). These data provide evidence that different timing and duration of BPA exposure differently affect extra-hypothalamic GnRH3 neural systems and social/locomotor behavior in adult medaka (Supported by NSF Career Award 1253126 to SR).

Disclosures: **T. Inagaki:** None. **J.A. O'leary:** None. **S. Ramakrishnan:** None.

Poster

060. HPG Axis I

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Topic: F.03. Neuroendocrine Processes

Support: NSF GRFP

P50HD28934

Title: Firing activity of gonadotropin-releasing hormone (GnRH) neurons across postnatal development in female mice is altered by prenatal androgenization

Authors: *E. A. DULKA[1], S. M. MOENTER[2];
[1]Mol. and Integrative Physiol., [2]Mol. and Integrative Physiology, Intrnl. Medicine, Obstetrics and Gynecology, Univ. of Michigan, Ann Arbor, MI

Abstract: Reproductive function is governed by the hypothalamic-pituitary-gonadal axis. GnRH neurons act as central regulators of reproductive function by releasing GnRH in a pulsatile manner. Pulses of GnRH control release of pituitary gonadotropins that regulate gonadal function. Disruption of GnRH pulses can cause infertility. Polycystic-ovary syndrome (PCOS) is the leading cause of infertility in reproductive-aged women. In patients with PCOS, release frequency of the pituitary gonadotropin luteinizing hormone (LH) is elevated suggesting an upregulation in GnRH release. In a prenatally-androgenized (PNA) mouse model, adults exhibit elevated LH levels, increased GnRH neuron firing, and increased excitatory GABAergic input to GnRH neurons. It is unknown when this increase in GnRH neuron activity occurs. In young girls with hyperandrogenemia, LH pulses are disrupted, suggesting that some aspects PCOS may

manifest prior to puberty. We hypothesized that 1) GnRH neuron activity increases across the postnatal period and 2) postnatal GnRH neuron activity is disrupted in the PNA model. To test if GnRH neuron firing rate changes across postnatal development in female mice, extracellular recordings (1hr duration) were conducted from GFP-identified GnRH neurons in acute brain slices at postnatal day (P) 7, 14, and 21 from control and PNA mice. PNA mice were generated by injecting dams with 250µg dihydrotestosterone on days 16-18 of gestation. Despite a lack of outward reproductive activity during the postnatal period, GnRH neuron activity was observed at all postnatal time points tested. In control mice, this activity increased with age, with firing rate higher at P21 than P7 (one-way ANOVA: $p<0.05$, n= 12-14 cells/group). In contrast to controls, firing activity in PNA mice did not increase across the postnatal period and was decreased at P21 compared to controls (two-way ANOVA $p< 0.05$ n= 11-14 cells/group). To further investigate the difference in firing rate observed at P21, the number and duration of action potential bursts, often associated with neurosecretion, were compared. Initial analyses found no difference in either parameter between control and PNA animals at P21. Together these data demonstrate that GnRH neurons are active even before puberty in female mice and that this activity increases over postnatal development. The observation that PNA treatment decreased GnRH neuron activity at P21 suggests that early GnRH activity may contribute to the adult PNA phenotype. Future experiments will address mechanisms underlying differences observed at P21 and if postnatal GnRH neuron activity is required for proper establishment of adult reproductive function.

Disclosures: **E.A. Dulka:** None. **S.M. Moenter:** None.

Poster

060. HPG Axis I

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Topic: F.03. Neuroendocrine Processes

Support: NIH Grant R01 HD41469

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NIH Grant T32 HD079342

NIH Grant T32 GM007863

Title: Gonadotropin-releasing hormone (GnRH) neuron excitability is increased during estradiol positive feedback

Authors: *C. E. ADAMS[1], S. SCHNELL[1,2], S. M. MOENTER[1,3,4];
[1]Mol. and Integrative Physiol., [2]Dept. of Computat. Med. and Bioinformatics, [3]Dept. of Intrnl. Med., [4]Dept. of Obstetrics and Gynecology, Univ. of Michigan, Ann Arbor, MI

Abstract: GnRH neurons form the final common pathway for neural control of fertility. GnRH triggers luteinizing hormone (LH) and follicle-simulating hormone (FSH) release from the pituitary; LH and FSH stimulate ovarian follicle maturation. During most of the reproductive cycle, estradiol suppresses GnRH neuron firing rates and GnRH/LH release (negative feedback) but switches to stimulatory action to induce a surge of GnRH and LH release (positive feedback); the LH surge initiates ovulation. In ovariectomized mice implanted with estradiol (OVX+E), GnRH neuron firing rates are suppressed in the morning by estradiol negative feedback (OVX+E AM, -FB) and elevated in the afternoon by estradiol positive feedback (OVX+E PM, +FB); no time-of-day dependent shift is observed in OVX mice. Many changes in ionic conductances of GnRH neurons are regulated by estradiol in a time of day-dependent manner. It is unclear if or how changes in ionic conductances isolated in voltage-clamp experiments drive the increase in GnRH neuron firing rates during positive feedback. We hypothesized that changes in ionic conductances alter baseline membrane potential. To test this, we measured membrane potential of GFP-identified GnRH neurons in acute brain slices prepared from OVX+E and OVX mice in the AM vs PM using an on-cell method. No difference in membrane potential was observed among groups ($p>0.1$). We thus hypothesized changes to ionic currents render GnRH neurons more excitable during positive feedback vs negative feedback. To test this, we studied GnRH neuron response to extrinsic stimuli (500 ms current injection, 0-40 pA, 2 pA steps) in the same animal models. Ionotropic $GABA_A$ and glutamate receptors mediating fast synaptic transmission were blocked to stop spike initiation due to activation of these receptors. The minimum current required to initiate spikes was lower during +FB relative to -FB and OVX AM/PM neurons ($p<0.05$). Once firing was initiated, GnRH neurons fired more spikes at each current step from 20-40 pA ($p<0.05$). Latency to first spike was compared in traces having 4-7 spikes; latency decreased during +FB (OVX+E PM 127±11, OVX+E AM 184±16, OVX PM 221±11, OVX AM 220±13 ms; $p<0.05$). Firing threshold of the first spike was hyperpolarized during +FB (OVX+E PM -47.5±1.1, OVX+E AM -39.1±1.7, OVX PM -35.6±1.7, OVX AM -37.5±2.6 mV; $p<0.05$). These data suggest GnRH neuron excitability is increased during positive feedback, thus both estradiol and some time of day-dependent cue are needed. We postulate increased excitability is due to reduced subthreshold potassium currents to increase spike initiation and decrease spike latency, and modifications of sodium channels to alter threshold.

Disclosures: C.E. Adams: None. **S. Schnell:** None. **S.M. Moenter:** None.

Poster

060. HPG Axis I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 60.12/KK7

Topic: F.03. Neuroendocrine Processes

Support: NIH R01 HD042634

Title: Characterization of the HPG axis in Fgfr3-deficient mice

Authors: *S. J. BONELLI, L. R. BROOKS, S. N. KALAVITY, S. I. KAVANAUGH, P.-S. TSAI;
Integrative Physiol., Univ. of Colorado Boulder, Boulder, CO

Abstract: The hypothalamic-pituitary-gonadal (HPG) axis coordinates communication between the brain and the gonad to initiate the onset of puberty and reproductive function in mammals. Defects in this axis have significant implications for the ability of mammals to reproduce. An example, hypogonadotropic hypogonadism, is a condition in which the hypothalamus or pituitary harbors a defect in the ability to secrete the hypophysiotropic neurohormone gonadotropin-releasing hormone (GnRH) or gonadotropins (FSH and LH), respectively. Neurons that secrete GnRH express two of the four receptors of the fibroblast growth factor (Fgf) signaling family, Fgfr1 and Fgfr3. Previously, Fgfr1 has been shown to be required for the proper birth and specification of GnRH neurons during prenatal development, but Fgfr3 does not appear to be required prenatally. Instead, it is hypothesized that Fgfr3 functions to maintain the GnRH neuron population postnatally. The goal of this work is to examine the function of the HPG axis in transgenic mice harboring a global heterozygote knockout of Fgfr3 ($Fgfr3^{+/-}$ mice) at postnatal day (PN) 30, 60, and 120. To assess hypothalamic function, GnRH neuron numbers and relative expression of GnRH mRNA are quantified in wildtype (WT) and $Fgfr3^{+/-}$ mice. Concentrations of FSH and LH in the pituitary and plasma of WT and $Fgfr3^{+/-}$ mice are quantified to assess the function of the pituitary, and the gonadal function is explored via immunohistochemistry for cleaved caspase-3 within the ovary of WT and $Fgfr3^{+/-}$ mice. Previous findings show a significant decrease (43%) in GnRH neuron number in $Fgfr3^{+/-}$ mice compared to WT at PN60. However, current data shows no significant difference in GnRH mRNA expression at PN60 or PN120, suggesting that the GnRH system may compensate for a deficit in GnRH neuron number by upregulating GnRH transcript levels.
Key words: GnRH, fertility, transgenic mouse, HPG axis, Fgf signaling, Fgfr3

Disclosures: S.J. Bonelli: None. **L.R. Brooks:** None. **S.N. Kalavity:** None. **S.I. Kavanaugh:** None. **P. Tsai:** None.

Poster

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Topic: F.03. Neuroendocrine Processes

Support: U54 HD012303

R01 HD072754

R01 HD082567

T32 GM008666

P42 ES010337

Title: The effects of FOXL2 mutations on FSHβ transcription

Authors: *S. M. NEWTON, M. J. SUNSHINE, P. L. MELLON;
Dept. of Reproductive Med., Univ. of California San Diego, LA Jolla, CA

Abstract: Appropriate regulation of follicle-stimulating hormone (FSH) is required for normal female fertility. Previous studies have shown that the synergistic induction of FSHβ transcription by activin and progesterone requires the binding of SMAD proteins and the FOXL2 transcription factor. Human mutations in the FOXL2 gene have been identified in premature ovarian failure (POF) and blepharophiosis ptosis epicanthus inversus syndrome (BPES). To understand the involvement of FOXL2 in these reproductive disorders, we modeled human FOXL2 point mutations associated with BPES and/or POF in the mouse FOXL2 expression vector. We characterized the effects of these mutations on FSHβ induction in a gonadotrope cell line. Our results show that FSHβ expression is altered in the presence of FOXL2 mutants. FSHβ induction is differentially affected by the FOXL2 mutants in response to hormone treatments. Future experiments will involve the testing of FOXL2 binding patterns with proteins involved in the activation of FSHβ. These studies bring new insight into the pathophysiology of human reproductive disorders.

Disclosures: S.M. Newton: None. **M.J. Sunshine:** None. **P.L. Mellon:** None.

Poster

060. HPG Axis I

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Program#/Poster#: 60.14/KK9

Topic: F.03. Neuroendocrine Processes

Support: NIH HD41469

Title: Gonadotropin-releasing hormone (GnRH) neuron firing activity is higher on the afternoon of proestrus than diestrus during the estrous cycle of mice

Authors: *M. A. SILVEIRA[1], S. M. MOENTER[2];
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Abstract: GnRH neurons form the final central output regulating fertility. GnRH induces release of luteinizing hormone (LH) and follicle-stimulating hormone by the pituitary; these hormones activate gonadal steroidogenesis. Estradiol exerts negative feedback on GnRH neuron function for the most of reproductive cycle. Estradiol feedback switches to positive on the afternoon of proestrus to generate the GnRH surge. Increased GnRH release during positive feedback induces the LH surge, which triggers ovulation. Many electrophysiological studies of the neurobiological mechanisms for estradiol feedback have been done in a mouse model in which ovariectomized estradiol-replaced (OVX+E) mice exhibit daily LH surges. In this model, GnRH neuron activity is dependent on estradiol and time of day, with positive feedback increasing activity in the afternoon. Estradiol is not, however, the only ovarian factor that may play a role in the GnRH/LH surge during the reproductive cycle. Contributions from other factors may account for the observation that LH surge amplitude is lower ($p<0.01$) in OVX+E mice in the daily surge model than in proestrous mice. We monitored GnRH neuron firing activity as a function of the estrous cycle to test the hypothesis that GnRH neuron firing rate is elevated on the afternoon of proestrus compared to diestrus. We also compared firing rates on proestrus to those observed in the afternoon in OVX+E mice. To monitor firing rate, targeted single-unit extracellular recordings were made from GFP-identified GnRH neurons in brain slices from females in diestrus (negative feedback) or proestrus (positive feedback). GnRH neuron firing rate is elevated ($p<0.05$) on the afternoon of proestrus (0.3±0.09Hz, n=15) compared with diestrus (0.09±0.03Hz, n=15). The percentage of time cells are quiescent (≤1 event/minute, diestrus 71% vs. proestrus 43%; $p<0.05$) and the maximum duration of quiescence (43.3±4.6min diestrus vs 24.8±5.4min proestrus; $p<0.05$) were greater on diestrus, possibly suggesting decreased pulse frequency on diestrus compared to proestrus. Interestingly, the firing frequency of GnRH neurons on proestrus was not greater than that published for OVX+E mice during positive feedback (0.6±0.05 PNAS 102:15682), suggesting that peripheral estradiol alone can recapitulate

many of the positive feedback mechanisms increasing GnRH neuron activity. This suggests the lower LH amplitude observed in the daily surge model may not be attributable to the participation of other ovarian factors in changing GnRH neuron activity, but may be due to reduced excitation-secretion coupling in GnRH neurons or reduced pituitary response in this model.

Disclosures: M.A. Silveira: None. **S.M. Moenter:** None.

Poster

060. HPG Axis I

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 60.15/KK10

Topic: F.03. Neuroendocrine Processes

Support: HD P5028934

Title: Prenatal androgenization alters prepubertal development of gonadotropin-releasing hormone (GnRH) neuronal network function and connectivity

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Abstract: GnRH neurons are the final pathway for the central network that regulates reproduction. GnRH signals the pituitary to release gonadotropins, which regulate ovarian follicle maturation and steroid production. The GnRH/pituitary system is persistently hyperactive in women with polycystic ovary syndrome (PCOS), a common cause of infertility. This over-activity drives irregular menstrual cycles and elevated androgen levels characteristic of PCOS. When and how the neuroendocrine dysfunction seen in PCOS arises is an open question. Recent evidence of hyperandrogenemia in early pubertal girls suggests that clinical precursors of PCOS are observed at a young age. Mice that are exposed prenatally to elevated androgens (PNA) have disrupted cycles and hormone changes similar to women with PCOS. In adult PNA mice, GnRH neurons receive increased GABAergic neurotransmission, which is excitatory, and have increased firing activity. We hypothesized that PNA increases connectivity of GABAergic synapses onto GnRH neurons before the onset of puberty. We used control and PNA mice aged 7-28 days and adults to study GABAergic transmission to GnRH neurons during prepubertal development and define when differences in PNA emerge. PNA mice were generated by injecting dams with 250µg dihydrotestosterone on days 16-18 of gestation. Whole-cell voltage-clamp recordings of GABAergic postsynaptic currents (PSCs) in GFP-identified GnRH neurons

were made while blocking ionotropic glutamatergic receptors. In control and PNA females, GABAergic PSC frequency, but not amplitude, increased during postnatal development ($p<0.01$). PSC frequency was increased in 21-day-old PNA mice compared to controls (n=6-7 cells/group $P< 0.05$). We tested if increased PSC frequency in PNA females is due to increased number of synaptic release sites and/or increased activity of afferents by blocking action potentials with tetrodotoxin. The remaining activity-independent 'miniature' (m)PSCs are proportionate to the number of release sites. mPSC frequency was greater in PNA than control mice ($p<0.01$), suggesting increased synaptic connectivity. These data suggest that PNA accelerates prepubertal development of GABAergic transmission to GnRH neurons. These changes may contribute to the increase in excitatory drive observed in adult PNA mice and perhaps dysfunction of the GnRH network in PCOS.

Disclosures: T. Berg: None. **S.M. Moenter:** None.

Poster

060. HPG Axis I

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Program#/Poster#: 60.16/KK11

Topic: F.03. Neuroendocrine Processes

Support: NIH Grant R01 HD039916 to M.N.L.

Title: Optical clearing of the intact rat brain permits three-dimensional imaging of the arcuate kisspeptin/neurokinin B/dynorphin (KNDy) neuron population

Authors: *A. M. MOORE[1], K. A. LUCAS[1], L. M. COOLEN[2], M. N. LEHMAN[1]; [1]Neurobio. & Anatom. Sci., [2]Physiol. and Biophysics, Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract: Kisspeptin/Neurokinin B/Dynorphin (KNDy) neurons in the arcuate nucleus play a key role in the central regulation of fertility. The ability to study the morphology and connectivity of the complete KNDy circuit over different reproductive states is likely to reveal unknown features of the population. In recent years, optical tissue clearing techniques have been developed to image neuronal populations in three-dimensions (3D) without sectioning the brain. To date, the ability to image neuronal populations in the intact mammalian brain has largely been limited to the mouse. We aimed to modify 3Disco (3D Imaging of Solvent-Cleared Organs, Ertürk et al. 2012, Nature Protoc.) and iDisco (Renier et al. 2014, Cell) clearing protocols to achieve immunolabelling and subsequent optical clearing of brains from larger species. We provide here a preliminary report for immunolabelling, clearing and imaging of the intact adult

rat brain. Perfusion fixed brains (4% paraformaldehyde) from ovariectomized adult Sprague Dawley female rats (5-6 months of age) were rendered transparent through optimizing the incubation length and concentration of the clearing agents tetrahydrofuran, dichloromethane and dibenzyl ether (n=2/3 per variable). Upon establishment of a reproducible clearing protocol, immunofluorescent labeling of hypothalamic neurotransmitters and neuropeptides in the whole brain was performed. Whole rat brains were incubated in mouse anti-tyrosine hydroxylase (1:5000, Millipore) for 6 days to label dopamine neurons (n=2). Whole rat brains (n=2) and 1 cm thick sagittal sections containing the hypothalamus (n=2) were incubated with rabbit anti-kisspeptin (1:250, gifted by Dr. Caraty) and guinea-pig anti-neurokinin B (1:250, gifted by Dr. Ciofi) for 6 days to identify KNDy neurons. Ultramicroscopy imaging of $1cm^3$ brain volumes was performed using a bi-directional lightsheet microscope (LaVision Biotec) combined with InspectorPro software at 6.3x magnification and a 4µm optical interval to achieve cellular resolution. Mosaic z-stack images were stitched together using Fiji software and 3D reconstructions made using Imaris software to visualize dopamine neuron populations throughout the brain and KNDy neurons in the rostral to caudal extent of the arcuate nucleus. We are now extending the application of this protocol to image the KNDy neuron population in sheep hypothalamic blocks. In conclusion, we report here an optimized technique that provides versatile analysis of neuroendocrine circuits and broad opportunity to study a variety of cell populations throughout the central nervous system of multiple animal models.

Disclosures: **A.M. Moore:** None. **K.A. Lucas:** None. **L.M. Coolen:** None. **M.N. Lehman:** None.

Poster

060. HPG Axis I

Location: Halls B-H

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Program#/Poster#: 60.17/KK12

Topic: F.03. Neuroendocrine Processes

Support: HD41469

Title: Estradiol-dependent suppression of gonadotropin-releasing hormone (GnRH) neuron firing activity by corticotropin-releasing hormone (CRH) in female mice

Authors: *C. PHUMSATITPONG[1], S. M. MOENTER[2];
[1]Mol. and Integrative Physiol., [2]Mol. and Integrative Physiol, Intrnl. Medicine, Obstetrics and Gynecology, Univ. of Michigan, Ann Arbor, MI

Abstract: GnRH neurons in the hypothalamus are the final central regulators of the reproductive system. GnRH regulates the production and release of luteinizing hormone (LH) and follicle stimulating hormone (FSH), which then activate gonadal cells leading to gametogenesis and steroidogenesis. GnRH neurons integrate various inputs that modulate action potential firing and thus secretion of GnRH. Stress typically has inhibitory effects on reproduction. CRH is released during the stress response and has been shown to be involved in stress-induced suppression of fertility/reproductive function. Intracerebroventricular injection of CRH decreases LH pulse frequency and amplitude and CRH antagonists reverse the inhibitory effects of stress on reproduction. We hypothesized that CRH inhibits GnRH neuron firing activity. To test this, mice were ovariectomized and either implanted with a capsule producing a physiological circulating level of estradiol (OVX+E) or not treated further (OVX) to examine the influence of estradiol on response to CRH. Targeted extracellular recordings were used to record firing activity before (con) and during bath application of CRH from GFP-identified GnRH neurons in brain slices; recordings were done in the afternoon when estradiol has a positive feedback effect to increase GnRH neuron firing. In OVX mice, CRH treatment (100 nM) did not affect the firing rate of GnRH neurons (con 0.90±0.46 Hz, CRH 0.84±0.59 Hz, n=6, $p>0.05$). In contrast, CRH decreased GnRH neuron firing activity in OVX+E mice with 6/9 cells responding to CRH as defined by a more than 25% reduction in firing rate from the control period (con 0.77±0.39 Hz, CRH 0.25±0.14 Hz, n=6, $p<0.05$). Inhibition by CRH is reversible as firing rates returned towards their control frequency after washout (wash period 0.51±0.37 Hz, n=6, $p>0.05$ compared to CRH). Preliminary data revealed pretreatment with 200 nM astressin, an antagonist that blocks both type 1 and 2 CRH receptors, prevented the suppressive effect of CRH on GnRH neurons (con 1.48±0.77 Hz, astressin 1.62±0.75 Hz, and astressin+CRH 1.41±0.84 Hz, n=3, $p>0.05$) suggesting that the effect of CRH on GnRH is mediated through CRH receptors. These results indicate that CRH inhibits GnRH neuron activity and that estradiol is required for CRH to exert this inhibitory effect. Further studies will aim to understand the specific neurobiological mechanisms of CRH effects on GnRH neurons by testing direct action on GnRH neurons and indirect action via modifying upstream neuronal input to GnRH neurons.

Disclosures: C. Phumsatitpong: None. **S.M. Moenter:** None.

Poster

060. HPG Axis I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 60.18/KK13

Topic: F.03. Neuroendocrine Processes

Support: CAPES (Brazilian agency)

Fapesp (Brazilian agency)

Title: Study about the modulation of estrogen and food condition on the interaction of leptin and nitric oxide to control LH and FSH secretion.

Authors: *L. OLIVEIRA[1], C. RODRIGUES FRANCI[2];
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Abstract: Leptin is a mediator of interaction between the mechanisms to control the reproductive function and the energy balance. However, their receptors are not express in neurons that produce gonadotropin-releasing hormone (GnRH) in the hypothalamus, indicating probably an indirect action through interneurons. Among likely neurons that modulate the secretion of GnRH neurons are NO (nitric oxide) neurons. Our aim was determine whether this interaction between leptin and NO, modulated by estrogen and/or food condition, may influence the secretion of Luteinizing Hormone (LH) and Follicle-stimulating Hormone (FSH). **Methods:** Wistar rats were subject to ovariectomy and stereotaxic implantation of intracerebroventricular (icv) cannula, a week before the experiment. From the fifth day, they were treated with estradiol cypionate (10 μg / rat) or vehicle (vegetable oil, 0.1 ml / rat). One group was kept fasting for 48 hours before the experiment and the other normally fed. On the day of experiment, the animals received icv microinjection of L-NAME (500μg/1μl; Sigma) or vehicle, and an hour later received isotonic saline (control) or leptin (3μg/1μl) icv microinjection. After two hours, they were decapitated to collect the blood that was centrifuged, and the plasma was separated and stored at -20ºC to measure hormones by radioimmunoassay. **Results:** The LH and FSH plasma concentration in fasting animals is lower than the fed group and leptin treatment restored and increased this content, respectively. However, the L-NAME treatment prevented this restoration and increased, highlighting the importance of NO. In control animals there was a higher plasma concentration compared to the groups treated with L-NAME in animals fed and fasted, and lower compared to the group fed leptin while in fasting was no significant difference, showing that probably in fasted there is another factor acting in the HPG axis, maybe NPY. In the estrogen-treated groups, the LH plasma concentration was higher than those treated with oil and FSH was lower, that can be explained by the estrogen negative feedback effect.

Disclosures: L. Oliveira: None. **C. Rodrigues Franci:** None.

Poster

060. HPG Axis I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 60.19/KK14

Topic: F.03. Neuroendocrine Processes

Support: Marsden Grant 14-UOO-077

Title: Development of gabaergic altered brain wiring and plasticity in a mouse model of polycystic ovary syndrome (pcos)

Authors: *M. S. SILVA, M. PRESCOTT, R. CAMPBELL;
Physiol., Univ. of Otago, Dunedin, New Zealand

Abstract: Polycystic Ovary Syndrome (PCOS) is the most common neuroendocrine disorder resulting in female infertility and is typically associated with hyperandrogenism. Disruptions in brain circuits regulating Gonadotropin Releasing Hormone (GnRH) neurons are hypothesized to underlie some of the pathophysiological features of this disease. Specifically, increased GABAergic innervation of GnRH neurons has been identified in a prenatally androgenized (PNA) mouse model of PCOS. The present study aimed to determine when these circuit abnormalities develop and whether they can be modified by androgen blockade in adulthood. GABA inputs onto GnRH neurons were evaluated in brain sections collected from prepubertal [postnatal day (PND) 25], control and PNA GnRH-GFP female mice. Vesicular GABA transporter (VGAT) appositions to GnRH neurons (10-12 neurons/animal) were quantified using immunofluorescence and confocal microscopy. Prepubertal PNA mice (n=5), lacking any rise in plasma testosterone levels, presented significant enhanced GABAergic input to GnRH neurons compared to controls (n=4). Adult PNA and control females were treated with flutamide (25 mg/kg), an androgen receptor antagonist, or an oil vehicle for 20 days, from PND 40 to PND 60. As expected, VGAT appositions were significantly increased in PNA+oil (n=5) animals compared with control+oil (n=4) or control+flutamide (n=4) groups. In contrast, VGAT contact onto GnRH neurons in PNA+flutamide (n=7) females was restored to control levels and this was coincident with restored estrous cyclicity. These findings indicate that disruptions in the GABA-GnRH network in a PCOS-like condition are programed before the onset of puberty. However, blockade of androgen actions can ameliorate GABA-GnRH circuit abnormalities. These results provide important insights into the development and treatment of conditions such as PCOS that are evoked by prenatal androgen exposure.

Disclosures: **M.S. Silva:** None. **M. Prescott:** None. **R. Campbell:** None.

Poster

060. HPG Axis I

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Topic: F.03. Neuroendocrine Processes

Support: NIH Grant K12GM074869

NIH Grant NS073574

Title: δ-$GABA_A$ receptors protect against the adverse effects of stress on reproductive function

Authors: *L. C. MELON[1], J. MAGUIRE[2];
[2]Neurosci., [1]Tufts Univ. Sch. of Med., Boston, MA

Abstract: Stress has significant negative impacts on reproductive function. About 30% of women experiencing impaired fecundity are diagnosed with functional hypothalamic amenorrhea (FHA), or secondary amenorrhea due to metabolic, physical or psychological stress. The mechanisms underlying attenuation of Gonadotropin releasing hormone (GnRH) activity following these stressors are unknown. GnRH neurons express functional $GABA_A$ receptors ($GABA_A$ R) and pharmacological modulation of $GABA_A$ R has long been shown to disrupt gonadotropin release, ovulation and other reproduction related events. A recent report (Bhattari et al., 2011) supports the functional regulation of GnRH neurons by extrasynaptic δ containing $GABA_A$ R (δ$GABA_A$ R). As this $GABA_A$ R subtype is particularly sensitive to stress-associated neurosteroids, we sought to determine whether δ$GABA_A$ R played an essential role in communication between the stress and reproductive axes. We developed a GnRH specific δ$GABA_A$ R KO mouse (GnRH-δ KO) to investigate if this Cre-mediated deletion of δ$GABA_A$ R in GnRH neurons would affect sensitivity of the reproductive system to psychogenic stress. This manipulation did not alter pubertal onset in these females, as there was no difference in the age of vaginal opening or first estrous for WT and GnRH-δ KO females. Baseline estrous cycling was monitored for 21 days, with GnRH-δ KO females displaying a marginally longer cycle length. Though GnRH-δ KO females displayed a shorter time to first litter than WT, this may be due to shorter time to plug (receptivity to male etc.), as time to 2nd litter was similar across genotypes. To determine the effects of stress on the fecundity of these females, animals were put in restraint tubes for 30 minutes daily for 21 days. Interestingly, WT females showed no change in estrous periodicity following stress, whereas GnRH-δ KO mice displayed an increase in cycle length. Analysis of corticosterone levels at the end of this stress period suggests neuroendocrine adaptation may have occurred for both genotypes, as cort following the 21st restraint session were similar to basal levels. Following this period of chronic stress, females were paired with WT studs. Stress reduced the number of litters born and pups per litter for GnRH-δ KO females only. These data suggest that δ$GABA_A$ R my not be integral for baseline activity of the reproductive system yet may protect GnRH neurons from the adverse effects of stress on reproductive function. Future work will determine a role for δ$GABA_A$ mediated tonic inhibition in sensitivity of GnRH neuron activity to stress hormones. These findings could identify novel targets for the treatment of stress-associated infertility.

Disclosures: **L.C. Melon:** None. **J. Maguire:** None.

Poster

060. HPG Axis I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 60.21/KK16

Topic: F.03. Neuroendocrine Processes

Support: NIH Grant HD042634

Title: The rescue of declining GnRH neurons in FGF signaling-deficient mice is associated with changes in diverse gene expression

Authors: *P.-S. TSAI, S. I. KAVANAUGH, C. D. LINK, P. K. GONZALES, L. R. BROOKS; Dept of Integrative Physiol., Univ. of Colorado, Boulder, CO

Abstract: Vertebrate neuroendocrine systems respond dynamically to environmental cues to enhance adaptation and survival. Consistent with this notion, a defective postnatal gonadotropin-releasing hormone (GnRH) system can respond positively to environmental cues to restore the organism's declining reproductive function. Transgenic mice with GnRH neuron-specific deficiency in fibroblast growth factor (Fgf) signaling exhibited significant age-dependent decline in GnRH neurons and gonadal functions. However, if they were housed with opposite-sex (OS) littermates and allowed the opportunity for sexual interaction, their GnRH neurons and downstream gonadal functions were restored to normal levels. In this study, we used RNA-seq to interrogate genes that may mediate this experience-dependent restoration of the GnRH system. A total of 1,485 genes in the preoptic area (region housing GnRH neurons) were differentially expressed between same-sex (SS) and OS-housed transgenic mice. The upregulated genes include growth factors that are likely to be neurotrophic to GnRH neurons (Igf1, Igf2, Bdnf, Fgf10) and an R-type calcium channel excitatory to GnRH neurons (Cacna1e). The downregulated genes include Msx1, a GnRH transcriptional repressor and Nhlh2, a basic helix-loop-helix factor with a transcriptional role in GnRH neurons. These gene expression changes have largely been confirmed by quantitative PCR, and some exhibit significant sex differences. In sum, we have identified an array of genes whose expression is altered by sexual experience and may participate in the restoration of a suboptimal GnRH system. These results strongly support the extraordinary plasticity of the postnatal GnRH system. (Supported by NIH HD042634)

Disclosures: P. Tsai: None. **S.I. Kavanaugh:** None. **C.D. Link:** None. **P.K. Gonzales:** None. **L.R. Brooks:** None.

Poster

061. Oxytocin and Vasopressin

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 61.01/KK17

Topic: F.03. Neuroendocrine Processes

Support: NIH Grant HD049651

Title: Activation of KNDy neurons triggers hot flashes in mice

Authors: *A. A. KRULL[1,2,3], S. L. PADILLA[4,5], S. A. LARSEN[1], C. W. JOHNSON[3,4], A. J. CABRERA[1], J. BLAZIER[1], J. A. CORREA[1], R. D. PALMITER[4,5], D. K. CLIFTON[1], R. A. STEINER[1,2];
[1]Ob/Gyn, [2]Physiol. & Biophysics, [3]Neurosci. Grad. Program, [4]Biochem., [5]Howard Hughes Med. Inst., Univ. of Washington, Seattle, WA

Abstract: Hot flashes are vasomotor disturbances that create a perception that the body is over-heated and cause facial flushing and sweating. Menopausal hot flashes occur in response to a decline in plasma levels of estradiol (E2), which disrupts thermoregulatory centers in the brain. In response to reduced E2, estrogen-sensitive KNDy neurons (neurons expressing kisspeptin, neurokinin B, and dynorphin) in the infundibular/arcuate nucleus become super-activated—and may be the generator for hot flashes. We tested the hypothesis that KNDy neurons drive hot flashes in two ways. First, we assessed the ability of neurokinin B (NKB, one of the neurotransmitters expressed in KNDy neurons) to induce thermoregulation via vasodilation and cold-seeking behavior. We found that the NKB agonist senktide caused mice to migrate to a position on a thermal gradient 7.22 ± 1.49 ºC cooler than the position chosen by vehicle-treated animals. Also, senktide treatment elevated tail skin temperature by 4.35 ± 0.74 ºC compared to vehicle. Second, we experimentally activated KNDy neurons and studied the effects on adaptive thermal behavior and tail skin blood flow. Specific activation of KNDy neurons was accomplished by transducing *Kiss1*^{Cre} neurons in the arcuate hypothalamus with a virus containing a stimulatory DREADD receptor (AAV1-DIO-hM3Dq:mcherry). hM3Dq-expressing KNDy neurons can be activated by administration of the designer ligand, CNO. We found that CNO administration caused the animals to move to a position on a thermal gradient 4.18 ± 1.27 ºC cooler than the position they maintained after receiving vehicle alone, and elevated tail skin temperature by 2.75 ± 0.86 ºC. These observations suggest that acute activation of KNDy neurons in mice creates a sense of excessive warmth— a *murine* hot flash— causing the animal to behaviorally seek a cooler ambient temperature and exhibit adaptive peripheral vasodilation. We conclude that activation of KNDy neurons is likely the proximate source of menopausal hot flashes and that therapeutic measures to block KNDy neuronal signaling in women who suffer from hot flashes could prevent or attenuate these unpleasant vasomotor symptoms.

Disclosures: **A.A. Krull:** None. **S.L. Padilla:** None. **S.A. Larsen:** None. **C.W. Johnson:** None. **A.J. Cabrera:** None. **J. Blazier:** None. **J.A. Correa:** None. **R.D. Palmiter:** None. **D.K. Clifton:** None. **R.A. Steiner:** None.

Poster

061. Oxytocin and Vasopressin

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 61.02/KK18

Topic: F.03. Neuroendocrine Processes

Support: Florida State University

Title: Postnatal oxytocin production in infant mice

Authors: ***R. VAIDYANATHAN**, E. HAMMOCK;
Psychology, Florida State Univ., Tallahassee, FL

Abstract: Oxytocin (OXT) signaling through the oxytocin receptor (OXTR) facilitates species-typical social behavior and social recognition of a familiar conspecific. $OXT^{-/-}$ and $OXTR^{-/-}$ mice have similar social deficits. OXT production and OXTR expression in perinatal development are both sex-dependent in mice, with females showing earlier onset of OXT production and higher expression of *Oxtr* in the perinatal brain. Further, neonatal OXT manipulations have sex-specific effects, which may be influenced by underlying sex differences in OXT production and release, and/or sex differences in *Oxtr* expression. Because OXTR activation can promote OXT release, we hypothesized that it might also serve to enhance OXT production during development. In our experiments, we tested the hypothesis that congenital loss of OXTR would impair the development of OXT production in neonatal C57BL/6J mice in a sex-specific manner. In this study we describe a sexually dimorphic effect of OXTR inactivation on OXT production. Our preliminary results show that at postnatal day 8, male OXTR knockout mice but not female knockout mice, show a 50% reduction in *Oxt* mRNA levels compared to WT animals determined by qPCR. This further demonstrates that the development of OXT/OXTR signaling is sex-specific and suggests sex differences in the experience-dependent development of the OXT system.

Disclosures: **R. Vaidyanathan:** None. **E. Hammock:** None.

Poster

061. Oxytocin and Vasopressin

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 61.03/LL1

Topic: F.03. Neuroendocrine Processes

Support: The Florida State University

Title: Neural activation of oxytocin receptor expressing circuits during pre-weaning development.

Authors: *M. TABBAA, E. A. D. HAMMOCK;
Florida State Univ., Tallahassee, FL

Abstract: Oxytocin (OXT) regulates species typical social behaviors. Emerging data indicate that OXT interacts with early life experience to influence behavioral development and adult social phenotype. In an effort to further inform how early experience and central OXT signaling affect neural activation and subsequent brain development, we examined the neural response to oral administration of OXT during a sensitive period of cortical development that coincides with peak transient oxytocin receptor (OXTR) expression in the cortex of mice. OXT or vehicle were administered orally to transgenic EGFP:OXTR reporter mice, both males and females, on postnatal day (PND) 14 or PND 21. Two doses of OXT were chosen: a low dose based on the concentration of OXT the offspring would experience from the dam's breast milk and a high dose analogous to concentrations used intranasally in human clinical trials. Animals were perfused 90 minutes after dosing. Immunohistochemistry revealed dense EGFP staining in cortical layers II/III in PND 14 OXTR:EGFP transgenic mice, consistent with our previous OXTR radioligand binding data, as well as in expected brain regions known to express OXTR such as the olfactory bulb, piriform cortex, amygdala, nucleus accumbens, hippocampus, and hypothalamus, among others. As expected, these patterns of EGFP expression were not seen in mice negative for the EGFP transgene. Furthermore, immunostaining for c-Fos revealed dense neuronal activation, including activation of OXTR-EGFP positive cells in several brain regions. These data will identify neural circuits activated by OXT received during maternal-offspring interactions or clinical treatment during development.

Disclosures: **M. Tabbaa:** None. **E.A.D. Hammock:** None.

Poster

061. Oxytocin and Vasopressin

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 61.04/LL2

Topic: F.03. Neuroendocrine Processes

Support: Florida State University

Title: Oxytocin receptor in peripheral tissues of the neonatal mouse

Authors: *M. A. GREENWOOD, E. A. D. HAMMOCK;
Psychology, Program in Neurosci., Florida State Univ., Tallahassee, FL

Abstract: Oxytocin (OXT) is an integral part of the neural regulation of social behavior across mammalian species, with a prominent role in maternal behaviors. Maternal OXT acts within the mother to transform her behavior and physiology to be able to deliver, nourish, and nurture her offspring. OXT is found in maternal peripheral fluids such as amniotic fluid, saliva, and breast milk. Is it possible that these maternal sources of OXT can be detected by OXT receptors (OXTR) located in the infant periphery? The aim of this project was to assess peripheral sites of OXTR for potential genotypic and sex differences in the newborn mouse. Receptor autoradiography was performed on 20μm sagittal sections of whole neonatal (PD 0) male and female C57BL/6J mice using the ^{125}iodinated-ornithine vasotocin ([^{125}I]-OVTA) radioligand. A competition binding assay was used to assess the selectivity of [^{125}I]-OVTA for peripheral OXTR. Radioactive ligand (0.05nM [^{125}I]-OVTA) was competed against concentrations of 0nM, 10nM, and 1000nM excess unlabeled OXT. Neonates with a genetic deletion of the OXTR (OXTR KO) were also used as control tissue to determine signal specificity. Autoradiographs demonstrated the high selectivity of the radioligand for infant peripheral OXTR. OXTR were identified in the oronasal cavity, ciliary bodies of the eye, whisker pads, skin, adrenal gland, and anogenital region in the OXTR WT mouse, but were absent in OXTR KO. Nonspecific binding that could not be fully competed away with unlabeled OXT was seen in areas with a high lipid content such as the scapular brown adipose tissue and the liver. In addition, male OXTR KO mice displayed evidence of hyperadiposity in these regions. Collectively, these data confirm OXT targets in the periphery of the neonate and suggest a role for OXT in modulating peripheral sensory inputs and contact-dependent social development.

Disclosures: M.A. Greenwood: None. **E.A.D. Hammock:** None.

Poster

061. Oxytocin and Vasopressin

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 61.05/LL3

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Bridges to the Baccalaureate Program

Title: Sexually dimorphic oxytocin receptor expressing neurons in the hypothalamus

Authors: R. LEBLANC[1,2], K. SHARMA[3], M. HAQUE[3], K. NISHIMORI[2], *R. TERUYAMA[3]; [1]Baton Rouge Community Col., Baton Rouge, LA; [2]Grad. Sch. of Agr. Sci., Tohoku Univ., Sendai, Japan; [3]Dept of Biol. Sci., Louisiana State Univ., Baton Rouge, LA

Abstract: Oxytocin (OT) is a neurohypophysial hormone produced primarily in the magnocellular neurons of the paraventricular and supraoptic nuclei in the hypothalamus. OT plays an important role in reproductive physiology by inducing contractions of the uterus during parturition and the mammary gland during milk ejection. Recent studies revealed that OT also has important roles in social and reproductive behaviors, such as maternal and pair bonding. OT induces these behaviors by binding to OT receptors (OTRs) in various parts of the brain; however, the cellular characterization and distribution of OTR expressing neurons is not well described. Furthermore, differences in male and female reproductive behaviors suggest the presence of a sexually dimorphic distribution of OTRs in the brain. The present study was conducted to find sexually dimorphic OTR neurons in mice brains using OTR-reporter mice in which a part of the OTR gene was replaced with Venus, a variant of the yellow fluorescent protein, cDNA (OTR-Venus mouse). We found that a group of Venus cells that was exclusively expressed in the ventro-medial preoptic nucleus (VLPO) and the anterior region of the periventricular hypothalamic nucleus (Pe) in females, but not in these areas of males. Many of these Venus cells were bipolar neurons with thick processes extending into the ependymal layer of the third ventricle. To validate that the Venus cells express OTR, single-cell RT-PCR was performed on individually collected Venus cells dissociated from the hypothalamus of heterozygous Venus female mice. OTR mRNA was detected from Venus cells, suggesting that OTRs are expressed in Venus neurons in these mice. Because of the sexual dimorphic nature of the Venus neurons in the anterior Pe and VLPO, the presence of the female sex hormone receptor, estrogen receptor alpha (ERα), was evaluated by immunocytochemistry. All Venus neurons in the anterior Pe and VLPO were also immunoreactive to ERα. To support the hypothesis that OTRs neurons in the female anterior Pe and VLPO are regulated by estrogen, a group of OTR-Venus mice were ovariectomized and the presence of Venus cells in these regions was evaluated. Ovariectomy caused significant reduction in the number of Venus cells in these regions. Furthermore, estrogen replacement therapy restored the population of the Venus cells in

ovariectomized mice. These findings strongly suggest that OTR neurons in the anterior Pe and VLPO are sexually dimorphic and the expression of OTR is regulated by estrogen.

Disclosures: **R. LeBlanc:** None. **K. Sharma:** None. **M. Haque:** None. **K. Nishimori:** None. **R. Teruyama:** None.

Poster

061. Oxytocin and Vasopressin

Location: Halls B-H

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Program#/Poster#: 61.06/LL4

Topic: F.03. Neuroendocrine Processes

Support: NIH Grant HD084362

NIH Grant HD060117

Title: Oxytocin receptor distribution in the prairie vole (Microtus ochrogaster) neocortex

Authors: ***A. M. SEELKE**[1], A. DUCHEMIN[3], T. C. SIMMONS[1], S. FREEMAN[2], K. L. BALES[1];
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Abstract: The neuropeptide oxytocin (OT) is involved in the processes of labor and lactation, as well as social behaviors including the formation of pair bonds in prairie voles (*Microtus ochrogaster*). Prairie voles are socially monogamous and biparental rodents that exhibit well-characterized complex social behaviors, many of which are mediated by OT. The distribution of OT receptors in the prairie vole brain has been well documented in subcortical regions, including the limbic system and hypothalamus, however, the distribution of OT receptors in the neocortex has never been examined. He we describe the distribution of OT receptors through different regions of the neocortex. Subjects were 5 male and 5 female prairie voles. Their brains were removed, sectioned, and autoradiography for OT receptors was performed on the tissue. We measured the relative density of OT receptors in the nucleus accumbens (NAcc), primary somatosensory cortex (S1), primary auditory cortex (A1), primary motor cortex (M1), parietal association area (PAA), temporal association area (TAA), insular cortex (Ins), and limbic areas of the cortex (Lim). OT density was compared across areas and sexes using ANOVA. We found no significant sex differences, so all subjects were analyzed together. As expected, the NAcc exhibited high OT receptor density, but surprisingly, this value did not significantly differ from OT receptor density in the Ins, Lim, or Taa. The Paa and M1 exhibited significantly lower OT

receptor density, and the OT receptor density in A1 was lower still. Finally, the density of OTR in S1 was significantly lower than in all other areas. These results demonstrate a non-homogenous distribution of OT receptors throughout the cortex, with the highest density in association areas and the lowest density in primary sensory and motor areas. Oxytocin has recently been implicated in the induction of multisensory plasticity in the neocortex, and the high density of OT receptors in association cortex may be the force driving those effects.

Disclosures: **A.M. Seelke:** None. **A. Duchemin:** None. **T.C. Simmons:** None. **S. Freeman:** None. **K.L. Bales:** None.

Poster

061. Oxytocin and Vasopressin

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 61.07/LL5

Topic: F.03. Neuroendocrine Processes

Support: NIH T90 DE021990-02

R01 MH104641

Title: Oxytocin receptor activation depolarizes interneurons and induces GABA release in the rat olfactory cortex

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Abstract: Aberrant central oxytocin (OXT) signaling is implicated in a variety of neurodevelopmental disorders, including autism spectrum disorder (ASD), and is known to be involved in the modulation of complex social behaviors such as fear, mood, affection, and affiliation. However, relatively little is known about the physiology of central OXT signaling outside the hypothalamus (where OXT-synthesizing neurons reside). The olfactory cortex of rodents express OXT receptor (OXT-R), receives axonal projections from OXT-synthesizing neurons of the periventricular nucleus (PVN), and OXT release in this area is known to enhance social recognition in rodents. Whole-cell patch-clamp recordings reveal a subpopulation of OXT-responsive fast-spiking interneurons in the olfactory cortex which are directly depolarized by brief exposure to the OXT-R agonist TGOT. We further characterize these neurons electrophysiologically, morphologically, and immunohistochemically. We also report the same application of TGOT enhances spontaneous inhibitory post-synaptic currents (sIPSCs) onto adjacent pyramidal neurons. Together, these findings reveal a subpopulation of fast-spiking

interneurons as key components involved in the oxytocinergic modulation of neurotransmission in the olfactory cortex. These data represent a significant step toward better understanding how OXT modulates the flow of information in cortical networks to influence complex behaviors.

Disclosures: S.W. Harden: None. **C.J. Frazier:** None.

Poster

061. Oxytocin and Vasopressin

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 61.08/LL6

Topic: F.03. Neuroendocrine Processes

Support: PAPIIT 216214

CONACYT-CB-238744

CONACYT-CB-176919

Title: Hypothalamic vasopressinergic innervation to amygdala: functional implications in anxiogenesis.

Authors: *V. S. HERNANDEZ[1], O. R. HERNÁNDEZ-PEREZ[2], L. ZHANG[1];
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Abstract: The magnocellular hypothalamic vasopressin (AVP) containing neurons are known for their role in the hydro-electrolytic balance control via its projections to neurohypophysis. We have recently demonstrated that these neurons possess multi-axonal processes projecting to non-neurohypophyseal limbic regions including medial (MeA) and central amygdala (CeA). The amygdala is a complex structure involved in anxiety and fear processing where vasopressin immunopositive fibers have long been observed. However, the origin, distribution, co-localization with classical neurotransmitters, and behavioral consequences of their activation remain unclear. To assess these issues, we performed a series of experiments: immunohistochemistry (IHC), application of retrograde tracers Fluoro-gold (FG) into amygdala, electron microscopy, in-situ hybridization (ISH) for vasopressin mRNA, behavioral tests for anxiety under pharmacological manipulation, and Fos quantification. AVP-IHC showed that the MeA was the most densely AVP inervated region (+++) followed by the rostral part of the CeA (++).The posterior CeA and baso-lateral amygdala (BLA) showed only a sparse innervation (+). A detailed anatomical charting of AVP fiber distribution in amygdala will be presented. ISH experiment showed presence of sparse AVP mRNA expression in the MeA and the bed nucleus

of stria terminalis (intra-amygdaloid division BSTIA). Fibers of two diameters were clearly observed. Using IHC, we observed co-localization of vGlut2 with AVP in thick fibers and of GAD65-67 with AVP in the thin fibers. FG retrograde tracing from MeA-CeA resulted in numerous AVP+ magnocellular neurons labelled with FG in both hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei. Water deprivation for 24 hr (WD24) is known to potently up-regulate the hypothalamic vasopressinergic system with only a mild increase of plasmatic osmolarity. We used the elevated plus maze (EPM) to assess anxiety-like behavior. WD24 rats showed a reduced percentage of time spent in open-arms of the maze, from 28% ± 3.56% to 15.91% ± 1.67% (n=5, $p<0.01$). Bilateral microinjection of AVP into the CeA (1ng AVP/250nl/side) produced a similar increase in anxiety behavior (24% ± 2.20% (control group) to 10.47% ± 1.25% (AVP-treated); n=10, <0.001). Both cases were correlated with an increase in the number of neurons expressing Fos in CeA and MeA. Our results suggest that the hypothalamic hydro-electrolytic homeostatic system has a role in the modulation of the emotional behaviors processed by the amygdala.

Disclosures: V.S. Hernandez: None. **O.R. Hernández-Perez:** None. **L. Zhang:** None.

Poster

061. Oxytocin and Vasopressin

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 61.09/LL7

Topic: F.03. Neuroendocrine Processes

Support: MOST 103-2311-B-002-026-MY3

Title: The lateral retinohypothalamic tracts surround the vasopressin neurons in the supraoptic nucleus of hypothalamus

Authors: *P.-C. CHEN[1], C.-T. WANG[1,2,3,4];
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Abstract: The hypothalamic-neurohypophysial system (HNS) secretes two neuropeptides vasopressin (VP) and oxytocin (OT) into the cerebrospinal fluid and peripheral plasma, which is separated by the blood-brain barrier. These two neuropeptides maintain individual homeostasis and contribute to a wide variety of social behaviors. Previous studies showed the retinal ganglion cells (RGCs) in the superior temporal quadrant of the retina can project their axons to hypothalamus through two branches of retinohypothalamic tracts (RHT), medial RHT (RHTm)

and lateral RHT (RHTl). Although RHTm has been extensively studied, the functional role of RHTl remains unclear. RHTl provides a massive input to the lateral hypothalamus area (LHA), i.e, the retinorecipient region of the ventral zone of the rostral region of LHA (LHAavr). This region is a perinuclear zone of the supraoptic nucleus (SON), a hypothalamic component of the HNS, and has been suggested as a source of GABAergic inputs to the SON. Although LHAavr has been reported surrounding the caudal two thirds of supraoptic nucleus (SON), corresponding to the major distribution of VP neurons, the evidence is missing regarding whether the axon terminals of RGCs directly project to VP neurons. Moreover, whether these RHTl projections to the LHAavr display eye-specific segregation remains elusive. To address these questions, we used intravitreal injection of the cholera toxin subunit B (CTB)-anterograde tracer to label LHAavr in 8-week-old Sprague-Dawley male rats. After 3 days, we performed immunostaining to identify the relative distributions of VP and OT neurons in the SON. We found that similar to the dorsal lateral geniculate nucleus (dLGN) and optic chiasm (Och), the LHAavr mainly received the inputs from the contralateral eye. Conversely, the suprachiasmatic nucleus (SCN) that received the projections from RHTm showed a relatively slight contralateral prevalence. Moreover, some of CTB signals closely surrounded the VP immunoreactivity or even localized to the VP immunoreactivity. These results imply the RHTl may involve in regulating vasopressin release.

Disclosures: P. Chen: None. **C. Wang:** None.

Poster

061. Oxytocin and Vasopressin

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 61.10/LL8

Topic: F.03. Neuroendocrine Processes

Title: The spatial topography of oxytocin and vasopressin neurons in the paraventricular nucleus of the hypothalamus in the prairie vole

Authors: *R. J. ORTIZ, A. N. PERRY, B. S. CUSHING;
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Abstract: Extensive research has demonstrated the critical role of the neuropeptides oxytocin (OT) and vasopressin (AVP) in regulating high levels of prosocial behavior, with the prairie vole (*Microtus ochrogaster*) serving as one of the primary model systems. The critical roles of OT and AVP in the regulation of pair bond formation and alloparental behavior in voles were first established over 25 years ago. Despite this, we still know very little about the spatial topography of OT and AVP neurons within the vole brain. This is largely due to an emphasis placed on

species differences in patterns of OT and AVP receptor binding, which can be quite striking. However, the assumption of a conserved organization of OT and AVP neurons in the brain is largely unfounded and mostly superficial. Like other rodents, OT and AVP are primarily synthesized in the paraventricular nucleus of the hypothalamus (PVH) and the supraoptic nucleus (SON). However, unlike the well-characterized rat PVH, the distribution of OT and AVP neurons in the vole PVH is not as well differentiated- much more like the pattern seen in mice. However, we hypothesized that the spatial topography of OT and AVP neurons in the vole PVH would differ from that of mice and rats, due to their unique physiology and prosocial behavior. To test this hypothesis, we used fluorescent immunostaining techniques to characterize the spatial topography of OT and AVP neurons in the same sections throughout the rostrocaudal extent of the vole PVH. While single labeling studies suggest a poorly differentiated organization of the vole PVH, our dual labeling studies revealed a more highly-ordered spatial distribution of OT and AVP neurons. Additional studies are under way to examine functional subdivisions within the vole PVH using retrograde tracers to identify neuronal populations projecting to the posterior pituitary, hypophyseal portal system and brainstem. The results of these and other experiments will provide a more accurate topographical reference for the vole PVH that will facilitate our understanding of how OT and AVP regulate prosocial behavior.

Disclosures: R.J. Ortiz: None. **A.N. Perry:** None. **B.S. Cushing:** None.

Poster

061. Oxytocin and Vasopressin

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 61.11/MM1

Topic: F.03. Neuroendocrine Processes

Support: Autism Research Trust, Oxytocin inhalation project

MRC Studentship to RB

Title: Connectome resilience, the effects of oxytocin administration on functional connectivity during resting-state fMRI in women.

Authors: *R. BETHLEHEM[1], M. LOMBARDO[1,2,3], M.-C. LAI[1,4,6,5], B. AUYEUNG[1,7], J. DEAKIN[8,9], S. SOUBRAMANIAN[8,9], A. SULE[8], S. BARON-COHEN[1,10];
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Psychology, Sch. of Philosophy, Psychology and Language Sci., Univ. of Edinburgh, Edinburgh, United Kingdom; [8]Dept. of Psychiatry, Univ. of Cambridge, Cambridge, United Kingdom; [10]CLASS Clin., [9]Cambridgeshire and Peterborough NHS Fndn. Trust, Cambridge, United Kingdom

Abstract: Background: Numerous studies to date have shown Oxytocin (OT) to be an important modulator of social and emotional behavior. Modulation of cortical-subcortical networks have been hypothesized as a key underlying neural circuit. Few studies to date have used resting-state functional connectivity to assess OT's effect on these networks. None have reported this type of approach in women. The present study used a data-driven approach (graph theory & independent component analyses) to assess potential differences pre- and post acute intranasal oxytocin administration. Specifically, we hypothesized that oxytocin might trigger a general shift from cortical to subcortical processing as evidenced by a change in the connectome modularity structure between conditions.
Methods: In a double-blind placebo-controlled crossover study 25 women received intranasal OT on one, and placebo on another occasion prior to a 10-minute resting-state multi-echo fMRI scan. Data were pre-processed using multi-echo independent component analysis (ME-ICA) that has been shown a robust method for dealing with noise artefacts in rs-fMRI data. Subsequently, functional time-series were parcellated, using an anatomical parcellation and adjacency matrices were constructed from pairwise Pearson correlations. Graph analysis were performed to assess whole brain network properties (transitivity, modularity, efficiency, small-worldness and versatility) and local or nodal properties (degree, nodal efficiency, nodal versatility and betweenness centrality).
Results: Using graph theory analyses we show a moderate shift in modularity structure, where oxytocin drives the split of a new (yet unstable) subcortical module. Overall network properties showed resilience in other measures; small-worldness, transitivity, assortativity and efficiency, under OT conditions. At nodal level there were small differences in nodal degree that showed a partial correlation with a shift towards subcortical areas. However, these nodal differences, albeit significant across network densities, did not survive multiple comparisons corrections.
Conclusions: In sum, these findings indicate the human functional connectome to be resilient against external influences of OT in resting-state conditions. This fits with the increasing awareness that oxytocin's effect on brain dynamics are strongly dependent on context and the specific individual.

Disclosures: R. Bethlehem: None. **M. Lombardo:** None. **M. Lai:** None. **B. Auyeung:** None. **J. Deakin:** None. **S. Soubramanian:** None. **A. Sule:** None. **S. Baron-Cohen:** None.

Poster

062. Stress: Sex Differences

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 62.01/MM2

Topic: F.04. Stress and the Brain

Support: NIH Grant R15 MH100689

Indiana University Provost's Travel Award for Women in Science

Title: Early life stress modulates adult dendritic spine density in basolateral amygdala in a sex-specific manner

Authors: *R. A. SKIPPER[1], K. M. SWANSON[1], C. L. WELLMAN[1], M. R. HERBST[2], J. J. QUINN[2];
[1]Dept. of Psychological and Brain Sci., Indiana Univ., Bloomington, IN; [2]Dept. of Psychology, Miami Univ., Oxford, OH

Abstract: In humans, stress can lead to a host of debilitating outcomes, such as specific phobias, anxiety disorders, and post-traumatic stress disorder. This has been modeled in rodents, with acute stress resulting in enhanced contextual fear conditioning and dendritic retraction in the amygdala. However, little research has been done to understand the effects of early life stress on emotional learning in adults, or whether there are sex differences in this effect. This is problematic, as many stress-related disorders are more prevalent in women than men and emerge early in life. We have previously shown that preweanling stress alters fear conditioning, extinction, and dendritic plasticity in the basolateral amygdala (BLA) of adult rats. Here we show that early life stress also produces long-term changes in dendritic spine density. Male and female Long-Evans rat pups were either stressed or unstressed, with equal numbers of males and females in each group. On postnatal day (PND) 17, stressed rats received 15 mild footshocks; unstressed controls were exposed to the same context without footshock. All then matured without manipulation until adulthood. On approximately PND 90, rats either underwent contextual fear conditioning or remained unconditioned. One day later, all rats were euthanized and brains were stained using Golgi histology. Dendritic morphology and spine densities of BLA pyramidal neurons was assessed. Among unconditioned animals, unstressed females had significantly fewer spines on BLA pyramidal neurons than did unstressed males. Further, females showed a stress-induced increase in dendritic spine density, while males did not. This is consistent with preliminary data from fear conditioned groups suggesting that stressed females but not males show enhanced contextual fear conditioning, but no sex-specific changes in dendritic length or complexity. Thus dendritic spine plasticity may be a key factor mediating sexually dimorphic effects of early life stress on contextual fear learning. These findings are relevant to the effects of early life stress and improper fear regulation among humans, including

individuals with early-developing post-traumatic stress disorder or those recovering from childhood trauma.

Disclosures: **R.A. Skipper:** None. **K.M. Swanson:** None. **C.L. Wellman:** None. **M.R. Herbst:** None. **J.J. Quinn:** None.

Poster

062. Stress: Sex Differences

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 62.02/MM3

Topic: F.04. Stress and the Brain

Title: Sex-dependent effects of isolation rearing in tests of anxiety and depression.

Authors: *D. MUSKIEWICZ, D. JOSHI, F. RESENDIZ GUTIERREZ, N. HALL, F. S. HALL;
Pharmacol., The Univ. of Toledo, Toledo, OH

Abstract: Background: Social isolation of mice after weaning (isolation-rearing) has been suggested to produce a variety of pathological behavioral phenotypes. In particular, it has been suggested to produce behavioral impairments indicative of a hyperdopaminergic state and behavior indicative of reduced serotonin function. Isolation-rearing has been extensively studied; however, most research has ignored sex as a factor. Some data suggests that males and females respond differently to this experience, and may be associated with sex-dependent differences in the propensity to develop certain psychiatric conditions. **Methods**: At the 21 days of age, male and female C57BL/6J mice (N=10 per experimental condition) were housed singly or in groups of 3-4 mice. Subjects remained in these conditions for 8 weeks. At this time, they were subjected to several behavioral tests of anxiety and depression, including the elevated plus maze, open field, light-dark test, and forced swim. **Results**: Sex-dependent effects on some measures of anxiety and depression, but not all measures, were observed in these tests. Most notably, isolation-reared female, but not male, mice spent less time in the center of an open field, indicative of increased anxiety. Isolation-reared female, but not male, mice also spent significantly more time in the closed arms of the elevated plus maze than socially reared female mice. In the light dark test isolation-reared female, but not male, mice had fewer transitions between the light and dark zones. Finally, forced swim results indicate decreases in depressive-like behavior (immobility) in male isolates, but not females, when compared to their social counterparts. **Discussion**: The present experiments found evidence that isolation-reared female, but not male, mice were less active in the center of an open-field, indicative of increased anxiety. By contrast, male isolates, but not females, were shown to have significant reductions in

immobility in a forced swim test, indicative of reduced depressive-like behavior. Collectively these data suggest that early social isolation has distinctly different effects in male and female C57Cl/6J mice, and that the development of anxious phenotypes may be much more pronounced in isolated females, while isolation in males, but not females, may reduce depressive-like behavior. As an animal model of the consequences of early social experience in humans, the present data suggests that early social experience may influence the etiology of anxious and depressive phenotypes in a sex-dependent manner. The mechanisms that might underlie such differences remain to be elucidated.

Disclosures: D. Muskiewicz: None. **D. Joshi:** None. **F. Resendiz Gutierrez:** None. **N. Hall:** None. **F.S. Hall:** None.

Poster

062. Stress: Sex Differences

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 62.03/MM4

Topic: F.04. Stress and the Brain

Title: Sex-dependent effects of isolation-rearing on pre-pulse inhibition of acoustic startle responses

Authors: Y. SABER, *F. S. HALL;
Pharmacol., Univ. of Toledo Col. of Pharm. and Pharmaceut. Sci., Toledo, OH

Abstract: Background: Social isolation of mice after weaning (isolation-rearing) has been suggested to produce a variety of pathological behavior phenotypes. One of the prototypical effects of isolation-rearing, associated with increased dopamine function in the ventral striatum, is impaired pre-pulse inhibition (PPI) of acoustic startle responses. PPI deficits in isolation-reared mice have been thought to model certain aspects of schizophrenia and other frontostriatal disorders. Some data suggests that males and females respond differently to isolation-rearing, resulting in sex-dependent differences in the propensity to develop certain psychiatric conditions. These experiments examined sex-dependent effects of isolation-rearing on PPI. **Methods**: At the age of 21 days of age, male and female C57BL/6J mice (N=10 per experimental condition) were housed singly or in groups of 3-4 mice for 8 weeks. Then, using an SR-LAB (San Diego Instruments, CA) startle system, they were subjected to an initial test to assess startle responses across a range of stimulus intensities, 70 to 130 dB, and a subsequent test of PPI at 3 different PPI values, 3, 6, and 12 dB above background using a startle stimulus of 120 dB and background noise of 65 dB. **Results**: There were small differences in startle responses between isolation-reared and socially reared mice that were slightly larger in males, but not statistically significant

overall. Consistent with many previous observations, reduced PPI was observed to result from isolation-rearing. Differences in PPI were observed only for the lowest pre-pulse intensity (pp3*)*. However, these differences were only observed in isolation-reared male mice, compared to socially reared male mice. Not differences were observed between isolation-reared female mice and socially reared female mice. **Conclusions**: The present experiments found evidence that isolation-reared male, but not female, mice had deficits in sensorimotor gating as assessed by PPI. PPI deficits in isolation-reared mice are thought to be indicative of increased ventrostriatal dopamine function, and reductions in glutamatergic afferents to the ventral striatum. By contrast, other work in our laboratory has shown that female, but not male mice, exhibit behavior indicative of increased anxiety. Collectively these data suggest that early social isolation has distinctly different effects in male and female mice, and that deprivation of certain types of early social experience may contribute differently to the development of mental disorders in males and females. This lays the groundwork for determining the epigenetic mechanisms that might ultimately underlie these effects.

Disclosures: Y. Saber: None. **F.S. Hall:** None.

Poster

062. Stress: Sex Differences

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 62.04/MM5

Topic: F.04. Stress and the Brain

Support: AA021262

Title: Gaba signaling in hippocampus may be modulated by early life stress in a sexually dimorphic manner

Authors: *O. O. KALEJAIYE[1], T. OBISESAN[2], M. C. GONDRÉ-LEWIS[3];
[1]Anat., Howard Univ. Col. of Med., Washington, DC; [2]Howard Univ., Washington, DC; [3]Anat., Howard Univ., Washinton, DC

Abstract: The separation of a maternal figure from its offspring at key neurodevelopmental time points is associated with many long-term behavioral and neurochemical deficiencies that lead to neuropsychiatric disease in adulthood. Our previous studies implicate maternal separation (MS) stress as an underlying risk factor for anxiogenic, depressive, impulsive, and alcohol-drinking behaviors in rats. These behaviors are linked with action at the GABA receptor, and some pharmacological agents that act at the GABA site show behavioral rescue of affective and addictive-like symptoms. In MS, we have shown that baseline levels of GABA receptors are

increased in the amygdala and prefrontal cortex of naïve adult rats. However, the extent to which MS induces GABAergic dysregulation in the hippocampus, a major component of the limbic system, is unknown. Furthermore, the possibility of sexually dimorphic effects of MS on GABA receptor expression, and in GABAergic neuron development and survival is important to investigate. To this end, we analyzed expression of GABAAα1, GABAAα2 receptor subunits, and the GAD67 GABA producing enzyme, in subregions of the P14 hippocampus of rats subjected to chronic MS and their non-separated male and female counterparts. To test if MS induces changes in GABA circuitry long-term, we evaluated the number of GAD67-positive neurons at P70 in the medial prefrontal cortex and whole hipoccampus, but also in specific subregions of rostral and caudal dentate gyrus (DG) and CA3, using immunohistochemistry and unbiased stereological techniques. MS stress resulted in a significant reduction in GABAAα1 but not GABAAα2 or GAD67 in the DG of P14 females. CA1 and CA3 at P14 showed no statistical difference in expression for GABAAα1, GABAAα2, or GAD67. Stereological data imply MS exhibited a significant effect on GABA neurons in the DG. In controls, females had higher numbers and density of GAD67 neurons in the DG compared to males. However, MS caused a reduction in GABA neurons such that the difference between males and females disappeared. Our findings implicate that MS stress may have sexually dimorphic effects at specific time points in neurodevelopment. Such effects may be region specific and may contribute to the neurocognitive deficits and affective behavior of MS animals.

Disclosures: O.O. Kalejaiye: None. **T. Obisesan:** None. **M.C. Gondré-Lewis:** None.

Poster

062. Stress: Sex Differences

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 62.05/MM6

Topic: F.04. Stress and the Brain

Support: NIH Grant R01MH093981-03

NIH Grant 5T32NS007413-17

Title: Age and sex-dependent impact of repeated social stress on rat prefrontal cortical neuronal function and synaptic transmission

Authors: *K. R. URBAN[1], E. GENG[2], M. SUAREZ[3], R. VALENTINO[1];
[1]Gen. Anesthesia, Children's Hosp. of Philadelphia, Philadelphia, PA; [2]Neurobio., [3]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Chronic or repeated stress can lead to the development of psychiatric illnesses including depression, post-traumatic stress disorder, schizophrenia, and anxiety. These disorders are characterized by impaired cognition and are thought to arise from dysfunction in the circuitry of the prefrontal cortex. Previous studies have shown that an acute stressor can enhance glutamate transmission and thereby cognition, whereas chronic stress reduces glutamate transmission and impairs cognition. Notably, the bulk of existing research has been performed on adult, male rats, and has not attempted to elucidate distinct age-dependent or sex-dependent mechanisms of stress effects on cognition. Little is known about how age at the time of stress determines the outcome. In this study, male and female rats were exposed to 5 days of social defeat or control condition during early adolescence (32-38 days old), mid-adolescence (42-48 days old), or adulthood (68-72 days old). Twenty-four hours after the final defeat, 300 μM brain slices were obtained from the rats and excitability of layer 5 pyramidal neurons in the prefrontal cortex were recorded using whole-cell patch clamp. Repeated resident-intruder stress produced age- and sex-specific effects on PFC intrinsic and synaptic excitability. Neuronal excitability evoked by high current intensities was decreased in stressed male and female mid-adolescent rats, whereas neuronal excitability elicited by low current intensities was selectively increased in stressed male mid-adolescent rats. These effects were associated with stress-induced changes in action potential amplitude, half-width, threshold and neuronal input resistance. Additionally, social stress exposure generally decreased synaptic excitability as indicated by a decrease in the amplitude of spontaneous excitatory post-synaptic potentials, although these effects were most prominent in females. Together, the findings demonstrate depressive effects of social stress on intrinsic and synaptic PFC neuronal excitability that occur in adolescents of both sexes and adult females. These neuronal effects may underlie the ability of social stress to impair executive functions such as cognitive flexibility, and suggest a potential neuronal correlate for greater female susceptibility to stress.

Disclosures: **K.R. Urban:** None. **E. Geng:** None. **M. Suarez:** None. **R. Valentino:** None.

Poster

062. Stress: Sex Differences

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 62.06/MM7

Topic: F.04. Stress and the Brain

Support: Migraine Research Foundation (DK, KCB); NS 083010

CDMRP PR130373 (KCB).

Title: Sex, stress, and migraine: differential effects of stress on the migraine relevant responses of males and females

Authors: *D. KAUFMANN[1], K. C. BRENNAN[2];
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Abstract: Introduction: Stress is a common and potent migraine trigger. Two thirds of migraineurs are females, and hormonal fluctuations are also associated with migraine triggering. We wish to examine the interactions of sex and stress in animal migraine models. Methods: Twenty one adult males and twenty two adult female C57Bl/6 mice were randomly divided into chronic stress and control groups. A chronic behavioral stress paradigm was administered randomly, in two daily sessions for forty days. After the end of the stress paradigm anxiety measures were evaluated behaviorally using the open field test and elevated plus maze. Mechanical allodynia was evaluated in both groups before and after administration of 10 mg/kg nitroglycerin (NTG), followed by evaluation of cortical spreading depressions (CSD). Results: Chronically stressed male and female mice displayed anxiety behavior in both the open field and elevated plus maze test compared to their control counterparts. In the mechanical allodynia test chronically stressed males and females had a significantly lower baseline pain threshold compared to controls. Both control and stress males and females showed a significant reduction in pain threshold after NTG administration. Chronically stressed male mice did not show a change in CSD frequency compared to non-stressed controls; however chronically stressed females showed a significant increase in number of CSDs compared to controls. Conclusion: Our work outlines the effects of stress and sex differences on migraine relevant phenotypes. Chronic behavioral stress elicited a pain phenotype in both sexes, but sexually dimorphic responses were observed in cortical excitability. These phenotypes underline the importance of both stress and sex differences in migraine, and open the door for further mechanistic evaluation. Supported by Migraine Research Foundation (DK, KCB); NS 083010, CDMRP PR130373 (KCB).

Disclosures: D. Kaufmann: None. **K.C. Brennan:** None.

Poster

062. Stress: Sex Differences

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 62.07/MM8

Topic: F.04. Stress and the Brain

Support: NIH Grant DA08256

NIH Grant HL098351

NIH Grant HL096571

Title: Sex differences in the subcellular distribution of corticotropin releasing factor 1 in the rat hippocampus in response to chronic stress

Authors: *H. R. MCALINN[1], R. POULTON-KAMAKURA[1], A. G. DYER[1], B. S. MCEWEN[2], E. M. WATERS[2], T. A. MILNER[1,2];
[1]Feil Family Brain and Mind Res. Inst., Weill Cornell Med., New York, NY; [2]Harold and Margaret Milliken Hatch Lab. of Neuroendocrinology, The Rockefeller Univ., New York, NY

Abstract: The rat hippocampus presents sexually dimorphic responses to stress, and to opioids. Our previous studies demonstrated sex differences in the subcellular distribution of corticotropin releasing factor receptors (CRFR1) in hippocampal delta opioid receptor (DOR) dendrites. We recently found that chronic immobilization stress (CIS) impacts the subcellular distribution of DORs in females, but not males, in a manner that would promote drug-related learning. As CRF1 action in the stress response is activational and regulatory, this study examined sex differences in the influence of chronic stress on the subcellular distribution of hippocampal CRFR1. Male and diestrus (low estrogen levels) female rats were subjected to 10 days of CIS, followed by brain perfusion and fixation with acrolein and paraformaldehyde. Hippocampal sections were labeled for CRFR1 with silver intensified immunogold particles (SIG). The subcellular distribution of CRFR1 was analyzed using electron microcopy in dendrites of CA3 pyramidal cells in stratum radiatum and in interneurons in the hilus of the dentate gyrus. At baseline, control animals showed that significantly more CRFR1-SIG particles were on the plasmalemma of male CA3 pyramidal cell dendrites compared to females, suggesting more available CRF binding sites in the males. Moreover, the cytoplasmic and total density of SIG-labeled CRFR1 in hilar interneuron dendrites was greater in males than in females, indicating greater reserve pools of CRFR1 in the males. After CIS, the density of CRFR1-SIGs increased on the plasmalemma of CA3 pyramidal cell dendrites in males but did not change in any cellular compartment of the females. Further, the cytoplasmic and total density of CRFR1-SIGs in CA3 pyramidal cell dendrites was less in males compared to females after CIS. In the dentate hilus, the total density of CRFR1-SIGs decreased in interneuron dendrites in males after CIS but did not change in any cellular compartment in females. As a result, the density of SIG-labeled CRFR1 near the plasma membrane of interneuron dendrites was greater in males than in females after CIS. These results propose that at baseline, CA3 pyramidal cells in males can bind more CRF than females and that in response to CIS, these neurons in males may bind even more CRF. Likewise, at baseline, hilar interneurons in males have more CRFR1 than females and after CIS, CRF1 redistributes in these neurons to arrange for their insert into the plasmalemma. These findings offer a mechanism for the added sensitivity of hippocampal neurons to chronic stress in males that could attenuate learning and memory processes.

Disclosures: H.R. McAlinn: None. **R. Poulton-Kamakura:** None. **A.G. Dyer:** None. **B.S. McEwen:** None. **E.M. Waters:** None. **T.A. Milner:** None.

Poster

062. Stress: Sex Differences

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 62.08/MM9

Topic: F.04. Stress and the Brain

Support: RO1AG043467

Title: Sex differences and dynamics of neuroimmune and neuroendocrine responses to stressors

Authors: *D. LOVELOCK, A. S. VORE, T. DEAK;
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Abstract: Expression of inflammatory cytokines such as interleukin-1β (IL-1β) are impacted by stress exposure and have emerged as key factors regulating stress adaptation. Though our prior studies have examined the IL-1 response across the ovarian cycle in females, sex differences in the neuroimmune response to stress have yet to be examined. The goal of experiment 1 was to establish a timecourse of pro-inflammatory cytokine expression in response to footshock in both sexes. Adult male and female Sprague-Dawley rats (N=64, n=8/group) were exposed to intermittent footshock (1.0 mA, 5 sec each, variable ITI of 90 sec) for 60 or 120 min. Brains, serum, and peripheral tissue (spleen) were collected immediately after stress termination under no-stress conditions or 2 hr after stress cessation to assess functional recovery. As expected, females showed a greater peak CORT response than males, and in both sexes the CORT response was resolved by 2 hr post-stress. Splenic IL-1β was markedly increased at 1 and 2 hr and largely resolved by the recovery timepoint. Notably, no sex differences were observed in this splenic IL-1β response. To better understand adaptation in central cytokine responses to chronic stress, Experiment 2 tested whether prior habituation to a mild stress challenge (60 min of daily restraint for 5 days) would impact the IL-1β response evoked by 30 min of forced swim incurred immediately after the final session of restraint. We examined cytokine expression and indices of cellular activation (c-Fos) in the PVN, PFC, and HPC as well as plasma CORT concentrations using adult male Sprague-Dawley rats (N=40, n=8/group,). In (non-habituated) rats that experienced acute restraint followed by 30 min of swim, CORT was markedly increased, c-Fos expression was robustly induced in all brain regions examined, and IL-1β expression was induced specifically in the PVN (3.5 fold increase). Interestingly, prior repeated daily restraint substantially attenuated IL-1β and c-Fos expression in the PVN, suggesting a significant influence of recent stress history on central cytokine responses to stress. These findings indicate that recent stress history may differentially influence the neuroimmune response to a novel stress challenge in a site- and cytokine-specific manner.

Disclosures: **D. Lovelock:** None. **A.S. Vore:** None. **T. Deak:** None.

Poster

062. Stress: Sex Differences

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 62.09/MM10

Topic: F.04. Stress and the Brain

Title: Sex differences in- and stress effects on- microglial activation in brain regions critical for emotion regulation

Authors: *J. L. BOLLINGER, K. E. COLLINS, R. PATEL, C. L. WELLMAN;
Psychological and Brain Sci., Indiana Univ., Bloomington, IN

Abstract: Susceptibility to stress-linked psychological disorders differs between men and women. Brain regions critical for emotion regulation, including medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC), and basolateral amygdala (BLA) have been implicated in stress-linked psychopathology. Chronic stress can affect these regions in a sex-dependent manner, differentially remodeling neuronal morphology and disrupting behavior. Recent studies indicate a role for neuroimmune pathways and microglia in psychological health and disease. Activated microglia can regulate synaptic plasticity by releasing neuroactive factors and directly pruning dendritic spines. In male rats, stress increases microglial cell activation and inflammatory factor expression in mPFC, and this contributes to deficits in mPFC-mediated behavior. We recently demonstrated a dramatic sex difference in the neuroimmune profile of mPFC, alongside differential effects of stress on microglial activation and immune factor expression. To investigate the generalizability of sex-specific stress effects on microglial activation, we assessed microglial density and morphology in OFC and BLA in male and female rats following acute or chronic restraint stress. Control animals were unhandled except for weighing. On the final day of restraint, brains were processed for visualization of microglia via Iba-1 immunohistochemistry. Microglia were classified as surveillant, primed, reactive, or amoeboid, and counted stereologically. There were no basal sex differences in OFC or BLA. However, chronic stress reduced the proportion of primed to surveillant microglia in OFC in male rats only, suggesting decreased activation in males but not females. Acute and chronic stress reduced the proportion of primed to surveillant microglia in BLA in males, whereas acute but not chronic restraint reduced this proportion in females, indicating a sex-specific response to chronic but not acute stress. Thus, stress differentially alters the morphological activation state of microglia in brain regions critical for emotion regulation in males and females. These differences may contribute to the differential effects of stress on neural structure and function in males versus females.

Disclosures: J.L. Bollinger: None. **K.E. Collins:** None. **R. Patel:** None. **C.L. Wellman:** None.

Poster

062. Stress: Sex Differences

Location: Halls B-H

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Program#/Poster#: 62.10/MM11

Topic: F.04. Stress and the Brain

Support: R21MH099562

R01MH090264

P50 MH096890

NARSAD young investigator award

Title: Sub-chronic variable stress induced sex-specific effects on glutamatergic signaling in the nucleus accumbens

Authors: *G. E. HODES[1], A. BRANCATO[1,2], D. BREGMAN[1], F. H. AHN[1], S. J. RUSSO[1]; [1]Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; [2]Dept. of science for health promotion and Mother and child care, Univ. of Palermo, Palermo, Italy

Abstract: Men and women manifest different symptoms of depression and under current diagnosis depression is a predominantly female disease. Yet little is known of the mechanisms contributing to these important sex differences and how they may impact potential future therapeutics. Sub-chronic variable stress (SCVS) induces depression-like behavior in female mice, modeling females' higher susceptibility to stress and depression. Accumulating evidence indicates that altered neuroplasticity of excitatory synapses in the nucleus accumbens is a key pathophysiological feature of stress susceptibility. Here we investigated the effects of SCVS on pre- and post-synaptic aspects of excitatory synapses in the nucleus accumbens of female and male mice. Animals underwent 6 day-exposure to alternating stressors, including foot shock, tail suspension and restraint stress. Medium spiny neurons from the nucleus accumbens were filled with Lucifer Yellow and spine density and phenotype were examined using the semi automated program neuron studio. In a separate group of animals immunofluorescence staining was performed for vesicular glutamate transporter 1 and vesicular glutamate transporter 2, in order to label cortical and subcortical glutamatergic terminals. Immunostaining for PSD95 was employed to evaluate post-synaptic density. Females, but not males, demonstrated circuit specific pre-synaptic alterations that may contribute to stress susceptibility in the absence of post-synaptic alterations in PSD 95 puncta, spine density and phenotype. Taken together, our data indicate that susceptibility to stress in females, is associated with changes in the strength of distinct glutamatergic inputs to the nucleus accumbens, which contribute to the susceptible phenotype induced by SCVS in female mice.

Disclosures: **G.E. Hodes:** None. **A. Brancato:** None. **D. Bregman:** None. **F.H. Ahn:** None. **S.J. Russo:** None.

Poster

062. Stress: Sex Differences

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 62.11/MM12

Topic: F.04. Stress and the Brain

Support: NSERC 436204-2013

Title: Male experience, female investment and offspring anxiety behavior: where father's nature meets mother's nurture

Authors: ***A. KORGAN**, E. O'LEARY, J. BAUER, A. FORTIER, T. PERROT, I. WEAVER; Psychology and Neurosci., Dalhousie Univ., Halifax, NS, Canada

Abstract: Programming of reproductive strategies and defensive (stress) responses in the adult rat is influenced by early life maternal care. The detection of a potential mate's past experience with predators could inform the dam about the stress level of a potential mate, the likelihood of paternal effects in offspring, and potential ecological threats. We examined whether a male's previous exposure to predator (cat) odor influences a female's attraction toward these males, subsequent mother-infant interactions and the development of long-term emotional (defensive) responses in the offspring. Our results show for the first time that variation among males in their predator encounters may contribute to stable behavioral variation among females in courtship and maternal care, even when the females themselves are not directly exposed to a predator. Furthermore, paternal predator experience is associated with long-term effects on chromatin plasticity and anxiety-like behavior of the young adult offspring. These results, together with our previous findings, suggest that paternal predator exposure can influence offspring development both directly and indirectly, through altering maternal behavior.

Disclosures: **A. Korgan:** None. **E. O'Leary:** None. **J. Bauer:** None. **A. Fortier:** None. **T. Perrot:** None. **I. Weaver:** None.

Poster

063. Cortical Hemodynamics

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 63.01/DP05 (Dynamic Poster)

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NINDS Grant 1 R01 NS063226-06

NINDS Grant 1 R01 NS076628

NHLBI Grant 1 F30 HL128023 01

Title: Endothelial dysfunction diminishes conducted vasodilation leading to abnormal functional hyperemia in the awake mouse cortex

Authors: *M. A. SHAIK[1], S. H. KIM[1], D. N. THIBODEAUX[1], E. M. C. HILLMAN[2]; [1]Biomed. Engin., [2]Zuckerman Mind Brain Behavior Institute, Biomed. Engin. & Radiology, Columbia Univ., New York, NY

Abstract: Functional hyperemia refers to the increase in brain blood flow driven by increased local neuronal activity. Recent studies have provided evidence that endothelium-dependent conducted vasodilation is an active participant in brain neurovascular coupling. Studies in the peripheral vasculature have shown that endothelium-dependent vasodilation mechanisms can include fast, long-range endothelium derived hyperpolarization and slow, calcium-wave dependent nitric oxide and cyclooxygenase product activity. In anesthetized rodents, we have previously shown that localized endothelial disruption can interrupt propagation of endothelium-dependent vasodilation. However, to assess the role of different endothelium-dependent mechanisms on functional hyperemia, it is important to be able to spatiotemporally map changes in neurovascular coupling and assign them to different aspects of endothelial coupling.
To explore different aspects of conducted vasodilation, we developed a new paradigm to image the exposed superficial cortex of awake Thy1-GCaMP6f mice, longitudinally. Mice were implanted with long-term thinned skull cranial imaging windows and head fixation mounts under anesthesia and then allowed to recover prior to repeated imaging sessions. Neural activity was recorded via wide-field fluorescence, while simultaneously recorded hemodynamics were calculated from interspersed dual wavelength reflectance measurements. Animals were recorded in the resting state and during tactile whisker stimulation of different durations. Animal locomotion and behavior were recorded during imaging. To analyze this data, a mathematical model was developed to describe two components of the hemodynamic response - first, local coupling derived by convolving local neuronal activity with an estimated hemodynamic response function, and second, a superimposed conducted hyperemic component, derived from local coupling in a centralized, highly responding region. This analysis enables unbiased analysis of

neural activity, hemodynamics, local and propagated neurovascular coupling properties and the presence of unaccounted for hemodynamics or apparent uncoupling. This methodology allows analysis of local and conducted hyperemic components, and how they are affected by different inductions of endothelial dysfunction, either acute or longitudinal. Here, we demonstrate this approach in animals that have received an acute dose of the non-blood brain barrier-permeable COX-inhibitor ketorolac, finding that it significantly diminishes the local hyperemic response consistent with an effect on COX-dependent endothelial coupling.

Disclosures: **M.A. Shaik:** None. **S.H. Kim:** None. **D.N. Thibodeaux:** None. **E.M.C. Hillman:** None.

Poster

063. Cortical Hemodynamics

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

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Topic: D.06. Vision

Support: NIH R01-EY022157 (ADH)

Pew Charitable Trusts Biomedical Scholar Award (ADH)

McKnight Foundation Scholar Award (ADH)

Title: Genetic mechanisms regulating connectivity and function of non-image-forming parallel visual circuits

Authors: *O. S. DHANDE[1], A. H. PHAN[2], T. A. SEABROOK[3], J. T. WANG[3], P. L. NGUYEN[2], A. D. HUBERMAN[3];
[1]Dept. of Neurobiology, Stanford Univ., Stanford, CA; [2]Neurosci., Univ. of California San Diego, La Jolla, CA; [3]Neurobio., Stanford Univ., Stanford, CA

Abstract: How are parallel visual pathways built and how do their unique connectivity patterns influence visual processing? Retinal ganglion cells (RGCs) encode visual features such as contrast, motion and luminosity and convey that information to the brain in the form of distinct parallel pathways that each connect to unique sets of retinorecipient brain targets. The precise connectivity patterns and signals relayed by parallel optic pathways are critical for eliciting specific visually guided behaviors. Thus, understanding the mechanisms by which these pathways are built and establishing causal relationships between specific RGC subtypes and the behaviors they drive, is an essential but unresolved set of challenges. Using a microarray-based approach we assayed and compared the transcriptional profile of transgenically labeled RGC

subtypes including direction selective, luminance sensing, and alpha RGCs, paying particular attention to the transcription factors uniquely expressed by certain RGCs and not others. We discovered that transcripts for several T-box family transcription factors are highly enriched in intrinsically photosensitive RGCs that comprise the non-image forming (NIF) pathway; these same transcription factors are near absent in other RGC subtypes. Protein expression analysis of the developing retina supported our microarray analysis. We chose to focus on Tbx20, a transcription factor whose function in the nervous system is largely unknown. We generated mutant mice lacking Tbx20 in NIF RGCs using the Cre/loxP system. Loss of Tbx20 in these RGCs resulted in significant reduction in the number of intrinsically photosensitive RGCs and their central projections, specifically those projections that feed centers of the brain involved in driving pupillary light reflex (PLR). To test the functional integrity of the visual system we developed a suite of visual behaviors to probe the consequence of genetically ablating T-box-family-expressing RGC subtypes. To our surprise most behaviors tested including PLR were not significantly altered in Tbx20 mutant mice. However, when we removed Tbx20 together with the gene encoding the photopigment melanopsin, we observed near-complete loss of the PLR. These data suggest that Tbx20 RGCs play a role in driving PLR but their contribution is likely masked by a more dominant driving force from other melanopsin-expressing RGCs. The purpose and mechanisms of this redundancy are the focus of our current work and will be discussed.

Disclosures: O.S. Dhande: None. **A.H. Phan:** None. **T.A. Seabrook:** None. **J.T. Wang:** None. **P.L. Nguyen:** None. **A.D. Huberman:** None.

Poster

063. Cortical Hemodynamics

Location: Halls B-H

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NINDS Grant R01NS085200

NIMH Grant R01MH098003

Title: Mapping functional atlas of the awake rat brain: a resting-state fMRI study

Authors: *Z. MA[1], P. PEREZ[1], Z. MA[1], Y. LIU[1], C. HAMILTON[2], Z. LIANG[1], N. ZHANG[1]; [1]Biomed. Engin., [2]Neurosci. Program, Penn State Univ., University Park, PA

Abstract: Our understanding of human brain organization has been significantly advanced by connectivity-based parcellation approach. With the premise that each functionally specialized

brain region is characterized by a distinct set of connections, connectivity-based parcellation can partition the brain into functionally specialized parcels based on neuroimaging data. The organization of the brain can be further investigated by analyzing the whole-brain network constructed using the parcellation. Despite great advancements in human studies in recent years, the parallel research in animals are still significantly underexplored, which highlights a gap in understanding the organization of the animal's brain using this approach. To surmount this obstacle, in the present study, we obtained a functional atlas by parcellating the brain into functional parcels using resting-state fMRI data collected in awake rats. This functional parcellation exhibited higher averaged parcel homogeneity than the histology-based anatomical parcellation. More importantly, the majority of functional parcels was highly robust, reflected by high reproducibility across animals imaged on the same MRI scanner, or even on different scanners of different field strengths. A rat whole-brain functional network was constructed based on this atlas and then analyzed using graph theoretic approach. Our results showed that the hubs identified in the rat functional brain network are consistent with the hub regions of the rat structural brain network, suggesting the convergence of structural connectivity and functional connectivity in brain hubs. In addition, we found that the rat functional brain network was topologically organized in a similar manner as the human brain, characterized by a balanced brain specialization and integration. The translational value of the present study in terms of comparative functional neuroanatomy was highlighted by the homologous functional hub region of the cingulate cortex and a rich-club organization shared by both the rat and human functional brain networks. Given the high relevance between neuropsychopathology and miswired brain network, this study can also help the establishment of translational animal models for multiple neuropsychiatric diseases.

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Poster

063. Cortical Hemodynamics

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 63.04/NN3

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

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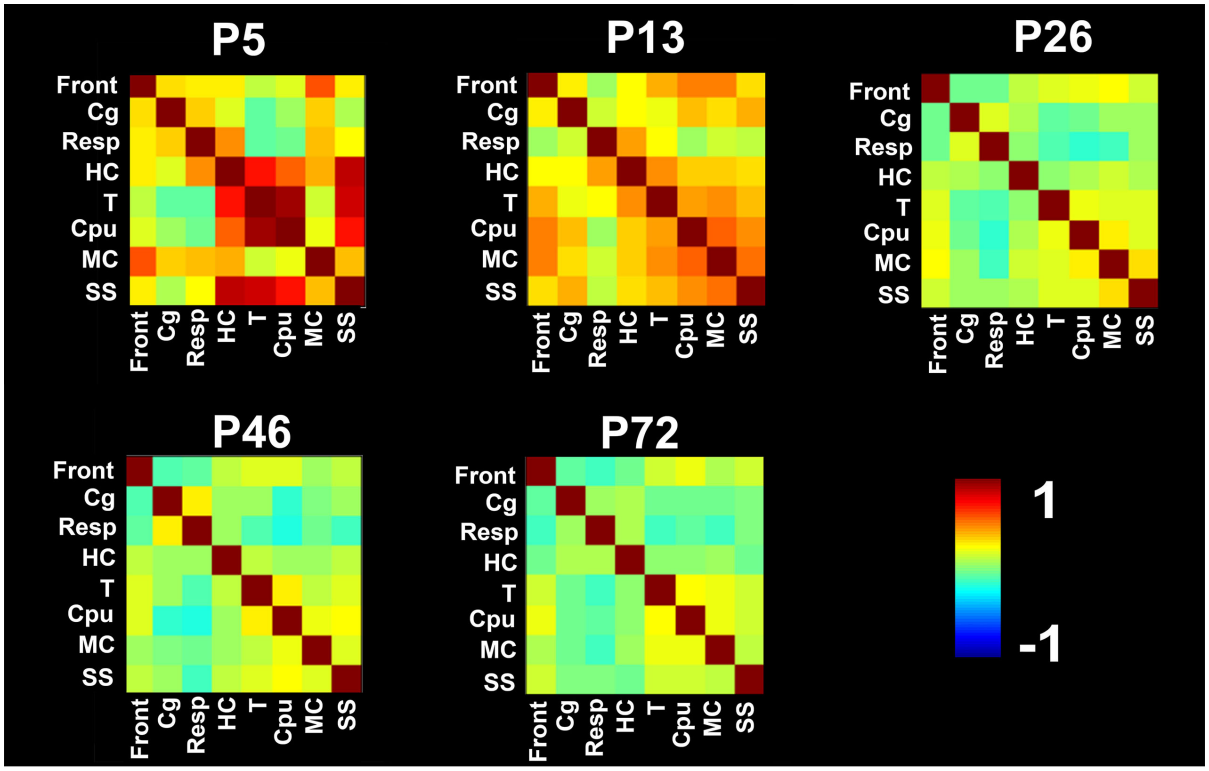
Molecular Imaging of Brain Pathophysiology (BRAINPATH) under grant agreement number 612360

Institute for the Promotion of Innovation by Science and Technology (IWT) under grant agreement 13160

Title: Investigating the development of functional and structural connectivity in the rodent brain during ontogeny

Authors: *D. SHAH, I. BLOCKX, C. ANCKAERTS, J. PRAET, M. VERHOYE, A. VAN DER LINDEN;
Bio-Imaging Lab/University of Antwerp, Antwerp, Belgium

Abstract: Aim: Although much is known about the development and plasticity of afferent pathways in the brain, the ontogeny of interregional connections is relatively unknown and research (optical and electrophysiological techniques) is often hindered by the difficulty of investigating multiple brain regions simultaneously in vivo. In this study, the development of functional and structural brain connectivity was studied using the combination of specific *in vivo* MRI techniques. **Methods:** Brain functional connectivity (FC) and microstructural tissue characteristics were examined in developing healthy wistar rats (ages P5, P9, P13, P17, P22, P26, P31, P36, P46, P56, P72, and P86, N=8/age group), using resting state functional MRI (rsfMRI) and diffusion tensor imaging (DTI), respectively. Functional and structural maturation was investigated in regions of different brain networks i.e. somatosensory, motor, default-mode-like network (DMN), thalamus, striatum and hippocampus. **Results:** At early stages of brain development, i.e. between P5 and P22, FC in brain networks was hypersynchronous and decreased with age, leading to the formation of stable networks starting at P22-P26 (Figure 1). Structural measures (mean, axial and radial diffusion) also stabilized with brain maturation and correlated with functional measures. **Conclusion:** The results of this study show how functional and structural connectivity develops in the maturing brain. In addition, it provides an important application to study various clinical disorders that may be the result of interference with normal ontogeny (e.g., schizophrenia, dyslexia, epilepsy, and autism).



Maturation of functional connections in the developing rat brain. Functional connectivity matrices show hypersynchrony of resting-state neuronal activity patterns at early stages of brain development, which stabilize with maturation. The x- and y-axis represent brain regions. The color scale represents the strength of functional connectivity. Abbreviations: Front=frontal cortex, Cg=cingulate cortex, Resp=retrosplenial cortex, HC=hippocampus, T=thalamus, Cpu=caudate putamen, MC=motor cortex, SS=sensory cortex.

Disclosures: D. Shah: None. **I. Blockx:** None. **C. Anckaerts:** None. **J. Praet:** None. **M. Verhoye:** None. **A. Van der Linden:** None.

Poster

063. Cortical Hemodynamics

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

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Title: A putative multiple-demand system in the macaque brain

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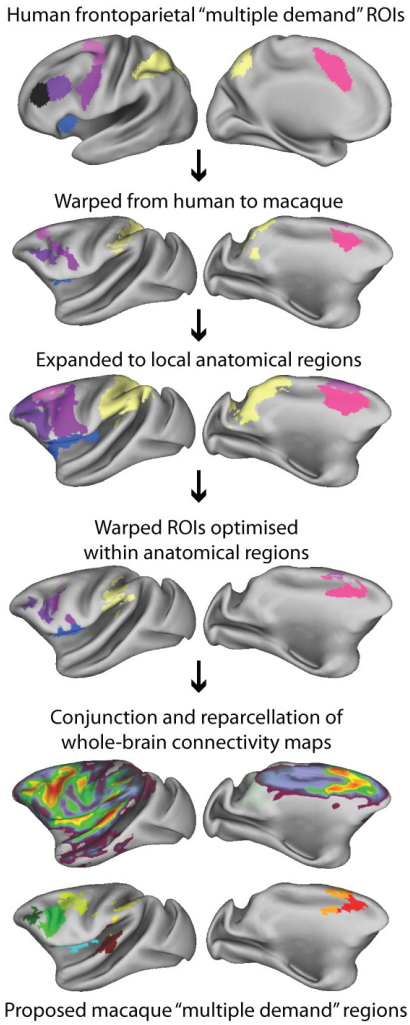
Abstract: In humans, cognitively demanding tasks of many types recruit common frontoparietal brain areas. Pervasive activation of this "multiple-demand" (MD) network suggests a core function in supporting goal-oriented behaviour. A similar network might therefore be expected in non-human primates who readily perform similar tasks after training. However, an MD network in non-human primates has not been described.
Single-cell recordings from macaque frontal and parietal cortex show some similar properties to human MD fMRI responses (e.g. adaptive coding of task-relevant information). Invasive recordings, however, come from limited pre-specified locations so do not delineate a macaque homologue of the MD system, and their positioning could benefit from knowledge of where MD foci lie. Challenges of scanning behaving animals mean that few macaque fMRI studies specifically contrast levels of cognitive demand. We therefore sought to identify a macaque counterpart to the human MD system using fMRI connectivity in 35 anaesthetised Rhesus macaques.
Putative macaque MD regions, mapped from frontoparietal MD regions defined in humans, were found to be functionally connected under anaesthesia. To further refine these regions, an iterative process was used to maximise their connectivity, cross-validated across animals. Finally, whole-brain connectivity analyses identified voxels that were robustly connected to MD regions, revealing seven clusters across frontoparietal and insular cortex, comparable to human MD regions, and one unexpected cluster in the lateral fissure.
The proposed macaque MD regions can be used to guide future electrophysiological investigation of MD neural coding, and in task-based fMRI to test predictions of similar functional properties to human MD cortex.



Disclosures: **D.J. Mitchell:** None. **A.H. Bell:** None. **M.J. Buckley:** None. **A.S. Mitchell:** None. **J. Sallet:** None. **J. Duncan:** None.

Poster

063. Cortical Hemodynamics

Location: Halls B-H

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Program#/Poster#: 63.06/NN5

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: 1 R01 NS076628

1 R01 NS063226-06

UL1 TR000040

Title: Comparison of wide-field optical mapping (WFOM) of neural activity and hemodynamics in awake mouse brain to resting state fMRI

Authors: *S. H. KIM, Y. MA, M. A. SHAIK, V. VOLETI, E. M. C. HILLMAN;
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Abstract: Resting state functional connectivity mapping (FCM) has become an increasingly popular method for human functional magnetic resonance imaging (fMRI). The blood oxygen level dependent (BOLD) signal measured in fMRI corresponds to local changes in deoxy-hemoglobin concentration. FCM is applied to fMRI BOLD data collected from subjects at rest to group brain regions of temporally correlated activity. While it is widely assumed that these BOLD signal maps directly reflect neuronal activity, the relationship between local neuronal activity and hemodynamic activity is not well understood, especially in the absence of stimulus. Interpretation of resting state FCM, therefore, requires a better understanding of the coupling between resting state hemodynamics and underlying neuronal activity.
In recent work, we developed a method for longitudinal wide-field optical mapping of both neural activity and hemodynamics over the superficial cortex of awake, behaving mice. Recordings of neural activity are achieved by macro-scale imaging of C57BL/6J-Tg(Thy1-GCaMP6f)GP5.17Dkim/J mice expressing calcium sensitive fluorescent protein GCaMP6f in excitatory neurons of layers 2/3 and 5. This technique has allowed us to develop model-based correspondence between spontaneous neural activity and resting state hemodynamics in the awake mouse brain. Here, we will describe measurements in which we compare these optical recordings of resting state neural activity and neurovascular coupling with resting state fMRI data acquired under light isoflurane in the same mice. Repeated wide-field optical measurements of the same mouse are achieved through an aseptic thinned-skull and headplate implant preparation. This approach allows precise explorations of the neural basis of fMRI data in health and disease.

Disclosures: S.H. Kim: None. **Y. Ma:** None. **M.A. Shaik:** None. **V. Voleti:** None. **E.M.C. Hillman:** None.

Poster

063. Cortical Hemodynamics

Location: Halls B-H

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Program#/Poster#: 63.07/NN6

Topic: D.06. Vision

Title: EEG dynamics suggest automatic detection of visual changes without attentional switch in hemianopic patients

Authors: *V. HADID[1], A. TRAN[1], D. K. NGUYEN[2], F. LEPORE[3];
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Abstract: Loss of posterior visual cortex in one hemisphere due to surgery or stroke leads to blindness in the contralateral hemifield, known as homonymous hemianopia (HH). Affected patients retain the remarkable ability to unconsciously perceive visual stimuli presented in their blind field, a phenomenon termed blindsight. There is still some controversy about the mechanisms underlying such unconscious vision. In fact, there is no consensus about how this phenomenon can be objectively evaluated. To understand its nature, we measured the cerebral dynamic of blindsight with EEG. An oddball paradigm was used to elicit the visual mismatch negativity (where no attention or consciousness is required) determined by subtracting the waves elicited by the rare stimuli from the frequent stimuli. It is mainly characterized by a negative parietal wave around 150-250ms, followed by a frontal attentional switch at 300ms. To be sure that the control group would not focus on the oddball stimulus, frequent and rare moving dots were presented in periphery while participants carried out a Stroop task in the central visual field. Participants also carried out other control tasks to ensure that the results were not due to other parameters, such as physical attributes. This approach allowed us to measure objectively their ability to automatically detect visual changes presented in the blind field, independently of the "consciousness problem". The results revealed a visual mismatch negativity wave in the control and in the hemianopic group between 165-250 ms that was maximal in the parietal regions, showing an automatic detection of the changes. However, compared to controls, in which the localized negativity was followed by a frontal positivity between 300 and 400 ms, hemianopic patients had a diffuse negativity and no frontal positivity. Moreover, controls showed a desynchronization of the alpha/beta band (8-20Hz) around 250 ms in the frontal areas, whereas patients showed lower desynchronization that was however found only in the low alpha band. Desynchronization of the alpha/beta band reflects a higher attentional demand that can be interpreted in our controls as an upcoming attentional switch, and in our hemianopic group as a lack of attentional capture to visual changes. In conclusion, our results showed a distinctive cerebral dynamic in hemianopic patients that reflects unconscious detection of specific visual changes in the blind field, but a lack of orientation of their attention towards it. In light of these results, rehabilitation should focus on stimulating attentional capacities towards the blind field to make visual information more accessible.

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Poster

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

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NINDS P30 NS-048056

NINDS NS-075321

NINDS NS-41509

NINDS NS-058714,

NIH NCRR UL1RR024992

Title: Abnormal resting state BOLD fMRI lag structure in idiopathic Parkinson disease

Authors: A. MITRA[1], *A. Z. SNYDER[2], M. C. CAMPBELL[1], A. TANENBAUM[3], J. M. KOLLER[3], J. S. PERLMUTTER[4];
[1]Washington Univ. Sch. of Med., Saint Louis, MO; [2]Radiol Dept, [4]Neurol., [3]Washington Univ. Sch. Med., Saint Louis, MO

Abstract: Parkinson disease (PD) is a progressive neurodegenerative disorder leading to motor disability and cognitive impairment. Degeneration of nigrostriatal dopaminergic neurons with consequent dysfunction of neural circuits involving the basal ganglia, thalamus, and cerebral cortex contributes to the clinical manifestations of PD. Prior studies of PD using resting-state blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) have assumed that functional connectivity (FC) is adequately understood in terms of instantaneously correlated activity. These studies have generated a variety of findings, including increased as well as decreased FC in multiple areas of the brain. By relaxing the assumption of synchronicity and focusing on temporal lags between time series, we recently demonstrated highly reproducible patterns of temporally lagged intrinsic activity in normal human adults (Mitra et al., 2014, 2015). We refer to this analysis technique as resting state lag analysis (RS-LA). A previous study of adults with autism spectrum disorder indicated that RS-LA is a much more sensitive marker of intrinsic activity abnormalities than conventional FC (Mitra et al., in press). Here, we report RS-LA as well as conventional FC results in adults with idiopathic PD (n=40; off meds overnight) and demographically matched control participants (n=25). RS-LA analysis revealed highly significant group differences in ventral midline thalamus with extension to the subthalamic nucleus (STN), and striatum. The direction of signal propagation between the

caudate nucleus and specific regions of the cerebral cortex was reversed in PD as compared to controls (cortex->caudate in controls, caudate->cortex in PD). Opposite PD-related changes in lag structure were found between thalamus-STN and specific areas of cerebral cortex. Conventional FC analysis of the same data showed only comparatively modest effects (moderately reduced FC in PD). These results demonstrate that the lag structure of intrinsic brain activity is more altered in PD than the correlation structure computed at zero lag, i.e., convention FC. One implication of these results is that propagated infra-slow activity in the BOLD-fMRI band (nominally, 0.01 to 0.1 Hz) plays an important, although as yet poorly understood, role in normal brain function.

Mitra et al., Cerebral Cortex, in press, doi: 10.1093/cercor/bhv294
Mitra et al., J Neuophysiol 2014, doi:10.1152/jn.00804.2013
Mitra et al., PNAS 2015, doi: 10.1073/pnas.1503960112

Disclosures: **A. Mitra:** None. **A.Z. Snyder:** None. **M.C. Campbell:** None. **A. Tanenbaum:** None. **J.M. Koller:** None. **J.S. Perlmutter:** None.

Poster

063. Cortical Hemodynamics

Location: Halls B-H

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: DGE 1106400

Title: Effects of continuous theta-burst transcranial magnetic stimulation on hemodynamic lag measured by BOLD fMRI

Authors: *D. J. LURIE[1], A. TAMBINI[2], C. GRATTON[4], J. POLINE[3], M. D'ESPOSITO[2]; [1]Psychology, [2]Helen Wills Neurosci. Inst., [3]Henry H. Wheeler Jr. Brain Imaging Ctr., Univ. of California, Berkeley, Berkeley, CA; [4]Dept. of Neurol., Washington Univ. in St Louis, St Louis, MO

Abstract: Continuous theta-burst TMS (cTBS) results in a sustained period of neural inhibition in targeted areas, and is often used to experimentally induce temporary “virtual lesions” in participants. Previous studies have observed local changes in Cerebral Blood Flow (CBF) at the site of cTBS stimulation. Likewise, reduction of CBF is also observed in patients with cerebrovascular disease, particularly in ischemic areas following stroke. Reduced perfusion is accompanied by a delayed BOLD hemodynamic response which can “lag” the rest of the brain

by up to 20 seconds. Thus, we hypothesized that cTBS may induce similar changes in hemodynamic lag under the coil. We tested this hypothesis in 27 healthy participants who underwent three sessions of cTBS and fMRI scanning. Each subject received cTBS on different days to left anterior insula/frontal operculum, left dorsolateral prefrontal cortex, and left primary somatosensory cortex. Resting BOLD and perfusion Arterial Spin Labeling (ASL) fMRI data were collected immediately before and after cTBS stimulation. We applied a cross-correlation based "lag mapping" analysis in which the BOLD time series from each voxel is compared to the average grey matter signal. By iteratively shifting the two signals relative to each other, it is possible to estimate the extent to which each voxel "leads" or "lags" the rest of the brain. Contrary to our hypothesis, we observed no consistent change in hemodynamic lag of the BOLD response at the site of cTBS stimulation. A previous analysis of the ASL scans from this dataset found high inter-subject variability in both the magnitude and direction (increase/decrease) of regional CBF changes under the coil. Even after accounting for this variability, we found no significant correlation between local changes in perfusion and changes in hemodynamic lag. These findings contribute to our understanding of the local (e.g. at the site of stimulation) physiological effects of cTBS. Moreover, these findings highlight important differences between true vascular lesions and the "virtual lesions" induced by cTBS.

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Poster

063. Cortical Hemodynamics

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: Hefftner Research Institute

Swiss Neuromatrix Foundation

Title: Investigation of the 5-HT2A/1A-agonist psilocybin on global and regional cerebral blood flow

Authors: *C. LEWIS[1,2], K. H. PRELLER[2], R. KRÄHENMANN[2], L. MICHELS[3], F. VOLLENWEIDER[2];
[1]Psychology, Arizona State Univ., Tempe, AZ; [2]Univ. of Zurich, Zurich, Switzerland; [3]Univ. Hosp. Zurich, Zurich, Switzerland

Abstract: Psilocybin, the active compound in psychedelic mushrooms, is a 5-HT2A/1A agonist. Seminal work in psilocybin neuroimaging research suggested hyper-frontal effects using positron emission tomography (PET) (Vollenweider et al., 1997; Gouzoulis-Mayfrank et al., 1999). However, recent work using arterial spin labeling (ASL) and blood oxygen level dependent (BOLD) functional MRI suggest global hypo-effects (Carhart-Harris et al., 2012). Therefore, we used ASL to investigate both global and regional effects of oral psilocybin on cerebral blood flow in a large sample to attempt to reconcile this seeming contradiction. In this placebo-controlled, double-blind study we used pseudo-continuous ASL perfusion MRI to measure regional CBF changes associated with two doses of oral psilocybin (low dose: 0.160 mg/kg; high dose: 0.215 mg/kg) in healthy controls (n = 29 in both groups, total N = 58) during a resting state scan. Participants from both the low and high dose groups reported profound subjective drug effects as measured by the Altered States of Consciousness Rating Scale (5D-ASC) with the high dose inducing significantly larger effects in four out of the 11 scales (Disembodiment, Complex Imagery, Elementary Imagery, and Audiovisual Synaesthesia). Controlling for sex and age, we utilized global normalization to infer the main effect of psilocybin on regional CBF and found psilocybin increases CBF in distinct right hemispheric frontal and temporal regions and bilaterally in the insula while decreasing CBF in left hemispheric parietal and occipital regions and the amygdala, globus pallidus, pallidum, insula, thalamus, and bilateral hippocampus ($p < 0.05$ FWE corrected). Using the traditional analysis of global effects, we found psilocybin significantly reduces CBF in various brain regions, including frontal, temporal, and parietal lobes, anterior cingulate, bilateral amygdale, insula, hippocampus, parahippocampus, caudate, and thalamus. Surprisingly, we were unable to find a significant dose effect of psilocybin on CBF, suggesting that the difference in subjective experience between the low and high dose groups is not coupled with CBF. Together, these results demonstrate a robust effect of psilocybin decreasing global CBF while having distinct regional right hemispheric frontal CBF increases and left hemispheric parietal and occipital decreases. This work was supported by Hefftner Research Institute and Swiss Neuromatrix Foundation.

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Poster

063. Cortical Hemodynamics

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Striatal dopamine transporter availability decreases throughout the day in healthy volunteers

Authors: *C. E. WIERS[1], E. SHOKRI-KOJORI[2], C. WONG[1], D. TOMASI[1], G.-J. WANG[1], N. VOLKOW[1];
[1]Lab. of Neuroimaging, Natl. Inst. on Alcohol Abuse and Alcoholism, Bethesda, MD;
[2]NIH/NIAAA, Bethesda, MD

Abstract: The Dopamine Transporter (DAT) has shown circadian fluctuations in animals, which have been associated with differences in dopamine release. However, circadian fluctuations of DAT have never been studied in humans. Here we investigated whether DAT availability was associated with the injection time of [11]Cocaine PET scans in healthy volunteers. We Based on preclinical findings that showed lower DAT in the afternoon than in the morning (Ferris et al., PNAS 2014) hypothesized that DAT availability would decrease with [11]Cocaine injection time. We moreover aimed to replicate that DAT availability decreases with age. A total of 130 healthy controls (15 females) underwent were imaged with an [11]Cocaine and PET scan for to measure DAT. Participants had a mean age of 34.3 years ± 8.1SD and a mean BMI of 25.6 ± 3.3SD. Mean injection time was 11.17 am ± .93SD, ranging from 9.05 am - 14.12 pm. Injection time was not associated with age, BMI or gender ($p>.05$). For hand- For the manually drawn striatal regions of interest (ROIs), [11]Cocaine injection time correlated negatively with Bmax/Kd in the putamen ($r=-.21$, $p=.017$) and ventral striatum ($r=-.22$, $p=.021$) but not caudate ($r=-.06$, $p=.52$). A The statistical paramateric mapping analysis corroborated similar these results, revealing that the: injection time correlated negatively with non-displaceable binding potential in the bilateral putamen: peak left = [-28, -10, 4], $t=3.56$, p-uncorrected<.0001; peak right = [28, -2, 6], $t=3.65$, p-uncorrected<.0001. As expected we also showed a significant negative correlation Age also correlated negativelybetween age and with Bmax/Kd in caudate ($r=-.42$, $p<.001$) putamen ($r=-.22$, $p=.011$) and ventral striatum ($r=-.35$, $p<.001$). The study confirms that in humans DAT in striatum decreases throughout the day in healthy volunteers, which suggests that diurnal variations of in DAT might underlie changes in striatal dopamine neurotransmission during the day throughout the day. The study further replicates the a negative association betweenfinding that DAT significantly decrease with age age and DAT in a larger sample than in used by previous studies.

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Poster

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Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant 1S10OD020039

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Title: Optimizing spatial and temporal resolution and coil selection for BOLD protocols on the Siemens 3T Prisma

Authors: *S. A. MCMAINS[1], R. W. MAIR[1,2];
[1]Ctr. for Brain Sci., Harvard Univ., Cambridge, MA; [2]Athinoula A. Martinos Ctr. for Biomed. Imaging Harvard Med. Sch., Charlestown, MA

Abstract: Siemen's new 3 Tesla scanner, the Magnetom Prisma, includes increased gradient strength, multiple standard parallel array coils, and a faster reconstruction computer. This latter feature allows routine use of slice-accelerated EPI with multiband (MB) RF pulses and simultaneous multi-slice (SMS) acquisition of BOLD images, which makes increased temporal or spatial resolution possible. Here, we set out to answer questions the typical user may have, such as: are more receive channels better (coil study) and how will data be affected by a short TR or small voxels (protocol study)? The coil study (n=6) consisted of 3 resting state functional connectivity (fcMRI) scans of 6min each with each of the 20, 32 and 64ch coils: noSMS ($3mm^3$ voxels (V), TR/TE 3000/30ms, flip angle (F) 85^o), SMS3 ($2mm^3$ V, TR/TE 2000/30ms, F 80^o, 7/8 partial fourier), and SMS8 ($2mm^3$ V, TR/TE 750/36ms, F 57^o, not with 20ch). The protocol study (n=8) used the 64ch coil and 4 different BOLD protocols: Standard ($3mm^3$ V, TR/TE 3000/30ms, F 85^o), Enhanced ($2.2mm^3$ V, TR/TE 2000/35ms, F 80^o), Fast ($2.3mm^3$ V, TR/TE 650/34.4ms, F 54^o), and HighRes ($1.5mm^3$ V, TR/TE 2000/30ms, F 80^o). For each protocol, subjects performed one fcMRI scan, and two 5min task scans where they saw blocks of familiar or unfamiliar words. Measurements of interest were timecourse signal-to-noise (tSNR) calculated for each voxel after motion correction and detrending, correlations between major network seeds (fcMRI scans), and average t-maps (task scans). For the coil study, tSNR did not vary by coil for noSMS scans. For SMS scans, tSNR was lowest for the 20ch ($p<.001$), and highest for the 32ch ($p<.05$). The tSNR findings were mirrored in the fcMRI correlation matrices. For the protocol study, tSNR correlated strongly with voxel size. In addition, tSNR was higher for Fast than Enhanced ($p<.001$), after correcting for the number of timepoints. For the fcMRI analysis, Fast and Enhanced had a slight advantage over Standard, while HighRes had the weakest correlations. For the task, maps were best for Fast and worst for HighRes. While the 64ch coil was out performed by the 32, likely because the extra channels are around the neck,

and the coil body is larger, the difference was small and might be outweighed by the fact more people can comfortably fit in the 64. When comparing protocols, Fast and Enhanced had an advantage over the more traditional Standard. HighRes had the worst performance, but the hit in fcMRI and task maps may be worth the reduction in distortion and increased spatial precision that comes from smaller voxels for certain studies. Regardless, new SMS protocols allow the entire brain to be covered with good temporal and spatial resolution at little cost.

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Poster

063. Cortical Hemodynamics

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Title: BOLD is thicker than white matter: Surgically disconnected temporal pole exhibits resting functional connectivity with remote brain regions.

Authors: *M. J. SUTTERER[1], D. E. WARREN[3], J. BRUSS[1], T. J. ABEL[2], A. JONES[4], H. KAWASAKI[2], M. W. VOSS[5], M. CASSELL[6], M. A. HOWARD, III[2], D. TRANEL[1]; [1]Dept. of Neurol., [2]Dept. of Neurosurg., Univ. of Iowa Hosp. and Clinics, Iowa City, IA; [3]Dept. of Neurolog. Sci., Univ. of Nebraska Med. Ctr., Omaha, NE; [4]Brown Univ., Providence, RI; [5]Dept. of Psychological and Brain Sci., [6]Dept. of Anat. and Cell Biol., The Univ. of Iowa, Iowa City, IA

Abstract: Functional connectivity, as measured by resting-state fMRI, has proven a powerful method for probing relationships between brain areas in the context of behavior, development, and disease states. However, the precise relationship between functional and structural connectivity remains unclear. For example, functional connectivity has been reported in the

absence of direct anatomical connections (Tyszka et al., 2011; Vincent et al., 2007). To investigate this vexing issue, we capitalized on an opportunity to examine functional connectivity between brain areas that were surgically isolated from the rest of the brain by resection of connecting tissue. We hypothesized that anatomical isolation of a brain region should eliminate functional connectivity with the rest of the brain, and therefore predicted that the disconnected brain regions would not show functional connectivity with remote brain regions. We tested this prediction by recruiting from a post-resection population to study resting state functional connectivity of the intact but surgically disconnected temporal pole. Anatomical and functional MRI images were acquired from patient participants (N=5) recruited from the Patient Registry at the University of Iowa. Each participant had undergone temporal disconnection surgery (3 left sided, 2 right sided) for treatment of epilepsy. Surgical disconnection was confirmed during the procedure and by subsequent inspection of postoperative structural MRI data. Resting-state functional connectivity (RSFC) was measured via coactivation of whole-brain fMRI data with the average resting BOLD signal from the disconnected tissue mask in each patient participant. Contrary to our prediction that disconnected regions would not show RSFC with remote brain areas, we observed significant RSFC between the disconnected temporal pole and other brain regions in each of the five cases. We compared these maps to normative functional connectivity maps by applying the same seed regions to resting-state fMRI data from 25 healthy comparison participants. While comparison participants showed a broader pattern of RSFC than the disconnected cases, we observed significant spatial similarity (measured by eta^2) in the RSFC profile between healthy comparisons and the disconnected cases. While these findings do not impugn the methodology employed by studies of resting-state functional connectivity, they raise important questions about the influences of neurovascular coupling on the BOLD signal and analyses of RSFC data in isolation from behavioral measures. Further investigation of these issues should improve the power and validity of projects based on RSFC.

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Poster

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1 R01 NS063226-06

UL1 TR000040

F30 HL128023 01

F31 NS084538 01

MSTP T32 GM007367 38

Title: Wide-field optical mapping of neural activity and cortical hemodynamics imaging during locomotion

Authors: *Y. MA, S. H. KIM, M. A. SHAIK, H. T. ZHAO, E. M. C. HILLMAN;
Biomed. Engin., Columbia Univ., New York, NY

Abstract: Understanding the relationship between neural activity and cortical hemodynamics, or neurovascular coupling is the foundation to interpret neuroimaging signals such as functional magnetic resonance imaging (fMRI) which measure local changes in hemodynamics as a proxy for underlying neural activity. Even though the stereotypical stimulus-evoked hemodynamic response pattern with increased concentrations of oxy- and total-hemoglobin ([HbO] and [HbT]) and a decrease in concentration of deoxy-hemoglobin ([HbR]) has been well-recognized, the linearity of neurovascular coupling and its dependence on brain regions and tasks haven't been thoroughly evaluated.
Longitudinal wide-field optical mapping of neural activity and cortical hemodynamics can be achieved across the entire, bilaterally exposed superficial cortex of awake, behaving mice. Neural imaging is achieved through wide-field fluorescence imaging in animals expressing genetically encoded calcium sensor (Thy1-GCaMP6f). Hemodynamics are recorded via simultaneous imaging of multi-spectral reflectance, and can also be supplemented by measurements of laser speckle flow. Our head-plate and restraint design allows animals to be implanted, recovered and then imaged repeatedly for 3 months. During imaging sessions, animals were allowed to stand or run freely on a saucer-wheel treadmill and could also receive timed whisker stimulation. Behavior of animals was also recorded using a near infrared camera during all imaging sessions. In prior work we have demonstrated that spontaneous changes in cortical blood volume in the resting state can be well-predicted by local changes in neural activity, given by absorption-corrected GCaMP fluorescence measurements. Here, we can use similar methods to compare coupling between bouts of locomotion versus resting state across the brain. Moreover, correlates of all aspects of observable behavior, and brain state interactions with the onset and cessation of locomotion can be explored.

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Poster

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Topic: D.06. Vision

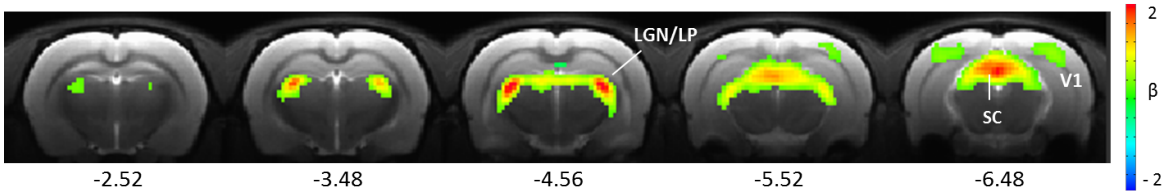
Support: NSC 101-2410-H-002-082-MY3

MOST105-2420-H-006-004-MY2

Title: Visual BOLD responses of dexmedetomidine-anesthetized rats to flashing light stimulation in an fMRI study

Authors: *D.-Y. CHEN[1], K.-H. CHEN[2], K.-C. LIANG[2];
[1]Dept Psychology, Natl. Cheng Kung Univ., Tainan, Taiwan; [2]Psychology, Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Light stimulus can elicit stable and robust electrophysiological activity of visual units in rodents or non-human primates even under deep anesthesia. However, fMRI BOLD responses were seldom used to assess visual activity in rodents under an anesthetic state. We build a light stimulation system equipped with an optical fiber (diameter 4 mm , length 5 m) and a white light LED as the light source. The flashing frequency (1, 5, 10 Hz with 50% duty cycle) and light intensity (0.1~23 cd/m^2) were administered to elicit BOLD responses of the visual system when male Wistar rats were anesthetized with dexmedetomidine (0.10 mg/kg/hr). A gradient-echo EPI was employed to obtain functional images with TR/TE = 3000/15 ms, seg = 2, in-plane resolution 312 x 312 micrometers, matrix size 80 x 80, and 10 slices with 1-mm thickness. A spin-echo EPI with TR/TE = 2000/35 ms and same geometry mentioned above was also used to provide complementary information. The testing stimulus was arranged into 5 blocks of 20 s-on/20 s-off cycles and synchronized with a 7T Bruker Biospec scanner via a custom-made LabView program. The light stimulus elicited strong BOLD responses on the lateral geniculate nucleus (LGN), superior colliculus (SC), and primary visual cortex (V1). The LGN preferred flashing in higher frequency and was sensitive to changes in light intensity, but V1 showed better BOLD response when low frequency was presented. A very weak light was sufficient to elicit SC activity, though neither frequency nor intensity preference was detected. As for the imaging sequences, SE-EPI showed better activity differentiation to various light intensities, while the BOLD activity detected by GE-EPI was saturated at a low intensity. The present study showed that light stimulation could elicit strong BOLD responses in the visual path of anesthetized rats, and two imaging sequences showed different response profiles with distinct features. These results provide a feasible setup and useful information to study the effect of learning and experience on visual pathway using BOLD fMRI experiments in the future.



Disclosures: **D. Chen:** None. **K. Chen:** None. **K. Liang:** None.

Poster

063. Cortical Hemodynamics

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 63.16/OO1

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Title: Correspondence of resting fMRI and electrophysiological connectivity dynamics following corpus callosotomy

Authors: ***E. M. YEAGLE**[1], M. ARGYELAN[2], P. MEGEVAND[4], J. HERRERO[3], S. BICKEL[5], V. DU[7], C. J. KELLER[6], D. GROPPE[8], M. MERCIER[9], L. ENTZ[10], A. D. MEHTA[2]; [1]Feinstein Inst. For Med. Res., Manhasset, NY; [3]Neurosci., [2]Feinstein Inst. for Med. Res., Manhasset, NY; [4]Neurol., Univ. Hosp. Geneva, Geneva, Switzerland; [5]Neurol., [6]Psychiatry, Stanford Univ., Palo Alto, CA; [7]Neurosurg., Hofstra North Shore LIJ Sch. of Med., Manhasset, NY; [8]Univ. of Toronto, Toronto, ON, Canada; [9]Ctr. de Recherche Cerveau et Cognition, Toulouse, France; [10]Natl. Inst. of Neurosci., Budapest, Hungary

Abstract: The functional specialization of the cerebral cortex depends in large part on its connectivity. While connectivity has traditionally been studied in humans with functional MRI, it may also be revealed by intracranial EEG (iEEG), which has a finer temporal resolution and the advantage of directly reflecting neuronal activity. A number of studies have investigated the correspondence between iEEG and MRI-based metrics of connectivity; however, the correspondence of these measures when connectivity is disrupted has yet to be examined. Corpus callosotomy, one treatment for intractable epilepsy, massively alters connectivity by severing the major fiber bundle connecting the cerebral hemispheres. This operation's disruption of inter-hemispheric connectivity has been documented using resting fMRI in a single patient (Johnston et al., 2008). To explore whether similar effects would be identified in electrophysiological measures of resting connectivity, we monitored three patients' resting intracranial EEG and mapped cortico-cortical evoked potentials (CCEPs), before and after undergoing anterior callosotomy.

Depth electrodes were placed bilaterally in frontal, parietal, and temporal lobes and insula in three epilepsy patients undergoing presurgical monitoring. All three patients then underwent anterior corpus callosotomy with electrodes intact, using stereotactic laser ablation. Electrodes were held in place with skull bolts, ensuring no migration during surgery. During clinical monitoring before and after callosotomy for each patient, we collected resting intracranial EEG (iEEG) during 3-6 minutes of quiet rest with eyes closed. We also mapped evoked potentials (CCEPs) using trains of bipolar stimulation at adjacent electrodes. Finally, we obtained 6 minutes of resting fMRI and DTI for each patient before electrode implantation and after electrode explant. To facilitate comparison with electrophysiology, BOLD time course extraction was performed using seeds at the location of each implanted electrode.
We found that inter-hemispheric iEEG connectivity, measured by resting iEEG and evoked potentials (CCEPs), was markedly altered following callosotomy in brain areas with projections to the lesioned section of the corpus callosum. These changes were paralleled by comparable effects in resting fMRI. Posterior to the callosotomy, both intra-and inter-hemispheric connectivity were relatively unchanged. These results provide further support for the correspondence of electrophysiological connectivity measures with fMRI.

Disclosures: **E.M. Yeagle:** None. **M. Argyelan:** None. **P. Megevand:** None. **J. Herrero:** None. **S. Bickel:** None. **V. Du:** None. **C.J. Keller:** None. **D. Groppe:** None. **M. Mercier:** None. **L. Entz:** None. **A.D. Mehta:** None.

Poster

063. Cortical Hemodynamics

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 63.17/OO2

Topic: F.06. Brain Blood Flow, Metabolism, and Homeostasis

Support: NSERC RGPIN 419235-2013

Alexander Graham Bell Canada Graduate Scholarships

Title: Accessing cortical inhibitory processes through the delay of the fMRI BOLD response.

Authors: *S. PROULX, R. FARIVAR;
Ophtalmology, McGill Univ., Montreal, QC, Canada

Abstract: Blood Oxygenation Level Dependent (BOLD) used in functional Magnetic Resonance Imaging (fMRI) allows an indirect measure of brain activity, but is blind to the ongoing interplay between excitation and inhibition. A recent study showed inhibition to not only reduce, but also

delay the BOLD response. Orientation of visual stimuli can be predicted from the spatial pattern of BOLD response *amplitudes* in the early visual cortex, and as inhibition is implicated in the cortical processing of orientation, we predicted that the pattern of BOLD *delays* will also allow to decode the orientation of the driving stimulus.

Amplitude and *delay* of BOLD responses induced by oriented visual stimuli were measured in 4 healthy subjects in a cyclic fMRI paradigm (Figure1). A Support Vector Machine (SVM) linear classifier was used to predict orientation of the two grating stimuli from either the V1 brain pattern of *amplitude* or *delay* of BOLD. Leave-one-out cross validation and random permutation tests (500 permutations) were performed to assess classification performance and significance. We obtained high and significant accuracies for prediction of stimulus orientation using both the *amplitude* and the *delay* of BOLD (Table1). Interestingly, similarly measured responses to a plaid stimulus, where cross-orientation suppression enhances inhibition, were delayed relative to grating stimulus in all subjects. These results confirm that the delay of BOLD responses carries neuraly-relevant information, and suggest that inhibition is implicated, implying that inhibitory processes *are* accessible with standard non-invasive fMRI exams.

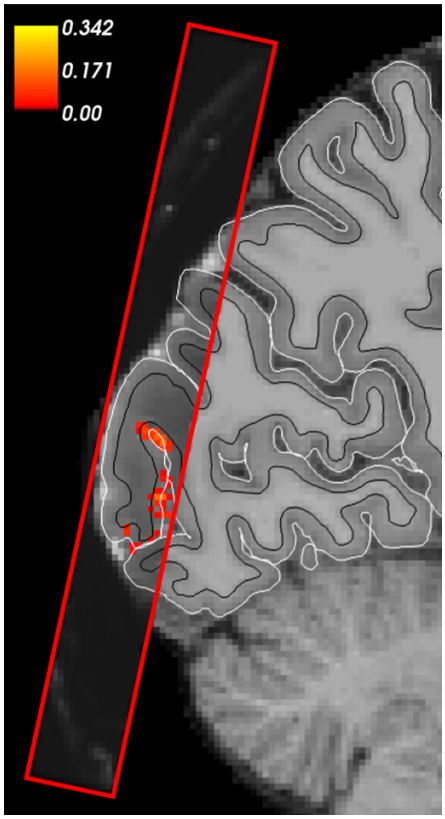


Figure 1: High-resolution BOLD fMRI at 3T with a custom 32-channel coil dedicated to the occipital cortex (1x1x1mm^3 GE-EPI; 13 slices; 10% gap; TR=1s; TE=68ms; red box). Activation extracted through cross-correlation of a sinusoid at stimulus period (12sec), and R-squared displayed on the overlayed heat map.

Classification accuracies and significance

	Classification accuracies (%; chance=50)	
Subjects	Amplitude	Delay
01ha	85*	86***
02jp	72	70
03sk	92**	78**
04sp	96***	79*
Group	**86*** **	**70*** **

Table1: *p<>

Disclosures: **S. Proulx:** None. **R. Farivar:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Güdform Neurotechnology Inc..

Poster

064. Molecular Mechanisms Underlying Sleep and Circadian Rhythms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 64.01/OO3

Topic: F.08. Biological Rhythms and Sleep

Support: University of Tennessee, Knoxville

Title: Low density lipoprotein receptor-related protein 1 (LRP1) regulates glutamate signaling in the mammalian circadian clock

Authors: *J. COOPER[1], R. A. PROSSER[2];
[1]Biochem. and Cell. and Mol. Biol., [2]Univ. of Tennessee, Knoxville, TN

Abstract: The mammalian circadian clock in the suprachiasmatic nucleus (SCN) exhibits daily, iterative changes in its responses to glutamate: nighttime glutamate induces SCN clock phase shifts; daytime glutamate does not. Mechanisms gating SCN phase shifting emulate those that

modulate neuroplasticity throughout the brain. LRP1 is a multifunctional membrane receptor that influences neuronal responses to glutamate through its endocytic and cell signaling actions. Here we investigate LRP1 as a regulator of circadian clock phase shifting. SCN brain slices from adult male C57BL/6 (WT) mice were treated with glutamate (1mM) +/- LRP1 inhibitors (500nM RAP or 75µg/mL anti-LRP1 antibody) at ZT 16 or ZT 23 (ZT 0=lights on, ZT 12 = lights off) for 10 minutes. The following day we recorded SCN single-unit neuronal activity (SUA) to determine the time of peak activity (an output signaling clock phase). Concurrent application of RAP or anti-LRP1 with glutamate prevents the normal shifts, while RAP alone has no effect on clock phase. One LRP1 ligand, tissue-type plasminogen activator (tPA), is known to influence neuronal plasticity both independently and via LRP1 interactions. We previously reported that tPA gates SCN phase shifting through proteolytic activation of BDNF (Mou et al 2009). Here we tested the hypothesis that LRP1 influences SCN phase shifting via interactions with tPA in experiments using tPA knockout ($tPA^{-/-}$; B6.129S2-$Plat^{tm1Mlg}$/J) mice. Continued entrainment and glutamate-induced phase resetting in SCN slices from adult $tPA^{-/-}$ mice suggest involvement of redundant pathways. Additional experiments indicate a second plasminogen activator and additional LRP1 ligand, urokinase plasminogen activator (uPA), participates in clock phase regulation. Addressing potential LRP1/tPA interactions, RAP continues to inhibit glutamate phase shifts in $tPA^{-/-}$ slices, indicating LRP1 may influence the clock independently of tPA. Preliminary western blot data indicate LRP1 is present in the SCN with no apparent circadian rhythm in total expression but potential changes in LRP1 phosphorylation on Y4507. Dynamic changes in LRP1 localization may underlie LRP1's role in SCN clock phase shifting, and future studies are aimed at assessing LRP1 cell surface localization. Given LRP1's diverse set of extracellular ligands and ability to mediate multiple cellular effects, exactly how LRP1 participates in circadian clock phase regulation remains a mystery. These results indicate that LRP1 contributes to the processes orchestrating circadian clock timing, and demonstrate the utility of the SCN circadian clock as a model system with which to study the neuronal functioning of LRP1.

Disclosures: J. Cooper: None. **R.A. Prosser:** None.

Poster

064. Molecular Mechanisms Underlying Sleep and Circadian Rhythms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 64.02/OO4

Topic: F.08. Biological Rhythms and Sleep

Support: CIHR Grant M00204

FRQS Bursary 30072

Title: Mapping the co-expression of clock proteins BMAL1 and PER2 with Enkephalin and Substance P in the rodent forebrain

Authors: *A. FREDERICK, J. GOLDSMITH, N. DE ZAVALIA, S. AMIR;
CSBN, Concordia Univ., Montreal, QC, Canada

Abstract: Despite rhythmic clock gene expression being found throughout the central nervous system, very little is known about their function outside of the suprachiasmatic nucleus. Understanding how clock genes are expressed across a variety of neural cell types is important to elucidating how these clock genes are regulated and how they may influence the function of each brain region. Using immunofluorescence and confocal microscopy, we quantified the co-expression of the clock proteins BMAL1 and PER2 with Substance P (SubP) and Enkephalin (Enk). Regions examined included the limbic forebrain (the dorsal striatum, ventral striatum, central nucleus of the amygdala, bed nucleus of the stria terminalis), the thalamus (medial habenula), the hypothalamus (paraventricular nucleus, arcuate nucleus) and the olfactory bulb. In most regions examined, PER2 and BMAL1 were homogenously expressed in nearly all cells (~90%), despite very different expression profiles of SubP or Enk in each nucleus. In nuclei that expressed both SubP and Enk, PER2 and BMAL1 were not preferentially co-expressed in one cell type or the other. The olfactory bulb was unique and did not express PER2 or BMAL1 throughout, and Enk was rarely found in the same cells that expressed the clock proteins (SubP was undetectable). This indicates that clock genes are not unique to specific cell types and further studies will be required to address which factors contribute to clock gene rhythmicity, focusing on how clock gene phase relationships, amplitudes and synchrony vary in different cell types.

Disclosures: **A. Frederick:** None. **J. Goldsmith:** None. **N. de Zavalia:** None. **S. Amir:** None.

Poster

064. Molecular Mechanisms Underlying Sleep and Circadian Rhythms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 64.03/OO5

Topic: F.08. Biological Rhythms and Sleep

Title: Continuous measurement of 28 neurotransmitters and other endogenous messengers for 24 hours in the striatum and prefrontal cortex by microdialysis in freely moving rats

Authors: N. MOORE[1], A. RASSOULPOUR[1], H. JANSSENS[1], L. YU[1], H. KOOIJKER[1], F. HELFRICH[1], J. ROESER[1], *M. G. VAN DER HART[1,2];
[1]Brains On-Line, South San Francisco, CA; [2]Psychiatry, UCSF, San Francisco, CA

Abstract: Circadian rhythms (CR) can be observed in several physiological systems throughout the body. In the brain, the suprachiasmatic nucleus (SCN) drives the 'internal clock' and further regulates the CR directly or indirectly throughout the body. Disruption of the CR can have enormous effects on mood and concentration, and is observed in individuals with mood disorders and neurodegenerative disorders. Understanding the resultant neurochemical changes that occur across the CR can help to inform therapeutic strategies to treat disorders related to a disrupted CR. While previous microdialysis studies have explored the neurochemistry underlying circadian patterns, these studies have primarily examined only a few neurotransmitters in a single brain region.
In the current study, we investigated the changes in a large number of neurotransmitters and other endogenous neurochemicals with a single sample using in vivo microdialysis. We placed a microdialysis probe in either the prefrontal cortex (PFC) or striatum (STR) and collected samples every hour for 24 hours. Across the 24 hour period, differences in neurotransmitter release were observed in the PFC and STR for ACh, DOPAC, HVA and 1-met-HA. The following analytes increased during at least part of the dark cycle; ACh, DOPAC, HVA in the PFC only, 1-MetHA in the STR only, GABA, Gly and HA in both the PFC and STR. The levels of Kynurenine, Kynurenic acid, Xanthurenic acid, tryptophan and 5-HIAA were attenuated in the dark cycle in both brain areas. These data demonstrate the feasibility of monitoring many neurochemicals within one sample for prolonged periods of time. Furthermore, we observed distinct neurochemical changes in the PFC and STR that are altered across the CR. Future work will explore neurochemical changes in brain regions more directly invoved in the CR, such as the SCN and the pineal gland, to gain a better understanding of the neurochemical changes that underlie, and perhaps may drive the CR.

Disclosures: N. Moore: None. **A. Rassoulpour:** None. **H. Janssens:** None. **L. Yu:** None. **H. Kooijker:** None. **F. Helfrich:** None. **J. Roeser:** None. **M.G. van der Hart:** None.

Poster

064. Molecular Mechanisms Underlying Sleep and Circadian Rhythms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 64.04/OO6

Topic: F.08. Biological Rhythms and Sleep

Support: CIHR 142458

Title: Dopamine regulation of clock gene expression in the olfactory bulb

Authors: *N. DE ZAVALIA, P. SOLIS, S. AMIR;
Psychology, Concordia Univ., Montreal, QC, Canada

Abstract: The olfactory bulb (OB) of rodents has been suggested to possess a self-sustaining circadian oscillator, which functions independent from the master circadian clock in the suprachiasmatic nuclei. It also contains the major forebrain dopamine (DA) system common to all vertebrates. The importance of DA in the regulation of circadian rhythms is suggested by clinical evidence of altered behavioral and physiological rhythms in patients with Parkinson's disease (PD) and by experimental findings in rodents showing that depletion of striatal DA, the primary neuropathology of PD, disrupts circadian rhythmicity.
The aim of this work was to study the regulation of the expression of major clock genes in the OB by DA.
First, we determined the rhythmicity and cellular expression of Per1, Per2 and Bmal1 in the OB by immunohistochemistry and western blot. PER1 was expressed in the glomeruli cell layer (GL), where it co-localizes with tyrosine hydroxylase expression. It was also expressed in the mitral (ML) and granular cell layers (GCL), while PER2 was only expressed in the ML. The levels of both proteins, PER1 and PER2, were increased at ZT 1 ($p < 0.05$). There was no rhythmical expression of PER1 in the GCL. BMAL1 was also expressed in the GL, GCL and ML. Subsequently, we studied the extracellular levels of DA in the OB by microdialysis and HPLC. DA was released rhythmically with a peak at the end of the light phase (ZT11-ZT12) and a trough at the end of the dark phase (ZT 23-ZT0). To decrease the levels of DA in the OB, we performed unilateral naris closure (UNO) in young adult rats (125-150 g). This method induced a significant down-regulation of the catecholamine biosynthetic enzyme tyrosine hydroxylase (TH) in DA neurons intrinsic to the OB 1 month after UNO (control: 109.3 ± 3.7 au, UNO: 78.8 ± 5.4 au, $p < 0.001$). Preliminary results showed a slight decrease of BMAL1 in the GCL, an increase in the GL and no change in the ML after UNO, although these changes were not found to be significant. The western blot showed an increase in the levels of BMAL1 and a decrease in the levels of PER2 in the OB, however these experiments have to be replicated.
These findings confirm the existence of a circadian oscillator in the OB and demonstrate significant circadian rhythms in proteins of major clock genes in subregions of the OB. They also demonstrate a circadian release of DA that contributes to the regulation of the expression of the major clock genes. Further studies have to be made to determine the exact extent of the DA contribution to the regulation of the circadian rhythms in the OB.

Disclosures: **N. De Zavalia:** None. **P. Solis:** None. **S. Amir:** None.

Poster

064. Molecular Mechanisms Underlying Sleep and Circadian Rhythms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 64.05/OO7

Topic: F.08. Biological Rhythms and Sleep

Support: Wellcome Trust Funded PhD Student

Title: Using *Drosophila melanogaster* as a model to study age related changes in the circadian clock

Authors: *J. A. CURRAN[1], K. TSANEVA-ATANASOVA[2], J. J. L. HODGE[1];
[1]Sch. of Physiology, Pharmacol. and Neurosci., Univ. of Bristol, Bristol, United Kingdom;
[2]Univ. of Exeter, Exeter, United Kingdom

Abstract: Circadian rhythms control a wide range of biological processes, ranging from metabolic activity to control of the sleep-wake cycle. It is well established that elderly individuals have increasing difficulties sleeping at night combined with generally going to sleep and waking up earlier. These age-related declines in circadian output are clearly observable in activity recordings in laboratory animals but what underlies these changes at a molecular and neuronal activity level are unknown. *Drosophila* makes an attractive system for studying this age-related decline in the clock output, with their short lifespan (~70 days) allowing for comparably manageable experimental timescales.
This study investigates how age impacts upon the circadian system of *Drosophila*, looking at changes in behavioural outputs, neuronal activity and gene expression. Behaviour was measured by recording the locomotor activity of male flies at various stages in the ageing process (1, 8, 15, 22, 29 days old), analysing the period and rhythm strength while in constant dark conditions. Changes in neuronal activity were investigated using whole-cell patch clamp electrophysiology to record from clock neurons (large ventral lateral neurons), with recordings made across the circadian day and at various ages. Finally changes in the molecular clock were investigated by analysing the relative expression of clock genes using quantitative RT-PCR.
At a behavioural level there was a significant age-related linear decline in the rhythm strength of circadian behaviour that was associated with an increase in period length. The linear decline in behavioural outputs of the clock demonstrate the suitability of *Drosophila* as a model to interrogate how ageing effects the circadian clock at other levels. Further experiments endeavour to understand how the disruption in behavioural rhythms are reflected by changes in the electrical activity of clock neurons.

Disclosures: J.A. Curran: None. **K. Tsaneva-Atanasova:** None. **J.J.L. Hodge:** None.

Poster

064. Molecular Mechanisms Underlying Sleep and Circadian Rhythms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 64.06/OO8

Topic: F.08. Biological Rhythms and Sleep

Support: FONDECYT 1111033

FONDECYT 1141233

Instituto Milenio P09-022-F

Title: Characterization of innexin hemichannels in the circadian peacemaker neurons of *Drosophila melanogaster*

Authors: *E. FRITZ[1], P. FERNANDEZ[1], J. CAMPUSANO[1], J. C. SAEZ[1,2];
[1]Pontificia Univ. Catolica De Chile, Santiago, Chile; [2]Ctr. Interdisciplinario de Neurociencia de Valparaiso, Valparaiso, Chile

Abstract: Introduction: In invertebrates, innexins (functional analogs to connexins in vertebrates) form gap junctions and hemichannels (HCs). These proteins are fundamental for cell-cell communication during development and adulthood. In Drosophila melanogaster, seven out of the eight innexin-encoding loci are expressed in the nervous system. However, to our knowledge no extensive study has been performed to determine their function and/or gene expression. Based on the importance of the conserved nature of circadian rhythms across all species, we analyzed innexin expression and HC activity in Drosophila melanogaster peacemaker neurons.
Materials and Methods: Single cell mRNA extraction and RT-PCR was performed in ventral lateral GFP-expressing PDF-positive (GFP-PDF^{+}) neurons to detect innexin mRNA. HC activity was measured by dye uptake of HC fluoresccent permeability tracers.
Results: Innexin 1, 6, 7 and 8 were detected. In cultured GFP:PDF^{+} neurons we found HC activity that increased by exposure to extracellular divalent cations free solution (DCFS) and the increase was inhibited by Lanthanum (La^{+3}).
Discussion: Our data indicates that ventral lateral PDF^{+} neurons of Drosophila melanogaster express functional HC. We hypothesis that innexin hemichannels may affect the circadian rhythms of Drosophila melanogaster.

Disclosures: **E. Fritz:** None. **P. Fernandez:** None. **J. Campusano:** None. **J.C. Saez:** None.

Poster

064. Molecular Mechanisms Underlying Sleep and Circadian Rhythms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 64.07/OO9

Topic: F.08. Biological Rhythms and Sleep

Support: Funded by NIH/NIGMS - Award Number P20GM103475-13 | Hosted by HPCf, UPR

Title: Developmental lead, ecdysone-responsive genes, and circadian sleep in the fruit fly

Authors: *A. VAZQUEZ-MONTES[1], W. MELENDEZ-PACHECO[1], C. VALENTIN-RAMOS[1], P. CARRION-COLON[1], J. AGOSTO-RIVERA[2], H. ORTIZ-ZUAZAGA[3]; [1]Biol., Univ. Del Turabo, Gurabo, PR; [2]Biol., [3]Computer Sci., Univ. of Puerto Rico, San Juan, PR

Abstract: Metal contaminants, such as lead (Pb^{+2}) are persistent environmental neurotoxicants producing cognitive, behavioral, and physiological impairments in the developing brain. Early Pb^{+2} exposure disrupts learning and memory processes, locomotor activity, and sleep cycle disturbances in Pb^{+2}-exposed children and rodents. Sleep is a hormone-dependent physiological process that is regulated by circadian rhythms and has been widely characterized in the fruit fly. Ecdysone steroid hormone regulates circadian rhythms, and ecdysone-responsive genes have been identified as important regulators of the molecular clock machinery of circadian neurons in the adult. The goal of this research is to characterize the effects of developmental exposure to Pb^{+2} in circadian sleep and ecdysone-responsive genes in the adult fruit fly. Our hypothesis is that early exposure to Pb^{2+} will impair ecdysone-responsive genes and circadian sleep later in non-exposed adults. Flies were exposed to control or Pb^{+2}-contaminated food during development, but not in the adult stage. Sleep was tested using infrared-based activity monitors during constant darkness. Circadian sleep disturbances were assessed using rhythm strength and standard sleep parameters. Ecdysone-responsive gene activities were detected from total RNA collected from whole individuals and non-exposed adults using fluorescence probes and Real Time PCR. Our data show that developmental exposure to Pb^{+2} disrupts developmental transcriptional activation of ecdysone-responsive genes and produces dimorphic sleep phenotypes in non-exposed adult flies.
Overall, our studies will give insights related to early low-level exposure of persistent contaminants, such as Pb^{+2} and their contribution in the etiology of circadian sleep disorders using the fruit fly as the model.

Disclosures: A. Vazquez-Montes: None. **W. Melendez-Pacheco:** None. **C. Valentin-Ramos:** None. **P. Carrion-Colon:** None. **J. Agosto-Rivera:** None. **H. Ortiz-Zuazaga:** None.

Poster

064. Molecular Mechanisms Underlying Sleep and Circadian Rhythms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 64.08/OO10

Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant R01 OD019037

Title: Functional imaging of sleep and wake states in zebrafish.

Authors: *A. ANDREEV[1], T. V. TRUONG[2], S. E. FRASER[2];
[2]Translational Imaging Ctr., [1]USC, Los Angeles, CA

Abstract: The zebrafish, with its small size and optical accessibility, has emerged as an attractive vertebrate, diurnal model system to decipher the basic mechanisms of sleep behavior, regulation, and function. We present our development of the toolset to allow brain-wide functional imaging and analysis, at single-celled resolution, of zebrafish larvae during sleep and wake behavior. As the sleep state is particularly sensitive to photo-induced effects imparted by the imaging itself, we first establish the necessary imaging conditions that minimally perturb the behaving animal, using two-photon selective plane illumination microscopy (2p-SPIM) as the modality of choice. Using 2p-SPIM, we capture zebrafish brain activity for periods up to 48 hours, measuring functional changes over different regions of the brain during natural sleep/wake cycle with cellular resolution. We characterize the brain activity due to visual and auditory stimuli to determine response thresholds during sleep and wake. Under this framework, we begin to characterize the similarities and differences between the natural sleep/wake states and similar states induced either via pharmacological means or optogenetic activation. Insights gained by our study will contribute to the understanding of the nature and function of sleep.

Disclosures: A. Andreev: None. **T.V. Truong:** None. **S.E. Fraser:** None.

Poster

064. Molecular Mechanisms Underlying Sleep and Circadian Rhythms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

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Topic: F.08. Biological Rhythms and Sleep

Support: JSPS KAKENHI 25670127

JSPS KAKENHI 15K15051

JSPS KAKENHI 24510069

JST A-step AS262Z00004Q

LRI of JCIA 13_PT01-01

Title: Pharmacological profiling of zebrafish sleep-awake states

Authors: *Y. NISHIMURA, S. OKABE, S. SASAGAWA, S. MURAKAMI, Y. ASHIKAWA, M. YUGE, K. KAWAGUCHI, R. KAWASE, Y. SHIMADA, T. TANAKA;
Pharmacol., Mie Univ. Grad. Sch. of Med., Tsu, Mie, Japan

Abstract: Sleep-wake states are impaired in various neurological disorders. Impairment of sleep-wake states can be an early condition that exacerbates these disorders. Therefore, treating sleep-wake dysfunction may prevent or slow the development of these diseases. Although many gene products are likely to be involved in the sleep-wake disturbance, hypnotics and psychostimulants clinically used are limited in terms of their mode of action and are not without side effects. Therefore, there is a growing demand for developing new hypnotics and psychostimulants with high efficacy and few side effects. Towards this end, animal models are indispensable for use in genetic and chemical screens to identify sleep-wake modifiers. As a proof-of-concept study, we performed behavioral profiling of zebrafish treated with chemical and genetic sleep-wake modifiers. We were able to demonstrate that behavioral profiling of zebrafish treated with hypnotics or psychostimulants from 9 to 10 days post fertilization was sufficient to identify drugs with specific modes of action. We were also able to identify behavioral endpoints distinguishing GABA-A modulators and hypocretin receptor antagonists and between sympathomimetic and non-sympathomimetic psychostimulants. This behavioral profiling can serve to identify genes related to sleep-wake disturbance associated with various neuropsychiatric diseases and novel therapeutic compounds for insomnia and excessive daytime sleep with fewer adverse side effects.

Disclosures: **Y. Nishimura:** None. **S. Okabe:** None. **S. Sasagawa:** None. **S. Murakami:** None. **Y. Ashikawa:** None. **M. Yuge:** None. **K. Kawaguchi:** None. **R. Kawase:** None. **Y. Shimada:** None. **T. Tanaka:** None.

Poster

064. Molecular Mechanisms Underlying Sleep and Circadian Rhythms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 64.10/OO12

Topic: F.08. Biological Rhythms and Sleep

Support: RANNIS 141788-053

Title: The effects of transient kcna2 knock-down on sleep-wake cycles in larval zebrafish

Authors: *K. KARLSSON, Dr.[1,2], S. SRDANOVIC[2], F. ÞÓR, 101[1], H. ÞORSTEINSSON[2]; [1]Reykjavik Univ., Reykjavik, Iceland; [2]3Z, Reykjavik, Iceland

Abstract: Relying on simple or genetically tractable animal models, a molecular vision of sleep that assumes there are cross-species common cellular sleep processes has been emerging. Recently a mutation in voltage-gated potassium channels has been shown to be associated with a short sleeping phenotype in rodents and flys. In order to replicate and extend these findings we use morpholino oliogonucleotides (MO) to knock-down Kcna2 in larval zebrafish and assess the effects on sleep-wake cycles. First, measuring arousal thresholds at four days-post-fertilization (dpf4) and dpf7 fish, we firmly establish criteria for sleep-wake measurements at these young ages. Second, three groups of fish were behaviorally assessed for sleep parameters: naïve fish and fish injected with control or Kcna2 MO: all groups underwent 24 hr behavioral monitoring of sleep-wake cycles at dpf4 and dpf 7 (i.e. while the MO is still active and after the effects have worn off). Third, the rate, duration and amplitude of spontaneous field potentials were measured at the level of tectum opticum and telencephalon at dpf4 and dpf7. We show that at dpf4 Kcna2 injected fish exhibit significantly less sleep behavior than do controls or naïve fish and exhibit higher rates and longer duration local field potentials. We show that MO knock-down of voltage-gated potassium channels results in a transiently short sleeping phenotype and increased rate of spontaneous activity due to global increasein membrane excitability. The current findings further establish zebra fish as an excellent model of sleep and are consistent with the notion that core neural pathways, common across phylogeny, control sleep-wake cycles.

Disclosures: **K. Karlsson:** None. **S. Srdanovic:** None. **F. Þór:** None. **H. Þorsteinsson:** None.

Poster

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Topic: F.08. Biological Rhythms and Sleep

Support: Conacyt- Mexico 249758

Fundación Pandea

Title: Glutamate regulates bmal1 protein in cultured bergmann glia cells

Authors: *L. D. CHI-CASTAÑEDA[1], S. M. WALISZEWSKI[2], R. C. ZEPEDA[2], L. C. R. HERNÁNDEZ-KELLY[1], M. CABA[2], A. ORTEGA[1];
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Abstract: Glutamate (Glu), the major excitatory amino acid, activates a wide variety of signal transduction cascades. This neurotransmitter is involved in photic entrainment of circadian rhythms, which regulate physiological and behavioral functions. The circadian clock in vertebrates, suprachiasmatic nucleus, is based on a transcription-translation feedback loop, in which Brain and muscle aryl hydrocarbon receptor nuclear translocator (ARNT)-like protein 1 (BMAL1) acts as transcriptional activator of others clock genes. This protein is expressed in nearly all suprachiasmatic nucleus neurons, and other brain and peripheral regions. This study aimed was to investigate the role of Glu in the molecular mechanisms involved in the processes of transcription/translation of BMAL1 protein. To this end, primary cultures of *Gallus gallus* cerebellar Bergmann glia cells were stimulated with glutamatergic ligands, the results indicate that BMAL1 levels increased in a dose- and time dependent manner. Additionally, the phosphorylation of serine residues in BMAL1 under Glu stimulation was studied and the results show an increase in the phosphorylation of this protein. The increased expression of BMAL1 is most probably the result of its stabilization after being phosphorylated by the cyclic AMP-dependent protein kinase and/or the Ca^{2+}/diacylglycerol dependent protein kinase. The results strongly suggest that Glu regulates translationally BMAL1 in glial cells, and that these cells are important for the regulation of circadian rhythms in the cerebellum.

Disclosures: L.D. Chi-Castañeda: None. **S.M. Waliszewski:** None. **R.C. Zepeda:** None. **L.C.R. Hernández-Kelly:** None. **M. Caba:** None. **A. Ortega:** None.

Poster

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T32 DK007004

Title: Ventral anterior homeobox 1 is required for suprachiasmatic nucleus development as well as circadian output in adulthood allowing female fertility

Authors: *H. M. HOFFMANN[1,2], C. TRANG[1,2], B. HEREFORD[1,2], K. BHARTI[3], D. K. WELSH[2,4,5], M. R. GORMAN[2,6], P. L. MELLON[1,2];
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Abstract: During the last century, our society has changed dramatically, and today many people work odd hours, travel across time zones, and have disrupted sleep patterns. These are all factors affecting circadian rhythms and leading to medical illnesses including diabetes, decreased cognitive capacity, and impaired fertility. Correct coordination and timing of hormone release in the hypothalamic-pituitary-gonadal (HPG) axis are required to maintain fertility. Female fertility is particularly sensitive to compromised circadian rhythms causing dysregulation of the HPG axis. Development of the suprachiasmatic nucleus (SCN), the brain's primary circadian pacemaker, is required for fertility. The SCN orchestrates the HPG axis by coordinating hypothalamic kisspeptin neurons and gonadotropin-releasing hormone (GnRH) neuron activity with peripheral tissues and light cycles. Mature GnRH neurons respond to kisspeptin by increasing GnRH release, which allows appropriate levels of sex steroids across the estrous cycle and the preovulatory luteinizing hormone surge. We here identify a novel homeoprotein required

for SCN development and function, Ventral Anterior Homeobox 1 (VAX1). H&E staining in the *Vax1 knock-out* mouse at P0 shows a complete loss of the medial portion of the hypothalamus, causing the partial or total absence of the medial preoptic area and anteroventral periventricular region, as well as the SCN. Indeed, VAX1 is necessary for expression of SCN markers: the SCN transcription factor, SIX homeobox 3, as well as two core SCN peptides, arginine vasopressin and vasoactive intestinal peptide, which demarcate the SCN shell and core regions respectively. Interestingly, in the adult brain, VAX1 is almost exclusively expressed in the SCN. Since the *Vax1* knock-out mouse is perinatal lethal, we generated $Vax1^{flox}$ mice and crossed them with $synapsin^{cre}$ mice, thus deleting VAX1 in mature neurons in the brain. $Vax1^{flox}$:$synapsin^{cre}$ mice had an impaired activity pattern and reduced activity of wheel running in constant darkness, which correlated with abnormal expression of SCN peptides and Per2::luciferase expression, indicating weak SCN output. In addition, $Vax1^{flox}$:$synapsin^{cre}$ females had prolonged estrous cycles and decreased fertility. Despite abnormal SCN output in $Vax1^{flox}$:$synapsin^{cre}$ males, they had normal fertility and sperm quality. This study provides a new model to address how weak SCN output impacts fertility, and confirms the high sensitivity of female, but not male, fertility to impaired SCN output.

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Poster

064. Molecular Mechanisms Underlying Sleep and Circadian Rhythms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 64.13/PP1

Topic: F.08. Biological Rhythms and Sleep

Title: Hypothalamic lesion of A11 area disturb clock genes

Authors: C. PIÑA-LEYVA[1], M. LARA-LOZANO[1], B. FLORÁN-GARDUÑO[1], *J. A. GONZALEZ-BARRIOS[2];
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Abstract: The A11 hypothalamic nucleus, also known as dopaminergic diencephalospinal tract, innervates the spinal cord. This descending tract is involved in the regulation of important mechanisms in the spinal cord such as nociception and locomotion, which are under circadian control. There are few studies focused on the physiological regulation of the clock genes in the spinal cord which control the circadian function. We evaluated the expression of the clock genes in the spinal cord and the subsequent alteration of the expression of these genes after lesion of

A11 nucleus by an injection of 6-OHDA (10 µg/µL). We used male Wistar rats to evaluate the expression of the transcripts per1, 2 y 3, bmal, clock, cry1 y 2 on cervical, thoracic and lumbar region of the spinal cord; the rats were lesioned by stereotaxic surgery, after 7 days we dissected the different regions of the spinal cord and extracted mRNA by a TRIzol® protocol. The expression of genes was evaluated by RT-qPCR using commercial taqman probes. The results shown that in the cervical region there are a downregulation the mRNA of per1, 2 and 3, bmal, clock, cry1 and 2, in the thoracic and lumbar regions there are downregulation of per1, 2 and 3, cry1 and 2 whereas upregulation of bmal and clock. Ours results show that dopaminergic depletion of the A11 nucleus alters the transcription of clock genes of the spinal cord of the rat, and suggest that the clock genes are regulated by dopamine receptors activation.

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Poster

064. Molecular Mechanisms Underlying Sleep and Circadian Rhythms

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Program#/Poster#: 64.14/PP2

Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant HD050470

Title: Predicting estrous state and pregnancy outcome: applications of high temporal resolution temperature rhythm analysis in female mice

Authors: B. L. SMARR[1], I. ZUCKER[2], *L. J. KRIEGSFELD[3];
[1]Psychology, [2]Psychology and Integrative Biol., [3]Psychology and The Helen Wills Neurosci. Inst., Univ. of California, Berkeley, CA

Abstract: Female reproductive cycles and pregnancy induce predictable changes to physiology and behavior that are associated with major health and quality of life issues. Although well described at the population level, these changes exhibit substantial interpersonal variance; this variance, combined with low temporal-resolution data, has left female reproduction remarkably under-described. The miniaturization of electronic sensors and data storage enable high-resolution monitoring of individuals across long time windows. These extensive data sets permit more subtle and sophisticated individualized predictors of physiological state. To explore the power of such strategies in animal models of reproductive functioning, mice were implanted with temperature probes generating one data point per minute for several months. Not only does this high temporal resolution approach permit a fine-grained analysis of course rhythmic processes

(e.g., circadian rhythms), but decomposition of each daily waveform can be applied to reveal quantifiable traits predictive of reproductive state. Here we take advantage of a number of quantitative analyses of these high-resolution, long-duration data sets to stage estrus and to accurately detect and predict the outcome of pregnancies within the first 12 hours after a night of pairing. The validation of this approach underscores the potential to reveal novel insights in time-series data, including development, puberty, and illness outcomes. These approaches also have the potential to inform human-subject investigations to render a higher-quality description of healthy female reproductive cycles, pregnancies, and menopause for use in personal, predictive, and preventive biomedicine.

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Poster

064. Molecular Mechanisms Underlying Sleep and Circadian Rhythms

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Program#/Poster#: 64.15/PP3

Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant MH087463

National Defense Science and Engineering Graduate (NDSEG) Fellowship, 32 CFR 168a

Title: $CBP^{Kix/Kix}$ mice have decreased gene model 129 (CHRONO) expression and increased free-running circadian period

Authors: *C. C. ANGELAKOS[1], S. G. POPLAWSKI[2], S. CHATTERJEE[2], G. PORCARI[2], J. B. HOGENESCH[3], T. ABEL[2];
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Abstract: The transcriptional coactivator CREB binding protein (CBP) is recruited by and heterodimerizes with the CLOCK/BMAL1 transcription factor complex to regulate clock gene activation. CBP overexpression enhances CLOCK/BMAL1-mediated Per1 activation, while CBP knockdown decreases circadian clock induction and oscillation. The mechanisms by which CBP regulates CLOCK/BMAL1-mediated transcription and circadian rhythms are not fully understood. We performed RNA sequencing in the hippocampus of mice carrying an inactivating triple point mutation in the CREB-binding (Kix) domain of CBP ($CBP^{Kix/Kix}$ mice), which blocks phospho-CREB binding to CBP. We found decreased expression of the circadian clock mediator

gene *Gm129* (CHRONO) in $CBP^{Kix/Kix}$ mice, which validated with quantitative PCR. CHRONO has recently been shown to regulate circadian rhythms by repressing CLOCK/BMAL1 function via direct interference of BMAL1-CBP binding, and *Chrono* knockout mice have prolonged free-running circadian rhythms. Activity monitoring of $CBP^{Kix/Kix}$ mice across the diurnal cycle in 12:12 hour light:dark revealed delayed activity onset as well as delayed peak activity in both male and female $CBP^{Kix/Kix}$ mice compared to sex-matched wildtype littermates. Similar to the prolonged circadian periods found in *Chrono* knockout mice, $CBP^{Kix/Kix}$ mice exhibited a 27-minute increase in free-running circadian period relative to wildtype littermates; however phase shifting in response to 15-minute light pulses at CT14 and CT22 did not differ between $CBP^{Kix/Kix}$ mice and controls. Quantification of CHRONO in the brain and liver of $CBP^{Kix/Kix}$ mice across the diurnal cycle are being performed. Together, these studies help to further elucidate the molecular machinery regulating the circadian clock and may contribute to our understanding of the contributions of epigenetic regulators to circadian rhythms.

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Poster

064. Molecular Mechanisms Underlying Sleep and Circadian Rhythms

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant R01NS072431

Title: The RNA editing gene, Adar, suppresses sleep by regulating glutamatergic synaptic plasticity

Authors: N. HOFFNER[1], J. ROBINSON[2], J. PALUCH[3], D. DICKMAN[3], *W. JOINER[2]; [1]Dept. of Neurosci., [2]Dept. of Pharmacol., Univ. of California San Diego, La Jolla, CA; [3]Dept. of Neurobio., USC, Los Angeles, CA

Abstract: Sleep is an essential, homeostatic process that is highly conserved throughout the animal kingdom. However, its functions are still poorly understood. One leading hypothesis for a core function of sleep suggests that synaptic potentiation during waking is offset by a homeostatic reduction in net synaptic strength during sleep to maintain overall synaptic function within a stable physiological range. However, molecular mechanisms supporting this hypothesis are lacking. Here we address the relation between sleep and synaptic plasticity in the context of the RNA editing gene, *Adar*, which we have identified as a critical regulator of sleep pressure in

Drosophila. Loss-of-function alleles and RNAi knockdown of *Adar* lead to selective destabilization of the waking state and increased sleep, effects we have mapped to a subset of glutamatergic neurons in the CNS. We also find that *Adar* mutants post-transcriptionally upregulate the vesicular glutamate transporter, VGLUT, leading to over-activation of NMDA receptors, and that these effects are required for observed increases in sleep. Furthermore, by electrophysiological and genetic analyses we show that in *Adar* mutants the reserve pool of glutamatergic synaptic vesicles is selectively expanded, and that this change is required for increased sleep. The net effect of this expansion is sustained neurotransmitter release under conditions that would otherwise result in synaptic depression. We propose that the shift in the balance from synaptic depression toward synaptic potentiation in sleep-promoting neurons underlies the increased sleep pressure of *Adar*-deficient animals. Our results provide a plausible molecular mechanism linking sleep and synaptic plasticity.

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Poster

064. Molecular Mechanisms Underlying Sleep and Circadian Rhythms

Location: Halls B-H

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Program#/Poster#: 64.17/PP5

Topic: F.08. Biological Rhythms and Sleep

Title: Symptomatic narcolepsy among inherited disorders, such as myotonic dystrophy

Authors: ***T. KANBAYASHI**[1,2], Y. OHMORI[1], A. IMANISHI[1], T. ONO[1], K. TSUTSUI[1], J. TAKAHASHI[1], E. NARITA[1], Y. SAGAWA[1], Y. KIKUCHI[1], M. TAKESHIMA[1], Y. TAKAHASHI[1], R. SASAKI[1], D. FUJIWARA[1], Y. SATOH[1], Y. TAKEKOSHI[1], R. AIZAWA[3], H. ONO[4], S.-I. UEMURA[5], S. SATO[1], S. IIJIMA[6], M. SATO[1], T. SHIMIZU[1,2];
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Abstract: The symptoms of narcolepsy can occur during the course of several neurological conditions. Inherited disorders, tumors and CNS autoimmune diseases were the three most frequent causes for symptomatic narcolepsy. Among inherited disorders, Niemann-Pick type C, Prader-Willi syndrome and myotonic dystrophy type 1 (MYD1) were mainly reported. MYD1 is the most common adult-onset form of muscular dystrophy. Excessive daytime sleepiness (EDS) is the most frequent non-muscular complaint in MYD1. As EDS tends to persist despite successful treatment of sleep disordered breathing (SDB) in MYD1 patients, available evidence

suggests that MYD related EDS is primarily caused by a central dysfunction of sleep regulation rather than by sleep fragmentation or SDB. Methods: The subjects were 14 patients with MYD1 (6 male and 8 female, 12-70y. mean 39.4y). Patients gave informed consent for the lumbar puncture. This study was approved by Akita Univ. ethical committee. We checked clinical symptoms, polysomnography, MRI/CT and measured orexin levels. Results: Orexin levels were decreased to low levels in 4 cases (<110pg/ml). Two cases were intermediate levels (110-200pg/ml). Eight cases were normal levels (>200pg/ml). All of them had EDS. PSG result revealed SDB, most patients have complications of severe obstructive sleep apnea syndrome. Six cases were treated with continuous or bi-level positive airway pressure. Conclusion: Since majority of patients with idiopathic narcolepsy show undetectable orexin levels (<40pg/ml), the degree of reduction in MYD1 was small in contrast to them. The EDS symptoms of this disease would not be caused only orexin dysfunction or SDB. Although orexin levels in other genetic neurological conditions with or without EDS are not systematically studied, further studies of the involvement of the orexin system in symptomatic narcolepsy and EDS are helpful to understand the pathophysiological mechanisms for occurrence of EDS.

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Poster

064. Molecular Mechanisms Underlying Sleep and Circadian Rhythms

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Topic: F.08. Biological Rhythms and Sleep

Support: EU-FP7 MC-ITN INSENS (#607616)

NARSAD / BBR II NARSAD/ BBR Independent Investigator Award (#20350),

Title: Effect of "sleep-in-a-dish" on DISC1 protein homeostasis

Authors: *C. KORTH;
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Abstract: **Background:** Sleep is a vital function when restorative processes occur. Disturbances in sleep-wake cycle and in circadian rhythms have been associated with many neurodegenerative

and psychiatric disorders. Previous studies show that sleep deprivation can induce tau phosphorylation, synaptic injury as well as impaired learning and memory in mouse models of Alzheimer disease (Rothman et al., 2012; Di Meco et al., 2014). An *in vitro* model that mimics cellular and molecular changes of sleep and wakefulness state was used (Hinard et al., 2012) to investigate disturbed proteostasis and the consequence for misfolded proteins clearance. Aggregation of DISC1 will be investigated, as it has been shown to be insoluble in brains of a subgroup of mental illness patients (Leliveld et al., 2008). **Methods:** Primary cortical cultures from tgDISC1 rats that exhibit insoluble full-length DISC1 (Trossbach et al., 2016) were treated with a mixture of waking neurotransmitters cocktail (Hinard et al., 2012), and used for biochemical analysis and for electrophysiological recordings, by multielectrode electrode arrays (MEA). **Results:** Electrophysiological recordings in cortical neurons from tgDISC1 stimulated with a waking cocktail show an induction of tonic firing that replaced the synchronized burst-pause firing pattern, as described previously as a sleep-like state (Hinard et al., 2012). Preliminary results indicate that blocking of burst activity leads to an increase in DISC1 aggregation as measured by quantitative Western blot. We will report more details of these analyses. **Conclusion:** We conclude that the "sleep-in-a-dish" model for simulating basic intercellular electrical oscillatory activity is suited to investigate clearance of misfolded proteins and thus a simple model for simulating the effects of sleep on protein homeostasis. References: Di Meco A, et al. *Neurobiol Aging* 2014;35:1813-1820. Hinard V, et al. *J Neurosci*. 2012 Sep 5;32(36):12506-17. Leliveld, SR., et al. *J Neurosci.* 2008 Apr 9;28(15):3839-45. Rothman SM, et al. *Neurobiol Aging* 2012 Apr;33:830.e1-12. Trossbach SV, et al. *Mol Psychiatry*. 2016 Jan 12.

Disclosures: C. Korth: None.

Poster

064. Molecular Mechanisms Underlying Sleep and Circadian Rhythms

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Topic: F.08. Biological Rhythms and Sleep

Support: Marie Curie FP7-IRG Grant PERISLEEP 268273

NeuroCure Cluster of Excellence

Swiss National Science Foundation

Title: Synchronization of cortical dendritic activity during sleep spindles in rodents

Authors: *J. SEIBT[1,2], C. RICHARD[2], J. SIGL-GLÖCKNER[3], N. TAKAHASHI[2], D. DENIS[4], C. BOCKLISCH[2], M. E. LARKUM[2];
[1]Univ. of Surrey, Guildford, United Kingdom; [2]Charité-Universitätsmedizin, Berlin, Germany; [3]Bernstein Ctr. for Computat. Neurosci., Humboldt Univ. of Berlin, Berlin, Germany; [4]Insitute of Physiol., Univ. of Bern, Bern, Switzerland

Abstract: Sleep has now been linked to brain plasticity at many levels, with converging evidences from the molecular, cellular and behavioral fields. Studies in humans and animals support of specific role for spindle oscillations (9-16Hz) in this process but the underlying physiology remain elusive. It has been suggested that spindle bursts promote calcium increase specifically in dendrites, a condition that would favor dendritic plasticity processes (1, 2). Although this hypothesis is supported by computational modeling (1), to date, evidence that such a relation exists during natural sleep is missing.
To address this issue, we measured calcium activity from layer 5 (L5) dendrites in the somato-sensory cortex using 1-photon (fiber-optic) and 2-photon imaging in naturally sleeping rodents. Calcium imaging was combined with electroencephalographic (EEG) recordings to monitor behavioral states and underlying network oscillations.
Our results show that activity of population of dendrites during slow-wave-sleep was specifically correlated with spindle-beta (9-30 Hz) power changes. Two-photon imaging of single dendrites further suggests that this relationship was largely explained by an increase in synchronization of dendritic activity during spindles. Interestingly, this effect was specific to dendrites as L2/3 and L5 cell bodies did not show such correlation.
Our results support the current hypothesis of a direct link between spindles and dendritic activity regulation and further reveal an important, yet unexplored, functional coupling between spindle and beta oscillations (15-30Hz). Further (and ongoing) experiments probing the influences of experience on this relationship will reveal important information on the physiology of spindles and their role in learning and memory.
References:
1. Contreras, D., Destexhe, A. & Steriade, M. Intracellular and computational characterization of the intracortical inhibitory control of synchronized thalamic inputs in vivo. J. Neurophysiol. 78, 335-350 (1997). 2. Sejnowski, T. J. & Destexhe, A. Why do we sleep? Brain Res. 886, 208-223 (2000).

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Poster

064. Molecular Mechanisms Underlying Sleep and Circadian Rhythms

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Topic: F.08. Biological Rhythms and Sleep

Support: John Baldwin Professor Endowment

Neuroscience Program

Lucy McCauliffe Research Grant

Title: Nicotine induction of orexin gene expression in SH-SY5Y cells is modulated by glucose

Authors: J. L. SABO[1], *J. K. MORRIS[2];
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Abstract: Narcolepsy is a chronic sleep disorder characterized by overwhelming day time sleepiness, cataplexy, sleep paralysis, hypnagogic hallucinations, premature REM sleep initiation, and is considered neurodegenerative due to loss of neuropeptide HCRT/ORX containing neurons within the LHA. Prepro-orexin contains two CpG-island rich regulatory regions that are possibly important for differentiation of orexin neurons. Epigenetic alteration of nucleotides in the prepro-orexin promoter induces embryonic stem cell differentiation to an HCRT/ORX neuron while sirt 1 histone deacetylase inhibited differentiation. Prenatal nicotine exposure increased HCRT/ORX positive neuron numbers. In addition, narcoleptic patients treated with nicotine therapy report fewer narcoleptic phenotypes than with amphetamine-based medication. Together, these findings suggest orexin differentiation may occur through several mechanisms, converging on genetic regulation of prepro-orexin gene expression. To determine if nicotine regulates the prepro-orexin gene, a pLuc-wtorx plasmid, containing the minimal mouse prepro-orexin promoter was stably transfected into SH-SY5Y cells and selected with Zeocin (100 µg/mL). SH-SY5Y cells were incubated in EMEM or DMEM, 10% FBS for 12 hours, then replaced by medium containing nicotine (0, 5, 10, 50, 100, 500, 1000 nM). Luciferase concentration of supernatant samples of eight independent wells per group were repeatedly measured (0, 4, 12, 24 hours) by 570 nm luminescence endpoint reading at 22°C, 31°C, and 37°C, and again 10 minutes later. Optimum assay conditions were determined to be at 22°C. Luminescent intensity of nicotine (10 nM) was 39% higher than baseline ($\alpha = 0.05$; $p = 0.026$). Therefore, nicotine has a regulatory effect on the prepro-orexin promoter of the pLuc-wtorx SH-SY5Y cell line. SH-SY5Y stably transfected cells with a control plasmid did not express significant levels of luciferase activity. High glucose (25 mM) DMEM inhibited prepro-orexin expression while low glucose (5.5 mM) EMEM allowed nicotinic upregulation of the prepro-orexin promoter. Orexin expression appears to increase under fasting conditions, which may

increase food foraging behavior in rodents. The ability of nicotine to induce HCRT/ORX expression does not override this mechanism. Thus, regulation of blood glucose levels might be important in narcolepsy management and nicotine treatment efficacy.

Disclosures: J.L. Sabo: None. **J.K. Morris:** None.

Poster

064. Molecular Mechanisms Underlying Sleep and Circadian Rhythms

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UT Departments of Anesthesiology and Psychology

UT Joint Institute for Biological Sciences

Title: Assessing the effects of sleep and wakefulness on the metabolome of mouse cortex using ultra-performance liquid chromatography coupled with high-resolution mass spectrometry

Authors: *A. K. BOURDON[1], G. SPANO[4], M. BELLESI[4], G. TONONI[4], C. CIRELLI[4], P. A. SERRA[5], H. A. BAGHDOYAN[2], R. LYDIC[2], S. R. CAMPAGNA[1,3];
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Abstract: Prefrontal cortical modulation of cognitive processing, alertness, and affective states is disrupted by sleep deprivation. These wide-ranging effects are likely to be modulated by a large number of molecules. Recent data demonstrate the power of an untargeted metabolomic approach for detecting arousal state-dependent changes in scores of known and thousands of unknown molecules in mouse prefrontal cortex (FASEB J 30:1231.1, 2016). The present study is testing the hypothesis that sleep deprivation alters the metabolome in the frontal association cortex (FrA; mouse homologue of prefrontal cortex) and the M1 region of motor cortex. Adult, male C57BL/6J mice (n=19) were implanted with a standard array of electrodes for objectively assessing states of sleep and wakefulness. After recovery and conditioning to being handled,

microdialysis samples were obtained from the FrA and M1 during three experimental conditions: 1) ad lib sleep from 09:00 to 15:00 (n=7 mice; sample n: FrA=166; M1=164); 2) sleep deprivation during the same time interval (n=6 mice; sample n: FRA=143; M1=144); and 3) sleep deprivation from 21:00 to 03:00 (n=6 mice; sample n: FrA 143; M1=144). Dialysis samples were analyzed off-line using ultra-performance liquid chromatography coupled with high-resolution mass spectrometry (UPLC-HRMS) and an established metabolomics method (Anal Chem 82:3212, 2010). Partial least squares-discriminant analysis revealed distinct groups of analytes that clustered as a function of brain region and the three experimental conditions outlined above. Variable importance projection scores identified more than 30 metabolites in FrA and more than 20 metabolites in M1 that were associated with sleep deprivation. Hierarchically clustered heat maps suggest brain-region specific, differential increases and decreases in metabolite levels among treatment conditions. In FrA, behavioral state-specific changes were observed in tyrosine metabolism, inositol metabolism, and valine, leucine, and isoleucine degradation. In M1, changes in behavioral state were associated with differential alterations in major energy pathways, such as metabolism of alanine, aspartate, and glutamate, the TCA cycle, and butanoate metabolism. This study demonstrates the feasibility of using UPLC-HRMS to quantify analytes obtained from mouse cortex by in vivo microdialysis. The approaches used in this study offer a novel opportunity for characterizing behavioral state-dependent changes in biomarkers of brain metabolism.

Disclosures: **A.K. Bourdon:** None. **G. Spano:** None. **M. Bellesi:** None. **G. Tononi:** None. **C. Cirelli:** None. **P.A. Serra:** None. **H.A. Baghdoyan:** None. **R. Lydic:** None. **S.R. Campagna:** None.

Poster

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Topic: F.08. Biological Rhythms and Sleep

Support: KAKENHI/26870547

Miyata Research Grant in E

Title: Effects of glycine injection into trigeminal motor nucleus on jaw-opening excitability duringsleep in rats

Authors: ***R. ODAI-IDE**[1], K. ADACHI[2], S. HINO[3], T. SHIMOYAMA[3], H. SAKAGAMI[2], S. WATANABE[1], G. J. LAVIGNE[4], B. J. SESSLE[5];

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Abstract: The excitability of the jaw-opening reflex (JOR) is depressed during quiet sleep (QS). Because the systemic administration of glycine reduces sleep latency and the incidence of micro-arousals and increases JOR excitability during QS in rats, it has been hypothesized that the glycine receptor system in the trigeminal motor nucleus plays a role in the maintenance of sleep quality. In the present study, we investigated the effects of the microinjection of glycine or vehicle (isotonic saline) on JOR excitability and sleep quality in rats. Under general anaesthesia, 5.5-week-old male Sprague-Dawley rats (n = 20) received wire implantations for electrocardiographic (EKG) recording, for electromyographic (EMG) recording from bilateral anterior digastric (AD) and masseter (MA) muscles, for electroencephalographic (EEG) and electrooculographic (EOG) recording, and for electrical stimulation of the genioglossus muscle. Guide cannulae were implanted into the AD region of the trigeminal motor nucleus (AP: -9.7 mm, Lateral: 1.5 mm, from bregma) for microinjection (0.2 μl/side) of glycine (0.1, 0.2 or 0.4 M) or saline. During recovery from surgery (approximately two weeks), rats were habituated to the observation environment. Sleep-related physiological (e.g., EMG, EOG, EEG and EKG) activities were scored with epochs of 5 sec. During the quiet awake (QW) period before sleep (QWBS), genioglossus stimuli (200 μs) were applied to define the threshold for inducing the JOR in 3 stimulation trials separated by more than 5 min intervals. Then the animal was allowed to sleep freely and the JOR threshold was determined 3 times during QS as well as QW after sleep (QWAS). After the microinjection of glycine or saline, the JOR excitability and sleep-related physiological activities were determined again across the sleep-awake state. After the investigation, the brain was removed under deep anaesthesia and the microinjection site was confirmed in brain slices (20 μm). Compared with saline, each dose of glycine (0.1, 0.2 and 0.4 M) significantly ($P < 0.05$) reduced the JOR threshold during QS. Sleep latency and the incidence of micro-arousals were reduced, but not significantly, by 0.4 M glycine microinjection compared with saline. In all groups (saline; glycine [0.1, 0.2 and 0.4M]), the distribution pattern of EEG frequency bands (δ, θ, α and β) was altered across the sleep-awake state and the δ band was significantly ($P < 0.05$) increased during QS compared with QWBS and QWAS. These findings suggest that the glycinergic system in the AD region of the trigeminal motor nucleus may be involved in the maintenance of sleep quality without affecting brain EEG excitability.

Disclosures: **R. Odai-Ide:** None. **K. Adachi:** None. **S. Hino:** None. **T. Shimoyama:** None. **H. Sakagami:** None. **S. Watanabe:** None. **G.J. Lavigne:** None. **B.J. Sessle:** None.

Poster

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Topic: F.08. Biological Rhythms and Sleep

Support: CIHR grant TGS-109219 to BRS

CIHR Studentship to VS

Title: Sleep deprivation induces changes in dopamine actions & D1 receptor expression in the rat hippocampus

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Abstract: Sleep deprivation is becoming increasingly common among teenagers and shift workers. For memory consolidation, sleep seems to be required. In rat hippocampus, the expression of metabotropic glutamate receptor 1α (mGluR1α) and γ-aminobutyric acid B receptor subunit 1 ($GABA_BR1$), and a hetero-dimerization between the two, are changed following sleep deprivation. Dopamine (D) receptors are also G-protein coupled. D is implicated in modulating sleep and is important in developing therapeutics in psychiatry. The objectives of this study are to examine whether the expression of D receptors and actions of applied D in the hippocampus change following sleep deprivation and, if so, whether these changes are reversible. Male Wistar rats were (a) allowed normal sleep (NS), (b) subjected to a 12-hour sleep deprivation (SD), or (c) sleep-deprived for 12 hours followed by a 24 or 48 hour sleep recovery (SR). Stratum radiatum stimulation-induced field excitatory post-synaptic potentials (fEPSPs), in the CA1 pyramidal layer in hippocampal slices, were measured in the presence of bath-applied D (25-400 μM) to construct a dose-response curve. In slices from NS animals, D depressed the fEPSP in a dose-dependent manner. In slices from SD animals, the depressant action was significantly decreased ($p<0.001$). SR for 24-48 hour was able to reverse this effect. Quinpirole, a D2 receptor agonist, did not have a significant effect on the fEPSP in concentrations of 1-400 μM. D1 receptor expression in the hippocampus was investigated with Western blot and immunohistohemistry. Both methods showed significantly decreased expression of D1 receptor following SD. D1 receptor levels recovered in 24-48 hrs of SR. In co-immunoprecipitation studies, no hetero-dimerization was observed between mGluR1α and D1 receptors. These results indicate that a 12 hr SD results in a down-regulation of D1 receptors and a reduction in D's depressant action in the rat hippocampal CA1 area. Both changes subside following a 24-48 hr

SR. These results have implications for therapies involving drugs targeting dopaminergic pathways in the mesolimbic system.

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Poster

064. Molecular Mechanisms Underlying Sleep and Circadian Rhythms

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Support: NIH OD011185

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Title: Noninvasive sleep monitoring in large scale screening of mouse knockouts (komp2) produces high hit rate with implications for sleep and behavioral studies

Authors: ***B. F. O'HARA**[1], M. SETHI[2], S. JOSHI[2], M. STRIZ[2], N. COLE[3], J. RYAN[3], S. RIZZO[3], M. E. LHAMON[4], A. AGARWAL[4], J. M. DENEGRE[3], R. E. BRAUN[3], V. KUMAR[3], K. D. DONOHUE[4], S. SUNDERAM[1], E. J. CHESLER[3], K. L. SVENSON[3];
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Abstract: Sleep is a critical process well-conserved across mammalian species, and perhaps most animals, yet its functions and underlying mechanisms remain poorly understood. A better understanding of genes and pathways that can influence sleep may shed new light on these functions. In this study, we report novel candidate genes identified by characterizing sleep-wake parameters for the large population of single-gene knockout mice at The Jackson Laboratory, a primary production and phenotyping center for the Knockout Mouse Program (KOMP2). Sleep-wake parameters were measured using a high throughput, non-invasive piezoelectric system that has been validated against EEG/EMG and human observations, and demonstrates a classification accuracy of over 90%. Knockout mice generated on a C57BL/6NJ (B6NJ) background, were monitored for sleep and wake parameters for five days under baseline conditions. Thus far, we have recorded over 5000 mice representing over 300 single gene knockout strains, and more than 1200 B6NJ control mice (males and females). Significant sleep-wake differences in both light and dark phases were found for a number of knockout lines compared to controls. Our study also integrated assessment of breath rates utilizing the piezoelectric system, uncovering a variety of

genes affecting cardio pulmonary traits. Utilizing multiple statistical approaches, more than 40 genes not previously implicated in sleep traits have been identified in this set of knockout mice, suggesting that a high percentage of gene products may influence sleep directly or indirectly. Additionally, sex differences were found for B6NJ mice and many of the knockout mouse strains. B6NJ female mice exhibited shorter bout lengths and less total sleep compared to males. Further studies investigating these genes and their correlation with other phenotypes and interaction with other known "sleep" genes has the potential to provide insight into the pathways regulating sleep and the functions associated with sleep. Incorporating breath rate as part of the screening protocol may be a promising supplemental tool in determining aberrant physiology in these knockout lines.

Disclosures: **B.F. O'Hara:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Signal solutions LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Signal Solutions LLC. **M. Sethi:** None. **S. Joshi:** None. **M. Striz:** None. **N. Cole:** None. **J. Ryan:** None. **S. Rizzo:** None. **M.E. Lhamon:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Signal Solutions LLC. **A. Agarwal:** A. Employment/Salary (full or part-time): Signal solutions LLC. **J.M. Denegre:** None. **R.E. Braun:** None. **V. Kumar:** None. **K.D. Donohue:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Signal Solutions LLC. **S. Sunderam:** None. **E.J. Chesler:** None. **K.L. Svenson:** None.

Poster

064. Molecular Mechanisms Underlying Sleep and Circadian Rhythms

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Topic: F.08. Biological Rhythms and Sleep

Support: NIH Grant R01NS064193

NIH Grant R21NS092926

Title: An intersectional strategy to target nitrergic neurons in the cerebral cortex

Authors: *M. R. ZIELINSKI[1,3], D. N. ATOCHIN[2,4], P. L. HUANG[2], D. GERASHCHENKO[1,3];
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Abstract: In the neocortex, neuronal nitric oxide synthase (nNOS)-containing neurons are divided into type I and type II. Type I cells are more intensely stained in nNOS immunohistochemical or NADPH diaphorase staining procedures and have larger somata than type II cells. Type I nNOS neurons have unique anatomical and physiological properties. This is the only type of GABAergic neurons in the cerebral cortex that form long-range projections within the cortex and between hemispheres. This is also the only currently known type of GABAergic neurons in the cerebral cortex that are specifically activated during slow wave sleep. Studying these neurons using currently available tools represent a challenge. Electrolytic or excitotoxic lesions of type I neurons cannot be performed because they are sparsely distributed throughout the cortex. Moreover, these neurons cannot be targeted using only one genetic marker. We targeted type I neurons in the cerebral cortex based on the overlapping expression between nNOS and somatostatin (SST). Male homozygous nNOS floxed mice were mated with hemizygous, SST-Cre female mice (B6N.Cg-Ssttm2.1(cre)Zjh/J; the Jackson Laboratory). Female offspring that were SST-Cre positive and heterozygous for nNOS floxed transgene were then mated with homozygous nNOS floxed male mice. The offspring from this mating, which were homozygous for the floxed nNOS transgene and either SST-Cre positive or negative, were perfused with formalin. Brains of these mice were processed for nNOS immunostaining. In the mice homozygous for floxed nNOS and expressing Cre under the control of SST promoter, intensely stained nNOS neurons were absent in the cerebral cortex. nNOS staining was also absent in the caudal putamen and olfactory bulb, but we did not observe any differences in nNOS immunostaining in other brain regions in these mice compared to the wild-type control mice. In the mice homozygous for floxed nNOS but SST-Cre negative, normal pattern of nNOS expression was observed. These results indicate that nNOS floxed/SST-Cre double transgenic mice represent an animal model of selective ablation of nNOS expression in type I cells in the cortex. Intersectional nNOS/SST strategy results in a highly selective targeting of type I cells in the cerebral cortex and thus is very useful for studying anatomical and physiological properties of type I neurons.

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Poster

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Topic: F.08. Biological Rhythms and Sleep

Support: NIMH Grant R00MH097792

Title: The neurochemical phenotype of lateral hypothalamic hypocretin/orexin and melanin-concentrating hormone neurons identified through single-cell transcriptional profiling.

Authors: *L. MICKELSEN[1], F. W. KOLLING IV[1,2], B. CHIMILESKI[1,2], C. NORRIS[2], C. E. NELSON[2], A. C. JACKSON[1];
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Abstract: The lateral hypothalamic area (LHA) orchestrates fundamental aspects of behavior including arousal, feeding, metabolism, stress and reward. Through its unique position at the intersection of multiple neural and humoral systems, the LHA drives essential behavioral programs that maintain homeostatic balance in physiology and behavior. Underlying the diverse functions of the LHA is an exceptionally heterogeneous population of neuronal cell types, typically defined by their neuropeptide expression. Two well-described neuropeptidergic neuronal populations, exclusively found in the LHA, are defined by their expression of hypocretin/orexin (Hcrt/Ox) or melanin-concentrating hormone (MCH). Hcrt/Ox and MCH neuronal populations are both important regulators of the sleep-wake cycle and metabolic function. Mounting evidence indicates that these cell populations may be diverse and that single markers may not adequately capture the complexity of their signaling repertoire. Outstanding questions remain concerning the neurochemical phenotype of Hcrt/Ox and MCH neurons and, in particular, their co-expression of other neuropeptides and the machinery for the synthesis and release of the fast amino acid neurotransmitters GABA and glutamate. In the present study, we undertook a single-cell transcriptional profiling approach to further our understanding of the neurochemical phenotype of Hcrt/Ox and MCH neurons. Using transgenic mouse lines that label each cell population, we optimized methods for the microdissection of brain slices, followed by fluorescence-activated cell sorting (FACS) into 96-well plates for the isolation of individual labeled cells. We then carried out gene-specific reverse transcription to convert mRNA into cDNA followed by qPCR to quantify the expression of 48 key genes at single cell resolution. These include housekeeping genes, neuronal and glial markers, neuropeptides, fast neurotransmitter components, transcription factors, receptors and calcium-binding proteins. Our single-cell transcriptional analysis of Hcrt/Ox and MCH neurons revealed unique expression patterns of neuropeptides and fast neurotransmitter components, demonstrating distinct neurochemical phenotypes. The transcriptional profiles of Hcrt/Ox and MCH neurons are consistent with known markers of each cell population while also exhibiting novel expression patterns. Identifying the underlying neurochemical phenotype of Hcrt/Ox and MCH neurons, and diversity within these cell populations, will further our understanding of how these neurons modulate postsynaptic excitability at their targets and participate in diverse behavioral outputs.

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Poster

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VA Merit Award 2I01BX001404

Title: Glutamate, nitric oxide, adenosine and sleep homeostasis: new development

Authors: A. A. LARIN, Y. KIM, S. A. KARPOVA, R. W. MCCARLEY, R. BASHEER, *A. V. KALINCHUK;
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Abstract: Recently we discovered a biochemical cascade which is triggered during sleep deprivation (SD) - initially in the basal forebrain (BF) and later in the prefrontal cortex (PFC) which receives dense projections from the BF. This cascade includes rapid production of intracellular inducible nitric oxide synthase (iNOS)-dependent NO followed by the increase in extracellular adenosine (AD). iNOS→NO→AD cascade is critical in promoting recovery sleep (RS); however, its triggers are unknown. We hypothesized that iNOS induction during SD is triggered by dramatic increase in extracellular glutamate (Glu). Here we investigated the temporal and pharmacological relationship between glutamatergic, nitrinergic and adenosinergic systems in the BF and PFC during long-term SD. Specifically, we: 1) examined the time course of Glu, NO and AD during 8h SD in the BF and PFC; 2) blocked Glu receptors (GluRs) in the BF during SD by infusing a selective inhibitors of NMDAR or AMPAR and measured the changes in SD-induced AD in the BF/PFC and RS; 2) stimulated GluRs in the BF by infusing NMDA or AMPA and measured the changes in AD in the BF/PFC and NREM sleep/delta power. Male rats were implanted with EEG/EMG recording electrodes and microdialysis guide cannulae targeting the BF and PFC for samples collection/drug infusion. Microdialysis samples were collected every 30 min during 8h SD and/or pharmacological manipulations. AD was measured using high performance liquid chromatography (HPLC) coupled with a fluorescence detector; Glu was measured using ultra HPLC (UHPLC) coupled with an electrochemical detector; NO (nitrate/nitrite) was measured using fluorescent assay kit. We observed that in the BF Glu dramatically increased at first 30 min-1 h of SD, followed by the increases in NO/AD at $1^{st}/2^{nd}$ h of SD, respectively. Further, Glu gradually decreased, while AD maximized at 4^{th} h of

SD and remained so till the end of SD. In the PFC, Glu increased within 2 h of SD and NO - at 3rd-4th h of SD; Glu returned to the baseline when AD increased at 5th h of SD. Next, a selective AMPAR inhibitor prevented AD increase during SD in both BF and PFC and attenuated NREM RS, while a selective NMDAR inhibitor did not show any effect on these parameters. Finally, the infusion of AMPA induced the increase in AD in both BF and PFC and NREM sleep/delta power, while NMDA was not effective. In summary, rapid increase in Glu during SD might be a trigger for the induction of iNOS/NO in both the BF and PFC, followed by the AD increase and RS generation. This effect is primarily mediated via AMPAR. The AD increase exerts a negative feedback on the extracellular Glu preventing its further rise during long-term SD which could be toxic for the brain cells.

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Poster

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Topic: F.08. Biological Rhythms and Sleep

Support: MINECO BFU2013- 43741-P

Title: Tuberomammillary histaminergic neurons modulate pontine tegmentum areas involved in wakefulness and REM sleep generation

Authors: *M. GARZON, A. DÍEZ-GARCÍA, A. NUNEZ;
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Abstract: Histaminergic neurons (HA) are exclusively localized in the hypothalamic tuberomammillary nucleus (TMN). Histaminergic system is involved in sleep-wakefulness cycle (SWC) regulation through promotion of cortical activation and wakefulness generation in wake-on centers, such as the locus coeruleus (LC). The aim of the present study was to examine in rats (1) the electrophysiological effect of TMN electrical stimulation on neuronal activity of wake-on centers such as the LC, as well as REM sleep-on centers such as the oral pontine reticular nucleus (PnO), and (2) the anatomical presence of immunohistochemically-identified HA axons and HA receptors (H1 and H3) in those former areas. Electrical stimulation of the TMN with single pulses elicited orthodromic responses in electrophysiologically characterized LC neurons. TMN stimulation with pulse trains produced an increase in LC neurons activity and a decrease in delta-wave activity of the EEG. In contrast, TMN stimulation with pulse trains produced a

decrease in PnO neuron activity. Both excitatory effects in LC and inhibitory effects in PnO were blocked by the systemic (i.p.) administration of the H1 receptor antagonist pirilamine. Immunohistochemical studies showed small varicose HA-immunolabeled axons through the oral pontine tegmentum, including both LC and PnO. H1 and H3 receptors were also identified in both nuclei. In addition, dense HA innervation was detected in the hypothalamic perifornical area (PeFHL), known to be involved in sleep-wake regulation by wakefulness enhancement as also does TMN. Thus, our results indicate that TMN HA neurons have excitatory actions on LC and inhibitory effects on PnO. This suggests that TMN HA projections to both brainstem areas could be part of a neuronal network modulating SWC and arousal by enhancement of wakefulness in LC and suppression of REM sleep in PnO. Synergic TMN and PeFHL actions on these nuclei are also likely mechanisms in sleep-wake modulation. Supported by MINECO BFU2013- 43741-P

Disclosures: **M. Garzon:** None. **A. Díez-García:** None. **A. Nunez:** None.

Poster

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Topic: F.08. Biological Rhythms and Sleep

Support: NIMH (R00MH097792)

Title: Electrophysiological phenotype and optogenetic silencing of histaminergic neurons of the hypothalamic tuberomammillary nucleus in a transgenic mouse line.

Authors: ***A. FUJITA**[1,2], P. BONNAVION[3], M. WILSON[1], L. E. MICKELSEN[1], L. DE LECEA[4], A. C. JACKSON[1];
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Abstract: Neurons that synthesize the monoamine neurotransmitter histamine (HA) exclusively reside in the tuberomammillary nucleus (TMN) of the posterior hypothalamus, extend fibers throughout the central nervous system and are interconnected with other neuromodulatory systems. Multiple lines of evidence suggest that HA neurons are well-positioned to drive transitions between global brain states and influence arousal and attention. *In vivo* single-unit recordings during the sleep-wake cycle have shown that putative HA neurons display slow pacemaking activity (1-5 Hz), which is tightly coupled to behavioral arousal, and exhibit a wake-

specific firing profile. Furthermore, loss-of-function manipulations have implicated HA transmission in cortical activation, behavioral arousal and cognitive function. Given the strong correlation between the excitability of HA neurons and behavioral arousal, we investigated the electrophysiological phenotype of identified HA neurons in brain slices and optogenetically modulated the excitability of HA neurons. In the present study, we undertook a characterization of HA neurons, identified in a transgenic mouse line, Tg(*Hdc*-Cre)IM1Gsat/Mmucd (GENSAT), that expresses cre recombinase in cells that express histidine decarboxylase (HDC), the synthetic enzyme and defining marker for HA neurons. We conducted an anatomical analysis of the specificity of this mouse line by crossing *Hdc*-Cre mice with a cre-dependent ROSA26-tdTomato reporter line to visualize expression of cre recombinase. Hdc-Cre::tdTom neurons were then observed via fluorescent and confocal microscopy and the specificity of this line was confirmed by immunohistochemistry using an anti-HDC antibody. We then carried out a systematic analysis of the passive and active membrane properties of both HA (tdTom+) and non-HA (td-Tom-) neurons in the TMN through cell-attached and whole-cell recordings at physiological temperature. Analysis of these data suggest that the membrane properties of HA cells are relatively uniform, while neighboring non-HA neurons displayed a heterogeneous range of membrane properties. Finally, optogenetic silencing through the selective, cre-dependent expression of archaerhodopsin (Arch) in HA neurons was examined both *in vitro* and *in vivo* as a first step in probing the role of the HA system in arousal. Together, these data suggest that the Hdc-Cre (GENSAT) mouse line may be a valuable transgenic tool for both the identification of HA neurons for electrophysiological recording and for the expression of cre-dependent viral reagents for the optogenetic interrogation of HA neurons and their role in physiology and behavior.

Disclosures: **A. Fujita:** None. **P. Bonnavion:** None. **M. Wilson:** None. **L.E. Mickelsen:** None. **L. de Lecea:** None. **A.C. Jackson:** None.

Poster

065. Appetitive and Incentive Learning and Memory

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 65.01/QQ2

Topic: G.01. Appetitive and Aversive Learning

Support: PSC/CUNY Grant 45-67301

Title: Adolescent exposure to nutritive or artificial sweeteners differentially alters the magnitude of adult sucrose-conditioned flavor preferences in BALB/c and C57BL/6 inbred mouse strains.

Authors: S. LA MAGNA, D. WARSHAW, K. OLSSON, G. FAZILOV, B. ISKHAKOV, A. BURAS, B. RAPP, *R. J. BODNAR;
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Abstract: Sucrose conditions flavor preferences (CFP) relative to saccharin in rodents. Murine genetic variance can determine the magnitude and persistence of such preferences and their underlying neurochemical substrates. Whereas some strains (e.g., BALB/c) display robust sucrose- and fructose-CFP, other strains (e.g., C57BL/6) display smaller sucrose-CFP and a lack of fructose-CFP. Moreover, these strains display initial preferences for non-nutritive sucralose-saccharin solutions relative to fructose that persists with experience with both solutions. Because adolescent exposure to nutritive (e.g., sucrose) and non-nutritive (e.g., saccharin) sweet solutions alters subsequent adult behavioral responsiveness, the present study examined whether early developmental (4 to 8 weeks of age) exposure to sucrose (10%), saccharin (0.2%) or water would differentially alter the magnitude and persistence of sucrose-CFP over 4 weeks in male and female BALB/c and C57BL/6 mice. At 4 weeks of age, 30 BALB/c (15 males (M), 15 females (F)) and 30 C57BL/6 (15 M, 15 F) received burettes of sucrose (10%, 5 M, 5 F of each strain), saccharin (0.2%, 5 M, 5 F of each strain) or water (5 M, 5 F of each strain) solutions in their cages in addition to pre-weighed food and water continuously for four weeks. One week later, all mice were food-restricted to 85-90% of their body weight, and received initial training with an unflavored saccharin (0.2%) solution. In CFP training, food-restricted mice alternately (10 sessions, 1 h) consumed a flavored (CS+, e.g., cherry) 16% sucrose solution and a differently-flavored (CS-, e.g., grape) 0.2% saccharin solution. Two-bottle CS choice tests (1 h) then occurred for five days per week over four weeks. Adolescent male mice of both strains across conditions gained significantly more weight than female counterparts. Adolescent sucrose consumption was significantly higher in BALB/c mice than C57BL/6 mice; the rank-order of adolescent sipper consumption for both strains was: sucrose > saccharin > water. One-bottle CS+/ sucrose training intake was significantly higher than CS-/saccharin training intake across all conditions in young adult BALB/c, but not C57BL/6 mice. Comparable magnitudes of sucrose-CFP in two-bottle choice tests were observed in BALB/c and C57BL/6 mice exposed to water as adolescents. In contrast, BALB/c mice exposed to sucrose or saccharin as adolescents displayed significantly higher magnitudes of sucrose-CFP preferences in two-bottle choice tests than C57BL/6 mice, indicating pronounced murine genetic variance in the effects of adolescent exposure to nutritive or non-nutritive sweeteners upon the magnitude of adult sugar-CFP.

Disclosures: S. La Magna: None. **D. Warshaw:** None. **K. Olsson:** None. **G. Fazilov:** None. **B. Iskhakov:** None. **A. Buras:** None. **B. Rapp:** None. **R.J. Bodnar:** None.

Poster

065. Appetitive and Incentive Learning and Memory

Location: Halls B-H

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Program#/Poster#: 65.02/QQ3

Topic: G.01. Appetitive and Aversive Learning

Support: NIH DA 033404

WSU Alcohol and Drug Abuse Research Program

Title: Prazosin alters fear and cocaine associated memories

Authors: *K.-A. E. REYES, R. P. TODD, B. A. SORG;
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Abstract: Drug addiction is a chronic disorder that creates persistent, unwanted memories. Reconsolidation of a memory is the process of retrieving (reactivating) a memory and then stabilizing it, thereby strengthening it. When a memory is reactivated, it can be disrupted using specific pharmacological agents that weaken the target memory. Here we examined the ability of prazosin, an alpha-1-adrenergic antagonist, to disrupt the reconsolidation of: a) memories associated with cocaine in a cocaine-induced conditioned place preference (CPP) task; b) memories associated with cocaine-induced self-administration; and c) memories associated with conditioned fear, a model used for post-traumatic stress disorder. Previous findings show that prazosin can enhance extinction, the process of diminishing a memory by creating a new memory that overrides the first, of CPP and fear conditioning (Bernardi and Lattal, 2010; Bernardi and Lattal, 2012). Here we sought to disrupt the reconsolidation of a cocaine-associated memory using both CPP and self-administration to understand the extent to which prazosin can disrupt appetitive memories and determine the ability of prazosin to disrupt the reconsolidation of an aversive memory using fear conditioning. Our findings show that prazosin enhances the extinction of an appetitive memory for a CPP task, disrupts reconsolidation of an aversive fear memory, and promotes reconsolidation in a self-administration model. These findings suggest that prazosin can potentially be used as a drug treatment for diminishing an addiction memory via extinction and can be used to disrupt the reconsolidation of stress related memories in rats.

Disclosures: **K.E. Reyes:** None. **R.P. Todd:** None. **B.A. Sorg:** None.

Poster

065. Appetitive and Incentive Learning and Memory

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Program#/Poster#: 65.03/QQ4

Topic: G.01. Appetitive and Aversive Learning

Support: NIH Grant R00 DA024719

NIH Grant F32 DK102294

Title: Investigation of the necessity of medial prefrontal cortex pyramidal neurons for food reward seeking and ingestion

Authors: *D. M. WARTHEN[1], A. BRUNAL[1], K. L. GASSMANN[1], N. P. ROGERS[1], N. K. SRINIVASA[1], L. S. ZWEIFEL[2], M. M. SCOTT[1];
[1]Pharmacol., Univ. of Virginia, Charlottesville, VA; [2]Psychiatry and Pharmacol., Univ. of Washington, Seattle, WA

Abstract: Dysfunction in the prefrontal cortex is associated with impulse control deficits and maladaptive reward seeking. We and others have shown that manipulation of neuronal activity in the medial prefrontal cortex (mPFC) alters reward seeking behavior in operant conditioning paradigms. Pyramidal neurons form the final output pathway from the mPFC, and therefore presumably mediate all effects of mPFC activity on behavior and cognition. While these neurons innervate a wide variety of targets throughout the brain, the necessity and sufficiency of specific subcortical mPFC projections in the regulation of feeding behaviors remains poorly understood. In prior work we showed that stimulation of mPFC-to-accumbens neurons using a G_q-coupled designer receptor (hM3d(Gq) DREADD) is sufficient to increase operant responding for a food reward in mice, without altering unconditioned food intake behavior. In our current work we assess the necessity of mPFC pyramidal neurons in general, while also selectively targeting those that project to the nucleus accumbens, in the regulation of food seeking and unconditioned feeding behavior. To produce neuron-selective silencing, we used an adeno-associated virus encoding a Cre recombinase-dependent Caspase-3 (AAV-flex-taCasp3-TEVp). When activated, this construct engages an apoptotic pathway, ablating the targeted cell. In initial experiments, we examined how a broad reduction in pyramidal neuron activity affects feeding, through delivery of AAV-flex-taCasp3-TEVp in combination with an AAV encoding Cre under control of the CamKIIα promoter to the PFC. To determine how specific subcortical projections from the PFC affect feeding behavior, we then selectively deleted mPFC neurons projecting to the nucleus accumbens, through mPFC injection of AAV-flex-taCasp3-TEVp and nucleus accumbens injection of canine adenovirus encoding Cre recombinase. Subsequently, we examined whether ablation of these neurons suppressed operant responding for food reward while also affecting binge-like overeating. Results from these studies complement our initial work, providing a clear

picture of the necessity and sufficiency of the PFC in regulating both the drive to feed and the act of food consumption.

Disclosures: **D.M. Warthen:** None. **A. Brunal:** None. **K.L. Gassmann:** None. **N.P. Rogers:** None. **N.K. Srinivasa:** None. **L.S. Zweifel:** None. **M.M. Scott:** None.

Poster

065. Appetitive and Incentive Learning and Memory

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 65.04/QQ5

Topic: H.01. Animal Cognition and Behavior

Title: Environmental and pharmacological modulation of novelty habituation in rats - The rising of self-grooming as a de-arousal indicator

Authors: *M. ROJAS[1,2], B. MÉNDEZ[2], J. FORNAGUERA[2,3], J. C. BRENES[4,2];
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Abstract: Habituation usually refers to as the decrease of a response after repeated or prolonged presentations of a given stimulus. This phenomenon has been observed along different life forms (e.g. amoeba, plants, rats, humans). In animals, habituation is considered as a non-associative learning process, which serves as a first-order attentional system filtering information depending on its biological salience. In rats, habituation is normally evaluated through the analysis of exploratory behavior displayed in a wide, bright, novel arena, as the open-field test (OF). There, risk-assessment (RA) behaviors, including vertical and horizontal exploration, are intensely emitted during the first minutes of the OF. As time passes and no threats are encountered, the RA behaviors diminish and self-grooming (SG) gradually increase. The appearance of a non-exploratory, self-oriented behavior suggests that the defensive system has yielded control to a de-arousal, compensatory system. In support to the later, we have found that treatments that increase OF habituation, such as environmental enrichment (EE) or repeated OF exposures, also increase SG. Such treatments, interestingly, differ qualitatively and quantitatively in the way they potentiate SG. In the first experiment, we will show how EE modulates OF-habituation, specifically how the transition from defensive-to non-defensive behaviors takes place. Also, we will describe the differences in time course, subtypes, and syntaxes of SG between EE- and non-EE rats repeatedly exposed to the OF. In a second experiment, we tested whether pharmacological impairment of OF-habituation after the administration (i.p.) of scopolamine (muscarinic antagonist) or MK-801 (NMDA antagonist) is able to retard or abolish the appearance of SG. Amnesic drugs were daily administered during four consecutive days 20-min

prior to a 15-min OF. On days five to seven, rats were given vehicle and tested as previously mentioned. Results will be discussed in terms of the translational value of SG and OF-habituation as targets for developing new pharmacological treatments and to uncover the neurobiological basis of neuropsychiatric disorders related with sensory gating deficits, increased defensiveness, and pronounced stereotypies as observed in autism spectrum disorders, schizophrenia, and obsessive-compulsive disorder.

Disclosures: **M. Rojas:** None. **B. Méndez:** None. **J. Fornaguera:** None. **J.C. Brenes:** None.

Poster

065. Appetitive and Incentive Learning and Memory

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 65.05/QQ6

Topic: H.01. Animal Cognition and Behavior

Title: Incentive motivation and neurobehavioral plasticity: the effects of unpredictable environmental enrichment in rats

Authors: *J. C. BRENES[1,2], M. SÁNCHEZ[3], M. ROJAS-CARVAJAL[3], J. FORNAGUERA[4], A. SEQUEIRA[5];
[1]Univ. of Costa Rica, Montes DE Oca, Costa Rica; [2]Neurosci. Res. Ctr., Univ. of Costa Rica, San Jose, Costa Rica; [3]Neurosci. Res. Ctr., [4]Biochem. Dept., [5]Natl. Inst. of Hlth. Res., Univ. of Costa Rica, Montes DE Oca, Costa Rica

Abstract: Environmental Enrichment (EE) is a model to study the relationships between behavior and experience-dependent changes in the brain. As compared to standard housing (SH), the idea behind EE is to ensure 1) greater levels of physical, social, and cognitive stimulation, 2) the opportunity of displaying more elaborated behaviors by fulfilling some ethological needs, and 3) the reduction of captivity stress. In this sense, having access to an EE cage may be rewarding for rats. It can be assumed that once EE is experienced, motivation to get enriched may gradually increase. Although the motivational properties of exercise have widely been tested in rodents exposed to running wheels, no evidence of such effects have been obtained in EE rats. It is well known that uncertainty about reward probability or magnitude may translate into stronger attribution of incentive salience to reward-related cues. To evaluate the motivation to get access to the EE cage, a group of rats were constantly kept in EE (CONEE), except during some hours three or four times per week, when rats were moved to standard cages in order to increase motivation for EE cage. Another group (RAMEE) was enriched in a semi-random manner and only during the 50% of total CONEE time (i.e. one month). Access to EE took place through a guillotine-controlled start box (30x30 cm) attached to the EE cages. There,

anticipatory activity and 50-kHz ultrasonic vocalizations (USV) were monitored twice per week before animals entered the cage. Both EE groups were tested simultaneously and were compared to a SH group. SH rats were also placed in the start box, but they had access to a standard cage. Animals were screened behaviorally (e.g., USV, open-field, and Barnes tests), and afterwards the expression of several genes (e.g., BDNF, CREB, p250gap) related to neural plasticity were measured in the medial prefrontal cortex, nucleus accumbens, and hippocampus. Data will be presented and discussed in terms of how uncertainty of motivationally relevant stimuli could boosted the effects of EE. Also, our data may provide evidence about how the benefits derived from EE may depend on individual differences in attributing motivational salience to such stimulation.

Disclosures: J.C. Brenes: None. **M. Sánchez:** None. **M. Rojas-Carvajal:** None. **J. Fornaguera:** None. **A. Sequeira:** None.

Poster

065. Appetitive and Incentive Learning and Memory

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Topic: G.01. Appetitive and Aversive Learning

Support: NIH DA033404

NIH DA016285

Title: Environmental enrichment alters perineuronal net staining in the rat prefrontal cortex following abstinence from sucrose self administration

Authors: *B. A. SORG[1], M. L. SLAKER[1], J. BARNES[2], J. W. GRIMM[3];
[1]Integrative Physiol. and Neurosci., Washington State Univ., Vancouver, WA; [2]Washington State Univ., Pullman, WA; [3]Behavioral Neurosci., Western Washington Univ., Bellingham, WA

Abstract: Perineuronal nets (PNNs) are aggregations of extracellular matrix molecules that form structures around a subset of GABAergic interneurons in the cortex. The staining intensity of the PNN marker, *Wisteria floribunda* agglutinin (WFA) is related to plasticity, where brighter WFA staining is associated with limited plasticity and dimmer WFA staining is associated with enhanced plasticity. Environmental enrichment (EE) reinstates plasticity during adulthood and diminishes both drug- and sucrose-seeking behavior in rodents. Long-term EE also decreases staining intensity of WFA within the visual cortex and cerebellum. We determined the impact of EE during abstinence on WFA staining intensity in the prefrontal cortex (PFC) of adult Long-

Evans rats trained to self-administer sucrose. PNNs within the prelimbic (PL), infralimbic (IL), and orbitofrontal (OF) regions of the PFC were assessed for staining intensity and number after 1 d or 30 d EE during abstinence followed by a cue-induced reinstatement test. Rats exposed to standard housing during 30 d abstinence from sucrose self-administration had brighter WFA staining within the IL compared to rats exposed to standard housing during 1 d abstinence. Rats exposed to EE prior to a cue-induced reinstatement test had brighter WFA staining in all regions examined compared with rats maintained in standard housing. The intensity of PNN staining within the PL corresponded to the total number of lever presses across all sucrose self-administration sessions. Conversely, naïve rats given 1 day of EE had decreased WFA staining intensity in the PL, no change in staining intensity the IL, and increased staining intensity in the OF. These findings demonstrate that PNNs within the PFC are dynamically influenced by time of abstinence, duration of EE exposure, and cue-induced reinstatement. These findings may have implications for reward-related plasticity and lasting changes in the PFC during abstinence.

Disclosures: **B.A. Sorg:** None. **M.L. Slaker:** None. **J. Barnes:** None. **J.W. Grimm:** None.

Poster

065. Appetitive and Incentive Learning and Memory

Location: Halls B-H

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Program#/Poster#: 65.07/QQ8

Topic: G.01. Appetitive and Aversive Learning

Support: NDSEG Fellowship (Department of Defense)

K08-DA037912-01 (NIDA)

NARSAD Grant 20829 (Brain and Behavior Research Foundation)

Title: Sign-tracking is difficult to extinguish and resistant to multiple cognitive enhancers

Authors: *C. J. FITZPATRICK[1], T. GEARY[2], J. F. CREEDEN[2], J. D. MORROW[2];
[1]Dept. of Psychiatry, [2]Univ. of Michigan, Ann Arbor, MI

Abstract: Loss of control over drug-seeking behavior and relapse in response to drug-related cues are hallmarks of addiction. Clinical studies have generally found that conditioned responses (CRs) to drug-related cues are resistant to extinction. Sign-tracking is a type of Pavlovian conditioned approach (PCA) behavior that can develop in response to repeated presentation of a conditioned stimulus (CS; e.g., a lever) preceding response-independent presentation of an unconditioned stimulus (US; e.g., food pellet delivery). Sign-tracking CRs are directed toward the CS, as opposed to goal-tracking CRs, which are PCA behaviors directed toward the US.

Moreover, sign-tracking is thought to underlie some aspects of addictive behavior, due to evidence that sign-tracking is difficult to suppress or control and that animals prone to sign-track also display more robust addiction-related outcomes such as cue-induced reinstatement of drug self-administration. We compared the effects of extinction training on sign-tracking and goal tracking CRs using a PCA procedure that exploits individual differences, such that identically trained rats developed one of three patterns of CRs: sign-tracking, goal-tracking, or an intermediate response (both CRs). We found that, while goal-tracking extinguishes completely within four days, sign-tracking behavior persists for over three weeks during extinction training. Moreover, two weeks after the final extinction session, sign-trackers but not goal-trackers show spontaneous recovery of their conditioned responding. In addition, we demonstrated that three different cognitive enhancers known to facilitate extinction of other learned behaviors—sodium butyrate (a histone deacetylase inhibitor), D-cycloserine (an NMDA receptor partial agonist), and fibroblast growth factor 2 (a pro-synaptic neurotrophic factor)—do not facilitate extinction of sign-tracking. These results indicate that sign-tracking is highly resistant to extinction training even when augmented with three classes of cognitive enhancers that are being investigated as pharmacotherapies for addicted patients. This work highlights one potential source of difficulty when attempting to control addictive behaviors.

Disclosures: **C.J. Fitzpatrick:** None. **T. Geary:** None. **J.F. Creeden:** None. **J.D. Morrow:** None.

Poster

065. Appetitive and Incentive Learning and Memory

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 65.08/QQ9

Topic: G.01. Appetitive and Aversive Learning

Support: NHMRC Grant 1047899

Title: The orexin system is not involved in operant palatable food seeking

Authors: *S. Y. KHOO[1], G. P. MCNALLY[2];
[1]Sch. of Psychology, Univ. of New South Wales, Sydney, Australia; [2]Sch. of Psychology, Univ. of New South Wales, Sydney, Australia

Abstract: Background: The orexin system has been implicated in appetitive motivation and particularly for highly salient reinforcers. Orexin receptor antagonists have been shown to reduce operant alcohol, cocaine and opioid seeking behaviour in rodents, however results involving natural rewards have been inconsistent. We therefore examined the impact of dual and single

orexin receptor antagonists on operant self-administration of palatable food. Method: Male Sprague-Dawley rats (n = 14) were implanted with chronically indwelling guide cannulae targeting the lateral ventricle. After recovery, the received 10 days of self-administration training where each active lever press (FR1) was reinforced with one 45 mg palatable food pellet (23% fat, 44% carbohydrate, F06162, Bioserv). The effect of the 30µg of the dual orexin receptor antagonist TCS1102 on self-administration was tested at FR1, FR5, FR10 and progressive ratio schedules. The selective OX_2 receptor antagonist, TCSOX229 (100µg), and the OX_1 antagonists ACT335827 (150µg) and SB-334867 (30µg), were then tested against FR10 self-administration. Results: Central administration of 30µg TCS1102 or its vehicle had no significant effect on operant responding for palatable food at FR1, FR5 or FR10 schedules of reinforcement. TCS1102 also had no effect on progressive ratio responding or breakpoint. The single receptor antagonists, TCSOX229, ACT335827 and SB334867 also had no effect on FR10 responding for palatable food. Conclusion: The orexin system is not necessary for palatable food seeking at multiple schedules of reinforcement. The orexin system is also not involved in motivation for palatable food, as measured by a progressive ratio. Additionally, this is unlikely to be due to any limitations of the dual orexin antagonist TCS1102, because commonly used single orexin receptor antagonists also did not affect FR10 responding.

Disclosures: **S.Y. Khoo:** None. **G.P. McNally:** None.

Poster

065. Appetitive and Incentive Learning and Memory

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Program#/Poster#: 65.09/QQ10

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant AA014925

Title: Neuronal correlates of goal-directed and habitual reward seeking in dorsal striatum

Authors: ***Y. VANDAELE**[1], J. M. RICHARD[1], P. H. JANAK[1,2];
[1]Dept. of Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD; [2]The Solomon H. Snyder Dept. of Neurosci., Johns Hopkins Sch. of Medecine, Baltimore, MD

Abstract: Balance between goal-directed and habitual response strategies is necessary for efficient and flexible decision-making. An inability to “break the habit” and re-engage in a goal-directed strategy when circumstances change may render a behavior impervious to negative consequences, and could be involved in several psychopathologies including alcohol and substance use disorders. While the dorsomedial (DMS) and dorsolateral (DLS) striatum are

necessary for goal-directed and habitual responding, respectively, the neuronal correlates of these two response strategies in the dorsal striatum remain unclear. To address this question, we obtained simultaneous *in vivo* single unit recordings in the DMS and DLS within an instrumental procedure, early and late in training. In this discrete-trial procedure, rats must wait for lever insertion and complete a sequence of 5 lever presses to obtain a reward (sucrose 20% or grain-based pellet). We compared neural activity in DMS and DLS during acquisition of the task, after overtraining, and during a satiety-induced devaluation test, in order to distinguish the relative contributions of these structure to the expression of goal-directed and habitual response strategies. Using statistical classification approach, we found that activity in DMS and DLS during learning and after overtraining is correlated with separable aspects of sequence learning. The largest group of DLS neurons progressively shown sustained activity throughout the full action sequence, from the initiation to the termination. In contrast, DMS activity is predominantly linked to the cue signaling reward availability, lever insertion, and is frequently inhibited during the sequence of lever presses. The findings overall suggest that activity in DLS neurons may be more closely tied to completion of action sequences. These findings raise the possibility that differential striatal encoding of sequence learning contributes to the development of outcome-insensitive habits.

Disclosures: Y. Vandaele: None. **J.M. Richard:** None. **P.H. Janak:** None.

Poster

065. Appetitive and Incentive Learning and Memory

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 65.10/QQ11

Topic: G.01. Appetitive and Aversive Learning

Support: NIDA IRP

Title: Blocking is unaffected by increasing outcome value via changes in satiety state - implications for the role of value-based errors and model free systems in regulating learning

Authors: *M. P. GARDNER, J. CONROY, G. SCHOENBAUM;
Cell. Neurobio. Res. Br., NIDA IRP, Baltimore, MD

Abstract: A major division among models of learning is how value prediction is tracked. This difference is evident in the distinct mechanisms underlying how a cue becomes associated with the value of a reward. Model-free theories consider a predictive cue to store reward value independently of the predicted outcome; whereas model-based theories propose a direct association between the predictive cue and a full representation of the reward. A large body of

evidence suggests that both of these systems drive behavior. In particular, we know that rats use model-based information to change their conditioned responding if the predicted reward's value changes. Here we test whether this same information can also serve to modulate learning by using an unblocking design. To do this, we trained mildly hungry rats that a cue predicted food reward. Subsequently we paired that cue with a novel cue when the rats were much hungrier. Because hunger should increase the value of the reward, the question was 1) whether this increase in value would be predicted by the first cue, allowing this cue to block learning to the added cue or 2) whether the ability of the first cue to predict reward value for the purpose of changing behavior would not translate over to modulate learning signals. In this latter case we would expected to observe a failure of blocking to the new cue. Here we will present initial pilot data that favors the former result. Specifically, with appropriate control conditions included, we found that learning for the new cue was blocked, consistent with the proposal that value predictions from model-based systems act generally and in rapid sequence to first guide behavior and then modulate error driven learning. Coupled with recent data showing that such model based predictions are reflected in dopaminergic error signals, these data provide concrete new evidence for the complexity of associative learning mechanisms. As a next step, we hope to take brain regions considered to contribute to encoding and retrieval of model-based information, such as the orbitofrontal cortex, offline during different critical periods of the task in order to determine whether behavior shifts to a model-free strategy.

Disclosures: **M.P. Gardner:** None. **J. Conroy:** None. **G. Schoenbaum:** None.

Poster

066. Dopamine in Reward: In Vivo Activity Recording and Manipulation Studies

Location: Halls B-H

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narSAD young investigator award

boettcher young investigator award

Title: *In vivo* electrochemical and optogenetic assessment of accumbal dopamine release events in a novel behavioral economics based food-seeking task

Authors: *S. SCHELP, K. J. PULTORAK, D. ISSACS, R. DAS, E. B. OLESON;
Univ. of Colorado, Denver, Denver, CO

Abstract: The mesolimbic dopamine system is strongly implicated in motivational processes. Currently accepted theories suggest that transient mesolimbic dopamine release events are involved in assessing the value of reward predictive stimuli and/or in generating motivated action sequences directed toward obtaining reward. During the pursuit of reward, critical associations are formed between the reward and otherwise neutral stimuli that begin to predict reward availability. Through these experiences, dopamine neurons, which initially represent the receipt of reward, begin to represent its earliest conditioned predictor (i.e., cue). The resulting concentration of dopamine release scales proportionally to the magnitude of reward predicted. Here, we are investigating the role of cue- and reward-evoked dopamine release on cue-motivated food seeking. To address this research question we developed a novel behavioral economics food-seeking task. In this task, food is provided to rats across 10 different unit-prices (i.e., response requirement/reward magnitude). Importantly, in this task, multiple pairings (>10/price/session; unlike with progressive ratio schedule) occur between each unit-price, reward and its predictive cue. Using fast-scan cyclic voltammetry we first determined that the concentration of accumbal dopamine time-locked to cue presentation decreases as a function of unit-price in this task. We next sought to assess the effect of optically augmenting release both at reward delivery and cue presentation. We selectively activated channelrhodopsin-2 expressing dopamine neurons within the ventral tegmentum during either cue or reward presentation (order counter balanced across animals). Our data reveal that optically facilitating dopamine release at the cue decreases motivation for food; whereas, facilitating release at reward delivery increases motivation for food. Interestingly, optically augmenting release at both cue presentation and reward delivery decreased response latency, consistent with an invigoration of responding that might be dissociable from value-based changes in motivation. It is possible that augmenting cue-evoked dopamine release decreases motivation in our task because we are violating the animal's expectation (i.e., the animal receives less than expected) and vice versa. Together these findings suggest that cue- and reward-evoked dopamine release play a causal role in action initiation, yet oppositely influence motivation in value-based behavioral economics based tasks.

Disclosures: S. Schelp: None. **K.J. Pultorak:** None. **D. Issacs:** None. **R. Das:** None. **E.B. Oleson:** None.

Poster

066. Dopamine in Reward: In Vivo Activity Recording and Manipulation Studies

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 66.02/QQ13

Topic: G.02. Motivation

Title: 99mTc-HMPAO SPECT imaging of brain-wide functional networks underlying optogenetic self- and passive stimulation in mice

Authors: *M. J. BROCKA[1], M. LIPPERT[2], A. KOLODZIEJ[2], T. WEIDNER[2], D. VINCENZ[2], J. TEGTMEIER[2], T. WANGER[2], A. OELSCHLEGER[2], F. STOEBER[2], F. ANGENSTEIN[2], J. GOLDSCHMIDT[2], F. OHL[2];
[1]Systems Physiol., Leibniz Inst. For Neurobio., Magdeburg, Germany; [2]Leibniz Inst. for Neurobio., Magdeburg, Germany

Abstract: Optogenetic methods for brain stimulation are increasingly applied in rodents for manipulating behavior and analyzing connectivity.The availability of appropriate transgenic mouse lines and established behavioral assays have contributed to widespread adoption of optogenetic methods, particularly for the study of neural correlates of motivation and reward. The VTA with its dopaminergic neurons is a key brain area for motivation and reward, and has been intensively studied using optogenetics. This self-stimulation has a strong appetitive and motivating value—if an animal can self-administer intracranial VTA stimulation, for example through lever pressing, it will do so even under adverse conditions. This self-stimulation therefore has a strong appetitive and motivating value. However, the VTA can also be stimulated passively — a condition in which no motivation is required. These two experimental paradigms can hence be used to investigate the difference in neuronal activity states of the VTA-reward network during a motivated state and an unmotivated state. Here we used a novel protocol for single-photon emission computed tomography (SPECT) imaging of regional cerebral blood flow (rCBF) to map the brain-wide activity differences resulting from optogenetic intracranial self-stimulation (optoICSS) and optogenetic intracranial passive stimulation (optoICPS). We expressed ChR2 targeted to dopaminergic midbrain neurons in the VTA of Th::Cre mice and trained the animals in an optoICSS task. Following training, mice were implanted with jugular vein catheters and intravenously infused with the rCBF tracer 99m-technetium hexamethylpropylene amine oxime (99mTc-HMPAO) during optoICSS, optoICPS, and baseline condition. We precisely matched the stimulation pattern and intensity for optoICSS and optoICPS to avoid any differences in neuronal stimulation. Mice were then imaged to measure the neuronal network activations during these three conditions. Compared to previous studies, which did not precisely match self and passive stimulation and used rather unspecific electrical stimulation, our findings show a surprisingly large similarity in VTA-related activity upon self and passive stimulation. The differences found are much more compatible with a higher level cognitive effect rather than with large brain wide differences in the two conditions.

Disclosures: **M.J. Brocka:** None. **M. Lippert:** None. **A. Kolodziej:** None. **T. Weidner:** None. **D. Vincenz:** None. **J. Tegtmeier:** None. **T. Wanger:** None. **A. Oelschleger:** None. **F. Stoeber:** None. **F. Angenstein:** None. **J. Goldschmidt:** None. **F. Ohl:** None.

Poster

066. Dopamine in Reward: In Vivo Activity Recording and Manipulation Studies

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Topic: G.02. Motivation

Support: NIH R01 DA019473

NIDA Diversity Supplement to DA019473

Klarman Foundation

Title: Exploring the neural mechanism by which optogenetic stimulation of ventral tegmental area dopamine neurons prevents extinction of cued approach behavior.

Authors: *C. M. REYES, S. M. NICOLA;
Albert Einstein Col. of Med., Bronx, NY

Abstract: When an environmental stimulus predicts a reward at a specific location, animals learn to approach that location in response to the stimulus. When the stimulus no longer predicts reward, the approach behavior extinguishes. Recordings from dopamine (DA) neurons during conditioned approach tasks have demonstrated that DA neurons in the ventral tegmental area (VTA) burst in response to the delivery of an unexpected reward; conversely, when predicted rewards are omitted, there is a characteristic pause in firing. This suggests that DA neurons encode reward prediction errors (RPEs), which serve to update the current state and alter the strength of cue-reward associations depending on the valence of the RPE (positive or negative). While RPEs presumably lead to changes in response probability, the neural mechanisms downstream of the RPE signal that mediate this behavior remain unknown.
Many NAc neurons exhibit cue-evoked excitations that are required for approach behavior. We hypothesized that negative RPE signals carried by the pause in VTA DA neuronal activity may result in decreased cue-evoked firing of NAc neurons in subsequent trials, lowering the probability of cued approach. To test this hypothesis, we used a conditioned approach task with a reward omission paradigm, and recorded from neurons in the NAc in *Th::Cre* rats that express channelrhodopsin in VTA DA neurons. During a 30 min baseline period, auditory cues predicted the availability of a sucrose reward contingent on entry into a receptacle. Sucrose reward was then omitted for the rest of the 90 min session, leading to a decrease in responding. Using multi-electrode recording of NAc unit firing, we found a reduction in the magnitude of cue-evoked excitations of NAc neurons on trials subsequent to the reward omission. Additionally, we found that optical stimulation of VTA DA neurons at the time when reward was omitted was sufficient to prevent extinction. By recording from NAc neurons during optical stimulation trials, we will be able to determine if the reduction in cue-evoked excitations is prevented, thereby suggesting a

mechanism by which phasic dopamine release modulates cue-evoked NAc neuronal excitations on subsequent trials.

Disclosures: **C.M. Reyes:** None. **S.M. Nicola:** None.

Poster

066. Dopamine in Reward: In Vivo Activity Recording and Manipulation Studies

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Topic: G.02. Motivation

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NARSAD young investigator award

Boettcher young investigator award

Title: *In vivo* electrochemical and optogenetic assessment of accumbal dopamine release events in a novel behavioral economics based footshock avoidance task

Authors: ***K. J. PULTORAK**, S. A. SCHELP, D. ISSACS, R. DAS, E. B. OLESON; Univ. of Colorado Denver, Denver, CO

Abstract: The mesolimbic dopamine (DA) system has historically been implicated in motivational processing under appetitive contexts. Within an appetitive context, mounting evidence supports that transient accumbal DA release events increase in response to reward predictive cues, represent intrinsic reward value, and causally modify reward-seeking. Less, however, is known regarding the role DA plays in an aversive context. Using fast-scan cyclic voltammetry (FSCV), we recently demonstrated that accumbal DA release events are increased in response to aversive stimuli both when animals are presented with a cue signaling the opportunity to actively avoid electric foot shock as well during successful avoidance behavior. Here we investigate whether DA concentration scales as a function of the value of footshock avoided and whether optical stimulation of DA neurons causally modifies the price animals are willing to pay to avoid footshock. In order to assess these research questions, we first developed a novel behavioral economics task in which rats are provided the opportunity to operantly avoid electrical footshock across epochs wherein unit-price (response requirement/mA shock avoided) increases on a semi-exponential array. In congruence with previous appetitive research, the concentration of DA observed at the avoidance-predictive cue as well as at successful avoidance

decreased as a function of increasing unit-price. To assess causality, we optogenetically activated channelrhodopsin-2 expressing DA neurons within the ventral tegmental area to selectively augment DA release at either cue onset or during successful avoidance. Our preliminary results suggest that augmenting release at cue onset decreases the maximal price paid to avoid footshock; whereas augmenting release at successful avoidance increases the maximal price paid to avoid footshock. Next, we sought to assess the role of anxiety in our behavioral economic avoidance task by pre-treating rats with the benzodiazepine, diazepam. While at low doses, diazepam increased the maximal price animals pay to avoid footshock, a decrease in avoidance occurred in a high dose range—an effect perhaps due to the sedative effects produced by high dosages. Together, these findings suggest that transient, accumbal DA release events play an integral role in the assessment of and behavioral response to aversive stimuli as well as suggest that the neuronal circuitry underlying anxiety strongly influence the valuation of avoidance.

Disclosures: K.J. Pultorak: None. **S.A. Schelp:** None. **D. Issacs:** None. **R. Das:** None. **E.B. Oleson:** None.

Poster

066. Dopamine in Reward: In Vivo Activity Recording and Manipulation Studies

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

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Topic: G.02. Motivation

Support: Canadian Institute of Health Research Institute of Neuroscience and Mental Health

Title: Modulation of nucleus accumbens dopamine efflux and reward-seeking behaviour in rats following electrical and optogenetic stimulation of the afferent glutamate projection from the ventral subiculum

Authors: *D. LINDENBACH, G. VACCA, A. HARATIKIA, J. K. SEAMANS, A. G. PHILLIPS;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: In large part, appetitive behaviours are motivated by dopamine (DA) released in the nucleus accumbens (NAc). Glutamate projections from the ventral subiculum (hippocampus) can modulate basal levels of DA in the NAc, providing a mechanism by which environmental cues processed by the hippocampus can motivate behaviour. Transient activation of the ventral hippocampus causes protracted changes in DA efflux in the NAc, facilitating relapse to substance abuse, recapitulating psychomotor symptoms of schizophrenia and modifying affect (Grace, 2010). Given the prolonged elevation of DA efflux observed following brief trains of

brain-stimulation to the ventral subiculum, we tested the hypothesis that enhanced dopaminergic tone may be mediated in part by changes in DA transporter function, which in turn may increase reward-seeking behaviour. The ventral hippocampus was stimulated electrically or optically using bipolar electrodes or channelrhodopsin, respectively. Efflux of DA in the NAc was measured via microdialysis; methylphenidate was used to pharmacologically block the DA transporter. Changes in behaviour were assessed with motion chambers and a progressive-ratio lever-pressing task for a fixed food reward. Both electrical and optical stimulation of the ventral hippocampus enhanced DA efflux in the NAc. Of particular interest, methylphenidate treatment attenuated stimulation-induced DA efflux. The fact that a DA transport blocker decreased DA release is striking and provides compelling preliminary evidence for the hypothesis that the ventral subiculum increases DA release by altering DA transporter function in the NAc. Preliminary behavioural results showed that electrical stimulation enhanced locomotion and lever-pressing for food reward. Surprisingly, optical stimulation did not impact spontaneous movement. Overall, these data suggest that activation of the ventral hippocampus-->NAc glutamate pathway potentiates DA signalling via action potential independent mechanisms. Modulating DA transporter activity may be a therapeutic target for the treatment of disorders including substances abuse, schizophrenia and depression.

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Poster

066. Dopamine in Reward: In Vivo Activity Recording and Manipulation Studies

Location: Halls B-H

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Support: NSERC RGPIN 308-2011

Concordia University Research Chairs Program

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Title: On the forms of learning supported by rewarding optical stimulation of dopamine neurons

Authors: *I. TRUJILLO-PISANTY[1], P. SOLIS[1], K. CONOVER[1], P. DAYAN[2], P. SHIZGAL[1];

[1]Psychology (CSBN), Concordia Univ., Montreal, QC, Canada; [2]Univ. Col. London, London, United Kingdom

Abstract: Model-free and model-based reinforcement learning have been proposed as the poles of a continuum stretching from acquisition of simple habits to the creation of internal models that support prospective planning. Given the association of phasic activation of dopamine neurons with the former pole, we consider learning established by optical activation of midbrain dopamine neurons in a novel behavioral paradigm.

A cre-dependent viral vector coding for channelrhodopsin 2 was injected into into the ventral tegmental area (VTA) of five TH::Cre rats. Optical-fiber implants were aimed at the VTA and anchored to the skull. The rats were trained to hold down a lever for 2 s to receive an optical reward: a 1-s train of 5 ms, 473 nm pulses delivered to the VTA. The 45 trials per session consisted of a 4-min interval during which a reward of constant strength could be earned by holding down a lever for 2 s. The trials were grouped into 15 repeating triads. On the leading trial of a triad, the optical reward was strong (a high optical pulse frequency). On the middle trial, either a weak, moderate, or strong optical reward was offered, whose strength was determined randomly. On the trailing trial, the optical reward was weak. Thus, the strength of the reward was predictable on leading and trailing trials but unpredictable on middle trials. One lever was extended on leading and trailing trials and another lever on middle trials.

Model-free learning is typically incremental and slow, but abrupt changes in behavior can occur when new information is interpreted on the basis of an internal model. In the present study, abrupt changes in response latency were observed once the stimulation strength on the middle trials had been sampled. This suggests that activation of dopamine neurons can be used as a signal of the state within the trial, rather than just to engender learning.

The rats also showed evidence of having learned the pairwise sequence of trial types. Although the environment appeared the same at the start of leading and trailing trials, the strength of the stimulation the subject would be able to obtain differed. The rats behaved as if they knew what the strength of the stimulation would be: Initial response latencies were low on leading trials and high on trailing trials.

Our results suggest that specific activation of midbrain dopamine neurons can produce learning more sophisticated than the simplest model-free variant. Experiments entailing prospective choice will be required to determine whether the effects of such stimulation extend to model-based reinforcement learning.

Disclosures: I. Trujillo-Pisanty: None. **P. Solis:** None. **K. Conover:** None. **P. Dayan:** None. **P. Shizgal:** None.

Poster

066. Dopamine in Reward: In Vivo Activity Recording and Manipulation Studies

Location: Halls B-H

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Topic: G.02. Motivation

Support: CIHR

Title: Effect of chemogenetic inactivation of midbrain dopamine neurons and their projections to the nucleus accumbens on cue-induced alcohol-seeking behaviour

Authors: *M. D. VALYEAR, I. TRUJILLO-PISANTY, F. LACROIX, P. SHIZGAL, N. CHAUDHRI;
Psychology/Center for Studies in Behavioural Neurobio., Concordia Univ., Montreal, QC, Canada

Abstract: Pavlovian cues that predict alcohol influence the vigour with which alcohol is pursued. We examined the impact of an alcohol-associated context and the contribution of dopamine receptors, midbrain dopamine neurons and their projections to the nucleus accumbens core (NAcC) to alcohol-seeking behaviour elicited by a discrete cue. Male, Long-Evans rats received 15% ethanol (EtOH) in the home-cage, followed by conditioning sessions in which a 10 s conditioned stimulus (CS; white noise or clicker; 15 trials/session) was paired with EtOH (0.2 ml/CS, 3 ml/session). Conditioning sessions occurred on alternating days in a distinct alcohol context where EtOH was dispensed into a fluid port during each CS trial. On the intervening days rats were exposed to a different non-alcohol context where a distinct auditory stimulus was played but EtOH was never delivered. In exp 1, the CS was presented without EtOH in both contexts. Alcohol-seeking elicited by the CS was elevated in the alcohol context and this effect persisted across several tests. Subsequent tests in the non-alcohol context showed that systemic injections (10 μg/kg sc) of a D1-like (SCH23390), but not D2/3 (eticlopride), receptor antagonist significantly attenuated alcohol-seeking elicited by the CS. We used this same training procedure with chemogenetics to determine if midbrain dopamine neurons were necessary for alcohol- (exp 2) or sucrose-seeking (exp 3) elicited by discrete CS presentations in a non-alcohol or non-sucrose context. In exp 2 and 3, male transgenic TH::Cre rats received infusions of a cre-dependent viral vector encoding the Gi-Coupled (hM4Di) designer receptor into the ventral tegmental area. The hM4Di receptor induces neuronal silencing when bound by clozapine-*n*-oxide (CNO). In exp 2 port entries elicited by the CS in the non-alcohol context were significantly attenuated by systemic injections of CNO (0, 10 or 20 mg/kg ip). In exp 3 systemic CNO (0 or 10 mg/kg ip) failed to significantly reduce CS port entries in the non-sucrose context. In exp 4 we targeted dopaminergic projections to the NAcC with the same methodology as exp 2 except that CNO (0 or 3 mM, 0.3 μl/hemisphere) was microinfused through intra-NAcC

cannulae. Inactivating the terminals of midbrain dopamine neurons in the NAcC was sufficient to reduce CS port entries in the non-alcohol context. Thus, the expression of alcohol-seeking elicited by a discrete cue depends crucially on the context in which the cue is encountered. When this contextual influence is controlled for, alcohol-seeking elicited by a discrete cue requires dopamine neurotransmission and dopaminergic projections to the NAcC.

Disclosures: M.D. Valyear: None. **I. Trujillo-Pisanty:** None. **F. Lacroix:** None. **P. Shizgal:** None. **N. Chaudhri:** None.

Poster

066. Dopamine in Reward: In Vivo Activity Recording and Manipulation Studies

Location: Halls B-H

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Program#/Poster#: 66.08/QQ19

Topic: G.02. Motivation

Support: DA025679 RAW

Title: Reduced dopamine signaling changes the patterned activity of nucleus accumbens neurons in response to rewarding taste stimuli

Authors: *C. CHAN[1], D. S. WHEELER[1], E. A. PANTHER[1], S. M. CONWAY[2], M. F. ROITMAN[2], R. A. WHEELER[1];
[1]Marquette Univ., Milwaukee, WI; [2]The Univ. of Illinois at Chicago, Chicago, IL

Abstract: Phasic changes in mesolimbic dopamine signaling guide appropriate behavioral responses to rewarding and aversive stimuli by modulating the activity of nucleus accumbens (NAc) neurons. The experience of a palatable tastant elicits a transient increase in dopamine concentration in the NAc, while unpalatable taste stimuli decrease transient release events. Coincident with the change in dopamine signaling, the patterned activity of NAc neurons differentially encodes rewarding and aversive stimuli. While the predominant neuronal response to palatable taste stimuli is inhibitory, aversive stimuli evoke largely excitatory responses. However, the direct influence of dopamine signaling on the encoding of rewarding stimuli by NAc neurons remains largely uncharacterized. Here we examined the effect of chemogenetic inhibition of ventral tegmental area (VTA) dopamine neuron activity on the NAc encoding of a palatable sucrose tastant. hM4Di DREADD expression was virally induced in the VTA dopamine neurons of male Long Evans TH-Cre positive rats. Electrophysiological recording arrays were implanted in the NAc for unit recording, and intraoral cannula were implanted for tastant delivery. At test, animals were exposed to blocks of 45 intraoral sucrose (20%) infusions (0.2 ml over 6 seconds) before and after administration of CNO (2mg/kg, i.p.) or saline vehicle

(0.9%) in a counterbalanced design. Four animals were tested in the current study, with 60 and 54 units recorded in CNO-treated and saline-treated conditions, respectively. Consistent with prior reports, intraoral sucrose infusions evoked predominantly inhibitory responses in phasically responsive NAc neurons. However CNO administration altered the neural encoding profile of the sucrose taste, significantly reducing the number of observations of inhibitory responses, while increasing the incidence of excitatory responses. Saline administration did not affect the neural encoding of sucrose. These observations demonstrate that chemogenetic inhibition of VTA dopamine neuron signaling significantly alters the neural encoding of sucrose. This ongoing study will continue to examine the effects of reduced dopamine signaling on the behavioral and neural responses during various reward-related tasks, including taste reactivity and reward omission.

Disclosures: C. Chan: None. **D.S. Wheeler:** None. **E.A. Panther:** None. **S.M. Conway:** None. **M.F. Roitman:** None. **R.A. Wheeler:** None.

Poster

066. Dopamine in Reward: In Vivo Activity Recording and Manipulation Studies

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Topic: G.02. Motivation

Support: DARPA REPAIR Project N66001–10-C-2008

Title: Dynamics of the reward signal in the primary motor cortex (M1).

Authors: *A. TARIGOPPULA[1], J. HESSBURG[1], D. B. MCNIEL[1], J. S. CHOI[1], B. T. MARSH[1], J. T. FRANCIS[1,2];
[1]Physiol. and Pharmacol., SUNY Downstate Med. Ctr., NYC, NY; [2]Biomed. Engin., Cullen Col. of Engineering, Univ. of Houston, Houston, TX

Abstract: We recently discovered that the primary motor cortex (M1) in non-human primates (NHPs) modulates with respect to the presence or absence of a juice reward at the end of the trial (Marsh et. al. 2015). Such a reward signal has also been observed in various deep brain (Schultz 2002) and cortical (Roesch and Olson 2003, 2004) structures. We investigated the dynamics of the reward signal in M1 under various reward probability structures and with multiple levels of reward. Experiments included (i) changing the percentage of the rewarding trials in a given session from 50% to 75% to 90%, (ii) structured sequence of rewarding and non-rewarding trials (R-NR-R-NR-R etc) and (iii) multiple levels of reward included one / two or three levels of juice reward at the end of a successful trial to see if M1 encoded the value of a trial.

We show that reward prediction signal (RPS) exists in M1. Increased certainty in the reward probability structure of the external environment modulates the RPS in M1. Modulation of the RPS represents the animal's confidence in understanding the reward landscape of the environment it is dealing with.

Disclosures: A. Tarigoppula: None. **J. Hessburg:** None. **D.B. McNiel:** None. **J.S. Choi:** None. **B.T. Marsh:** None. **J.T. Francis:** None.

Poster

066. Dopamine in Reward: In Vivo Activity Recording and Manipulation Studies

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Topic: G.02. Motivation

Support: NIH R01MH101697

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University of Michigan

Title: Local control of dopamine release may produce reward-prediction rather than reward-prediction-error signals.

Authors: *A. HAMID[1,2], A. MOHEBI[1,3], J. D. BERKE[1,3,2];
[1]Psychology, [2]Neurosci. Grad. Program, Univ. of Michigan, Ann Arbor, MI; [3]Neurol., Univ. of California, San Francisco, San Francisco, CA

Abstract: Dopamine (DA) is closely involved in both motivation and reinforcement-driven learning. However, the message conveyed by dopamine signals is under active debate. Many studies have found a relationship between dopamine cell spiking and reward prediction errors (RPE), consistent with a role in learning. Yet voltammetry and microdialysis measures indicate that DA conveys a motivational signal - the temporally-discounted, estimated value of work - better than RPE (Hamid et al. 2015 Nat Neuro). Furthermore, DA release from terminals is not a simple function of DA cell spiking, but is also modulated strongly by local microcircuitry including striatal cholinergic interneurons. We therefore hypothesized a dissociation between DA cell firing encoding RPE, and DA terminal activity encoding value. We used fiber photometry, GCaMP6f and TH:Cre rats to monitor calcium dynamics in VTA DA cells and accumbens DA terminals simultaneously. Rats performed three different tasks that probe reward expectations and RPEs: 1) Two-armed bandit; 2) Probabilistically-rewarded linear track running; 3) Pavlovian approach. Initial data suggest that that there is indeed a dissociation between the

activity of DA cell bodies and terminals, and that these can even evolve in opposite directions as rats anticipate a delayed cue. We propose that DA targets tailor DA release to their own computational requirements, potentially converting an RPE-like spike signal into a motivational message.

Disclosures: **A. Hamid:** None. **A. Mohebi:** None. **J.D. Berke:** None.

Poster

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Topic: G.02. Motivation

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IMHRO 19 (KD)

NIH MH103374 (SHS)

Title: Anticipatory and reward-responsive neural activity of mesocorticolimbic DA circuitry in mice performing a sample-to-match task

Authors: *M.-A. T. VU[1,3], C. J. BURRUS[3], M. VAGWALA[4], S. D. MAGUE[4], L. K. DAVID[4], J. WANG[4], G. E. THOMAS[6], R. ADCOCK[1,3,4], S. H. SODERLING[3,5], K. DZIRASA[3,4,2]; [1]Ctr. for Cognitive Neurosci., [2]Biomed. Engin., Duke Univ., Durham, NC; [3]Neurobio., [4]Psychiatry & Behavioral Sci., [5]Cell Biol., Duke Univ. Sch. of Med., Durham, NC; [6]Univ. of Maryland Baltimore County, Baltimore, MD

Abstract: The mesocorticolimbic dopamine (DA) system has been shown to be important for cognitive functions such as reward processing, learning, and memory. Classic studies have shown that DA signaling following reward outcomes reflects reward prediction error; other studies additionally suggest a role for anticipatory DA signaling during goal-directed behavior prior to reward outcome. In this study, we investigated both anticipatory neural activity and reward-responsive neural activity in a cued T-maze sample-to-match task. We used wildtype mice and mutant mice with conditional postnatal disruption of actin-related protein 2/3 (Arp2/3) in excitatory forebrain neurons. This genetic mouse model of schizophrenia has been previously shown to exhibit cognitive deficits and aberrant prefrontal cortical signaling resulting in striatal hyperdopaminergia. During task performance, local field potential (LFP) and extracellular action

potentials were recorded from 6 brain areas: prelimbic cortex (PrL), nucleus accumbens (NAc), dorsal medial striatum (DMS), dorsal hippocampus (dHPC), mediodorsal thalamus (mdThal), and ventral tegmental area (VTA). Receiver operating characteristic (ROC) analysis of cell spiking in wildtype mice showed that PrL and VTA anticipatory and reward-response activity differentiated correct from incorrect choices. Mutant mice showed altered responses in these task-relevant regions as well as in NAc. Specifically, the mutant mice showed less reward-response differentiation in earlier learning, and greater anticipatory differentiation in later learning. Taken together, these data implicate both anticipatory signaling and reward-responsive signaling of mesocorticolimbic DA circuitry in this task, and that hyperdopaminergia may lead to increased task accuracy.

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Poster

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Title: Nigrostriatal signal inhibits saccadic eye movement during countermanding task in monkeys

Authors: T. OGASAWARA[1,3], M. TAKADA[3], *M. MATSUMOTO[2,1];
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Abstract: Response inhibition is the ability to suppress planned or on-going motor actions that would lead to unwanted outcomes. Since the basal ganglia “indirect pathway” is supposed to control body movements in an inhibitory manner, this pathway would be a candidate that

regulates response inhibition. Here we investigated how the nigrostriatal pathway that modulates the indirect pathway contributes to response inhibition. We recorded single-unit activity from projection neurons in the caudate nucleus and dopamine neurons in the ventral midbrain while monkeys performed a saccadic countermanding task. First, the monkey was required to gaze a fixation point. Then, the fixation point disappeared, and a saccadic target was presented on the right or left side of the point. In 70% of the trials, the monkey was required to make a saccade to the target (no-stop signal trials). In the remaining 30%, on the other hand, the fixation point reappeared as a "stop signal" with a random delay after the onset of the saccadic target (stop signal trials). The monkey was then required to cancel a planned saccade. We first recorded the activity of 41 projection neurons in the caudate nucleus. Compared with the activity in no-stop signal trials (i.e., trials in which a saccade was made), a significant excitation was shown in six neurons (14%) in response to the stop signal in stop signal trials (i.e., trials in which a saccade was canceled), while a significant inhibition was exhibited in 10 neurons (24%). These excitatory and inhibitory neurons might be involved in suppression or facilitation of a planned saccade, respectively. We also recorded the activity of 70 dopamine neurons in the ventral midbrain. In contrast to the caudate neurons, many of them (30/70, 43%) showed a significant excitation to the stop signal, whereas only a few neurons (3/70, 4%) displayed a significant inhibition. This indicates that dopamine neurons preferentially contribute to suppression, rather than facilitation, of a planned saccade. Notably, the dopamine neurons with a significant excitation to the stop signal were observed mainly in the substantia nigra pars compacta, but not in the ventral tegmental area, that send massive projections to the caudate nucleus. To test the causal relationship between the ability of response inhibition and nigrostriatal dopamine signaling, we injected haloperidol, a dopamine D2 receptor antagonist, into the caudate nucleus. We found that the performance of canceling a planned saccade was significantly reduced in stop signal trials. Our findings suggest that nigrostriatal dopamine signaling may be important for suppressing a planned saccade in the saccadic response inhibition paradigm.

Disclosures: **T. Ogasawara:** None. **M. Takada:** None. **M. Matsumoto:** None.

Poster

066. Dopamine in Reward: In Vivo Activity Recording and Manipulation Studies

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 66.13/RR4

Topic: G.02. Motivation

Support: R21MH103806

Title: Subsecond dopamine signaling of reward and punishment is modulated by social context

Authors: *M. R. ROESCH[1], B. LEE[2], M. A. MYERS[2], B. S. CHAPPA[2], M. S. WALTERS[2], S. KANTOR[2], M. G. PECUKONIS[2], E. A. GREEN[2], D. POTEMRI[2], D. A. LAGOWALA[2], E. G. MELLINGER[2], H. T. GIRMA[2], R. T. TESFAY[2];
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Abstract: Dopamine (DA) release is thought to reflect reward prediction errors, increasing and decreasing to events that are better (e.g., reward) or worse (e.g., punishment) than expected. Here we ask how DA release is modulated by social context. Social context molds our perceptions and understanding of the world, yet we know little about how DA signals are modulated in social situations. To address this issue we designed a paradigm where 3 different auditory cues predicted 3 different events: reward, shock, or nothing. In this task, two rats were placed side-by-side in a single behavioral box separated by a wire mesh. One of these rats - the recording rat - was equipped with a fast scan cyclic voltammetry electrode in NAc to record subsecond DA release. Five seconds after the onset of the auditory cue, a 'directional' light illuminated indicating which rat would receive the predicted outcome. During control sessions the conspecific rat was removed. Ultrasonic vocalizations, food cup entries, freezing, orienting, and approach were used to determine the recording rats' mental state and outcome predictions. Consistent with previous work, DA release increased to cues that predicted reward and during reward delivery. Phasic increases to reward were higher when rats were together. This likely reflects a reduction in reward expectancy as evidenced by fewer anticipatory beam breaks. It might also be argued that elevated DA release reflects higher perceived value; however appetitive vocalizations did not support this hypothesis. Cues that predicted shock inhibited DA release non-discriminately across trial types; DA was low for both self and conspecific-shock trials prior to shock onset. However, during shock trials, DA release was modulated by social context in two ways. First, reductions in DA release to cues that predicted shock were weaker in the presence of the conspecific. During these trials, rats increased appetitive vocalizations and froze less often, suggesting that they were seeking social interaction and were less fearful. Second, DA release on shock trials increased quickly when shock was administered to the conspecific, suggesting that recording rats were using the emotional reactions of the conspecific to verify personal safety. We conclude that DA release is modulated by social context in that rats use social cues to optimize predictions about their own well-being. Social context also appeared to reduce fear, increase social calls, and weaken DA signals associated with predicted shock. Finally, our results suggest, at least in this context, that DA does not signal the valence of positive and negative events from the perspective of the conspecific (i.e., empathy).

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Poster

066. Dopamine in Reward: In Vivo Activity Recording and Manipulation Studies

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Topic: G.02. Motivation

Support: NIHNIDA Grant 11PAF05335

Title: The ventral tegmental area encodes differential behavioral strategies towards reward-paired cues

Authors: *L. FERGUSON[1,3], A. M. AHRENS[2], L. G. LONGYEAR[2], J. ALDRIDGE[2];
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Abstract: The ventral tegmental area (VTA) is important in mesocorticolimbic reward circuit. In this study we exploit individual behavioral differences in a Pavlovian approach task (Flagel et al., 2007) to examine the specific roles of dopamine and non-dopamine neurons in reward prediction error and in the attribution of incentive salience (i.e. the attribution of motivational value to reward predictive cues). In the Pavlovian task, an illuminated lever (conditioned stimulus, CS) is inserted into the cage for 8 seconds (the predictive cue). At lever retraction, a food reward (unconditioned stimulus) is delivered. Trials occur randomly. All animals learned the predictive nature of the CS, but some also found the cue to be attractive (attributed incentive value), approaching and interacting with the lever. These animals, "sign-trackers" (STs) were compared to animals that predominantly approached the location of reward receptacle ("goal-trackers", GTs). Dopamine neurons were characterized by spike waveform shape and firing rate and confirmed by a systemic apomorphine test. Firing rates and magnitudes of responses in relation to Pavlovian behaviors, cue presentation, and reward delivery were assessed.
We recorded from 89 dopamine and 90 non-dopamine neurons. Neural coding responses were characterized by firing rate changes to behavioral cues and by proportions of responsive cells (population coding). Population coding of GTs and STs differed in 2 important ways. Only dopamine neurons of STs responded during the lever interaction period, the last 7 seconds of lever presentation when STs engaged the lever. Dopaminergic coding of GTs and STs showed opposing responses to cue onset and cue offset. Cue onset predicts the pending reward while cue offset and cue engagement are redundant reflecting moments of greatest incentive value (period just prior to reward presentation). Dopamine neurons of GTs showed a significantly higher proportion of cells responding to cue onset, while STs had significantly higher proportion of cells responding to cue offset and interaction. Non-dopamine neurons of STs and GTs did not show significant differences to these cues in terms of population coding. Non-dopamine neurons from STs, however, did show enhanced firing rates to cue offset compared to cue onset. These are the first results to show that the different behavioral strategies expressed by rats are coded for

specifically by dopamine neurons in the VTA. These results support an important role for dopamine neurons in the attribution of incentive salience to reward-paired cues and underscore the consequences of potential differences in motivational behavior between individuals.

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Poster

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Topic: G.02. Motivation

Support: Simons Foundation 348880

Bial Foundation 413/414

Title: Dopaminergic network dynamics during behavioral exploration

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Abstract: We are constantly faced with the trade-off between exploiting past actions with known outcomes and exploring novel actions whose outcomes may be better. When environmental rewards are stable, it is preferable to perform actions known to be rewarding. However, when environmental rewards are changeable, it is adaptive to explore alternative actions and revisit previous actions whose value may have changed. This exploration-exploitation balance is thought to be coded by dopaminergic neurons of the substantia nigra pars compacta (SNc). However, little is known about the ways in which environmental changes impact action selection, and even less is known about SNc network dynamics during exploration. We developed a novel behavioral paradigm in mice to investigate how changes in environmental stability affect behavioral variability. Mice were placed in environments with three equidistant nose poke ports and had to explore the environment to discover which sequence of three nose pokes was rewarded. Actions were variable as mice explored to find the rewarded sequence, but became stable as they learned to exploit the rewarded sequence. We then performed calcium imaging in freely behaving mice during task performance, which allowed us to simultaneously record activity in large populations of genetically-identified neurons and track the same populations throughout training. We saw an evolution of single-cell responses associated with

task-relevant events over the course of learning. In addition, we observed changes in SNc network dynamics during periods of exploration versus periods of exploitation. These experiments support a role for dopaminergic networks in behavioral exploration.

Disclosures: **A.C. Koralek:** None. **R.M. Costa:** None.

Poster

066. Dopamine in Reward: In Vivo Activity Recording and Manipulation Studies

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Topic: G.02. Motivation

Support: NIH Grant R01DA038642

Parkinson's Disease Foundation - the Stanley Fahn Research Fellowship

Title: Analysis of dopamine-dependent circuit activity by functional and molecular MRI

Authors: ***N. LI**, A. P. JASANOFF;
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Abstract: The neurotransmitter dopamine (DA) has received special attention because of its roles in reward behavior and the control of movement. The relationship between DA release and broader circuit function is complicated by the diverse modulatory roles of DA acting on its multiple receptors. In this work, we aim to relate spatial patterns of striatal DA release to broader brain-wide activity patterns detectable by noninvasive neuroimaging. We apply a combination of molecular-level functional magnetic resonance imaging (fMRI) with an MRI-detectable DA sensor with conventional blood oxygenation level dependent (BOLD) fMRI to directly compare regional DA release and population neural activity in the rat brain. Data reveal three-dimensional multimodal activity profiles that vary as a function of stimulus properties. Comparison of these maps illustrates spatiotemporal relationships between dopamine dynamics and BOLD activation. Results from rodent studies will facilitate better understanding of dopamine's effects on distributed circuit function and inform interpretation of reward related striatal BOLD signals in multiple species. Ultimately these studies could contribute to diagnostic and therapeutic strategies for addressing DA-related neurological diseases such as addiction and Parkinson's disease.

Disclosures: **N. Li:** None. **A.P. Jasanoff:** None.

Poster

066. Dopamine in Reward: In Vivo Activity Recording and Manipulation Studies

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University of Michigan

Title: Dopamine signals the value of work selectively in accumbens core and ventral prelimbic cortex.

Authors: *J. R. PETTIBONE[1,3], J.-M. T. WONG[2], A. MOHEBI[1,3], R. T. KENNEDY[2], J. D. BERKE[1,3];
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Abstract: Dopamine (DA) is a key modulator of neural circuit functions in both the striatum and the frontal cortex. We recently demonstrated that DA release in ventral striatum signals motivational value, across both fast and slow timescales (Hamid et al. 2015 Nat Neuro). A critical remaining question is whether DA provides a single, global signal to all targeted striatal and cortical subregions. One alternative possibility is that striatum and cortex each receive distinct DA signals, and yet a third possibility is that DA release patterns are organized by cortical-striatal loops, to provide a common modulation of interconnected circuit components. We performed minute-by-minute microdialysis in various subregions of medial frontal cortex and striatum in rats performing a trial-and-error choice task, measuring a battery of 20 neurochemicals including DA, acetylcholine, serotonin, norepinephrine, adenosine, GABA and glutamate. Striatal and cortical subregions were sampled simultaneously within each session, and probes were advanced along a dorsal-ventral axis between sessions. We replicated our prior finding that striatal DA covaries with reward rate, a proxy for the estimated value of work, and that among small-molecule neurotransmitters this relationship is unique to DA. However we found that this DA value signal is specifically localized to accumbens core (R2= 0.12, p= 1.35e-16, n=7), rather than shell (n=8) or dorsal-medial striatum (n=11). Within frontal cortex we also found a clear relationship between DA and reward rate, but focally within ventral prelimbic cortex (R2= 0.10, p= 1.18e-14, n=7) rather than more dorsal (ACC, n=7; dorsal prelimbic, n=8) or ventral (infralimbic, n=4) subregions. These matching “hotspots” of value signaling in ventral prelimbic cortex and accumbens core show an intriguing correspondence to human brain areas encoding subjective value in fMRI studies (Bartra et al. Neuroimage 2013). We conclude that, rather than providing a uniform signal to all targets, or separate information to cortex and

striatum, DA release is tailored to the functional demands of specific cortical-basal ganglia loops.

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Poster

066. Dopamine in Reward: In Vivo Activity Recording and Manipulation Studies

Location: Halls B-H

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Topic: G.02. Motivation

Support: NIH

Title: The role of the ventral pallidum in a nucleus accumbens dopamine-dependent sensorimotor transformation

Authors: *J. D. LEDERMAN[1], S. LARDEUX[2], S. NICOLA[3];
[1]Psychiatry, Yeshiva Univ. Albert Einstein Col. of Med., Bronx, NY; [2]Psychiatry, [3]Dept. of Psychiatry, Albert Einstein Col. of Med., Bronx, NY

Abstract: Dopamine in the nucleus accumbens (NAc) is a well-established modulator of reward-seeking behavior. In a discriminative stimulus (DS) task, nearly half of all NAc neurons are excited by cues that predict sucrose reward contingent on approach to and operation of a lever. These excitations precede approach movement onset, predict movement onset latency, are reduced by blockade of dopamine receptors within the NAc, and are causal to approach movement initiation. Dopamine receptor blockade decreases, and activation increases, the probability of cued approach. The ventral pallidum (VP) receives a prominent GABAergic projection from the NAc. This projection is essential for translating limbic sensory, value and motivation signals into motor output, but how it does so is unknown. In this study we ask how VP neurons fire in response to reward-predictive cues, how this firing relates to the motor response, and how dopamine receptor blockade in the NAc alters this firing. Rats were food-restricted and trained on a discriminatory stimulus (DS) task. The DS was an auditory cue, presented at variable intervals, which directed the rat to approach and press a lever to obtain 10% sucrose reward. A lever press during the 10s DS resulted in reward delivery and cue termination. Trained animals were implanted with microelectrode arrays in the VP and infusion cannulae in the NAc. During a 45 min baseline period, animals performed the task while VP neurons were recorded. D1 antagonist was then infused into the NAc, and behavior and recording continued an additional 2 hr and 15 min. Video tracking of head-mounted LEDs enabled detection and

measurement of locomotion. Our results show that approximately half of the neurons in the VP were excited by DS presentation and these were typically those that are inhibited preceding locomotion. Changes in activity of VP neurons were highly correlated with both initiation and cessation of spontaneous locomotor movements (i.e., movements during the inter-trial interval that were typically not directed towards the lever) in that neurons that showed inhibition during movement onset were excited during movement cessation, and vice versa. The majority of DS-excited neurons were inhibited during spontaneous movement initiation. Bilateral infusion of D1 antagonists into the NAc reduced the number of neruons showing DS- and movement-related inhibitions and moderately decreased DS- and movement-related excitations. These results suggest that the dopamine-dependent DS-evoked excitation of NAc GABAergic projection neurons promotes approach movement initiation via inhibition of a subset of VP neurons.

Disclosures: **J.D. Lederman:** None. **S. Lardeux:** None. **S. Nicola:** None.

Poster

067. Motivation and Reward Mechanisms

Location: Halls B-H

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Topic: G.02. Motivation

Support: NARSAD

Title: Primate dorsal striatum signals uncertain object-reward associations

Authors: *J. K. WHITE, I. E. MONOSOV;
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Abstract: In order to survive, animals attend to and approach objects that have consistently predicted rewards. However, to learn, and to maximize foraging, animals must also approach objects associated with variable or uncertain rewards. To date, the mechanisms that direct behavior towards uncertain objects are not well understood. Reward-driven behaviors are in-part dependent on the caudate-putamen complex, also called the dorsal striatum (DS). Here we asked if DS contains a mechanism to mediate behaviors aimed at objects associated with uncertain rewards. We found that single neurons in the internal-capsule bordering regions of DS (icbDS), particularly in the putamen, signaled reward uncertainty associated with visual objects. We recorded neurons across the DS while monkeys viewed conditioned stimuli (CSs) that predicted juice rewards of varied amounts (0.25ml, 0.125ml, 0ml) and probabilities (100%, 50%, 0% 0.25ml). We discovered a subset of neurons in icbDS that selectively responded with strong excitation to CSs that predicted uncertain rewards (50%). Their uncertainty response often

included a strong ramp-like component that terminated at the time of the trial outcome. In a series of additional control experiments we also found that icbDS uncertainty neurons did not encode information about punishment or action-kinematics. Next, we tested if icbDS uncertainty responses depended on the presence of the CS object by varying the presentation of certain (100%) and uncertain (50%) cues such that the CSs were either present until trial outcome (2.5 seconds) or removed 1.5 seconds before trial outcome. We found that the icbDS uncertainty response was mostly ablated by removing the CS prior to the trial outcome. Because this finding indicated that icbDS uncertainty responses were strongly dependent on the uncertain object, we hypothesized that icbDS neurons may play a role in object-reward associative learning. Therefore, we next recorded from icbDS uncertainty neurons while monkeys learned three novel CSs associated with 100%, 50%, or 0% 0.25ml of juice. Before the monkeys learned the stimuli, icbDS uncertainty neurons responded similarly to all three CSs, with a sharp ramp-like excitation. As the CS-reward associations were learned, the response to certain cues (100% and 0%) was ablated but a strong response to the uncertain CS remained. In sum, we show that icbDS neurons signal uncertainty of object-reward associations - a critical variable for monitoring and learning from objects. These results demonstrate a novel role of the striatum, particularly of the putamen, in signaling uncertainty and in forming new associations between objects and rewarding outcomes.

Disclosures: J.K. White: None. **I.E. Monosov:** None.

Poster

067. Motivation and Reward Mechanisms

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Program#/Poster#: 67.02/RR11

Topic: G.02. Motivation

Title: Ventral tegmental area GABA neurons modulate motivation and attention to a cue predicting sucrose reward in a rat operant task

Authors: *C. E. BASS[1], P. M. FULLER[2], M. J. BRUNO[3], R. V. BHIMANI[3], J. PARK[3], K. HAUSKNECHT[3], R.-Y. SHEN[3], S. HAJ-DAHMANE[3], K. T. WAKABAYASHI[3];
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Abstract: Dopaminergic neurons projecting from the ventral tegmental area (VTA) to cortical and limbic regions such as the nucleus accumbens (NAc) regulate motivated behaviors including responding to cues associated with the availability of reward. Less understood is the role of VTA-GABAergic neurons in mediating this behavior. Up to 25% of VTA neurons are

GABAergic interneurons that inhibit DA neurons, while ~5% are GABA projecting neurons (GPN) that synapse in reward processing regions including the NAc. We hypothesized that VTA-GABA neurons serve to inhibit mesolimbic DA, decreasing the motivational significance of associated cues. We tested this hypotheses using a rat model of cue-mediated responding for a natural reward (sucrose). In this task, male Long Evans rats were trained to nosepoke for sucrose reward during a distinct, intermittent audiovisual cue that was presented approximately every 30 s during a 1-hr session. After a successful nosepoke, sucrose solution (10%) was delivered into a cup next to the nosepoke. During this task, several indices can be measured, such as the response ratio (the number of successful responses to a predictive cue), the latency to nosepoke during a cue (the time taken to enter the reward receptacle to consume reward), and the overall accuracy in the responding to the cue. Criterion performance was defined as 85% of all reward-predictive cues during a session. While previous studies using microinjections of pharmacological agents have demonstrated that VTA-GABA neurons play a role in reward-seeking and consumption in this task, the contribution of specific sub-populations of VTA-GABA neurons has not been explored. To address this, rats trained on this task were infused in the VTA with adeno-associated viruses (AAV) targeting GABA neurons to deliver excitatory Designer Receptor Exclusively Activated by Designer Drugs, (DREADDs) or channelrhodopsin-2 (ChR2). Immunohistochemistry revealed that ChR2 expression occurred in approximately 25% of the neurons which were also TH negative. In addition, *in vitro* whole cell recordings from VTA DA neurons revealed that optical stimulation (470 nm, 1 ms) readily elicits inhibitory synaptic currents (IPSCs) abolished by the $GABA_A$ receptor antagonist picrotoxin (100 μM). Finally, CNO stimulation of VTA-GABA neurons expressing excitatory DREADDs during the cued-reinforcement task resulted in a decrease in performance in all metrics of this task, suggesting that VTA-GABA neurons play a key role in cue-mediated reinforcement and attention for a natural reward such as sucrose.

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Poster

067. Motivation and Reward Mechanisms

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NIDA SC1DA034995

Title: Chunked action sequences are sensitive to reward devaluation.

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Abstract: Actions can become chunked together to form whole units of behavior. This is an especially important idea in instrumental conditioning in which sequencing actions together can save time and increase reward. Yet despite the possible increases in reward, it is also possible that chunking can cause each action to become driven by its preceding action rather than its consequence. This account provides a tantalizing explanation for why actions are sometimes insensitive to changes in reward value. Yet, there is no convincing empirical evidence that chunking results in insensitivity to reward devaluation (i.e. habits).
To test the hypothesis that action chunking leads to habits, we conducted an experiment in which rats pressed a left and right lever in sequence for a pellet reward. If a rat pressed the left lever and then the right lever, a pellet was delivered and the lever retracted for 1.5 seconds. Any other two-press sequence (left-left, right-left, right-right) resulted in retraction of the levers for a 5 second timeout and no reward. Prior to the beginning of the experiment, rats were assigned to either the extensively trained group (60 days of training) or the moderately trained group (20 days of training). The rats in the moderately trained group were trained to the point of asymptotic accuracy to prevent chunking, while the extensively trained group was trained well beyond asymptotic accuracy to ensure chunking. Following training, two reward devaluation tests were given. Prior to each test, rats were given unlimited access to either the pellet type that was earned during training (devalued) or a differently flavored pellet type that was not earned during training (valued). All rats were then given a 5 minute extinction test in which no rewards were earned for pressing the levers. Each rat was tested while sated on both pellet types over two different days. During training and across both groups, there was an increase in accuracy and speed across sessions, while there was a decrease in inter-response time (IRT) and sequence entropy. Results from the devaluation tests show that rats from both groups were goal-directed (i.e. not habitual). The frequency of left-right sequences was lower during the devalued extinction test. Interestingly, the mean IRTs indicate that the extensively trained rats were more goal-directed in that they showed a longer pause between left and right lever presses during the devalued test. The moderately trained rats did not show a difference in IRTs between valued and devalued test days. These results do no support the prediction that chunked actions become habitual. Rather, action chunking may lead to even greater sensitivity to reward devaluation.

Disclosures: E. Garr: None. **A.R. Delamater:** None.

Poster

067. Motivation and Reward Mechanisms

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Title: Nucleus Accumbens Shell neurons modulated by feeding do not encode palatability but they integrate external cues relevant to stop licking palatability but they integrate external cues relevant to stop licking

Authors: *M. A. VILLAVICENCIO CAMARILLO[1], A. I. HERNANDEZ-COSS[2], I. O. PEREZ[3], S. S. SIMON[5], R. GUTIERREZ[4];
[1]Ctr. De Investigación Y Estudios Avanzados, Mexico, Mexico; [2]Bioelectrónica, [4]Farmacología, [3]Ctr. de Investigación y de Estudios Avanzados, Mexico, Mexico; [5]Duke Univ., Durham, NC

Abstract: It is well known the role of Nucleus Accumbens Shell (NAcSh) in emotional responses to reward and consumption. Opiod manipulations in NAcSh alter behavioral affective reactions to sucrose and electrical stimulation of NAcSh disrupts oromotor behavior. However, it is not known how NAcSh integrates both, hedonic and motor components of consumption. To address this issue we designed 2 variants of a Brief Access Task (BAT) where we carefully controlled oromotor behavior (licking), while rats consumed graded-hedonic sucrose solutions. In a gustatory-cued variant of the task (gcBAT), a group of rats licked a spout to obtain in each trial 1 out 5 different sucrose concentrations (0, 3, 5.8, 11.7 or 20%) to obtain in each lick a 10-µL drop for 5 s. After this opportunity window (OW) additional licks did not deliver any liquid and rats had to notice the absence of reward to stop licking for 1-3 s in order to initiate a new trial. Therefore, rats had to emit bouts of rhythmic licking of similar length (~5 s) to receive variable sucrose solutions. We trained another group of rats in an auditory-cued variant (acBAT). Here a white-noise was turned on at the end of the 5-s OW and lasted up the required 1-3 s pause in licking was achieved. Then the auditory cue was added to guide rats to distinguish the end of the OW and helped them to stop licking faster than the gcBAT. We found that the auditory cue helped rats to refrained licking in 0.6 s while rats that only used the absence of reward as a cue lasted 1.33 s. Then, multichannel recording was made in the NAcSh during the 2 tasks. In both tasks, we found few neurons (<3 % in each task) that, controlling for licking behavior, encoded the differences in the hedonic value. Instead, neurons with modulations relative to consumption were more common (66 and 68 % in each task). From these neurons we found a subset that also

responded tonically after the OW, being more common in the acBAT than in gcBAT (51 vs 26 %). An additional subset of neurons (some consummatory-related) responded phasically with the reward termination. This kind of neurons where more frequent in the acBAT and responded in a more precise fashion. This stronger neuronal control may result in the faster reaction time for refrain licking observed in the acBAT, which will be evaluated in further experiments. So far, we showed neurons in NAcSh that either track oromotor activity, monitor external cues relevant for feeding maintenance or that integrate both, oromotor and contextual aspects of feeding. Our data indicate that NAcSh may act by signaling the pertinence of sustain feeding guided by external cues and that the circuits involved in monitoring consumption marginally do so based on palatability information.

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Poster

067. Motivation and Reward Mechanisms

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Support: NIH Grant MH090264

Title: Cell specific role of ΔFosB in aggressive behavior in male mice

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Abstract: Aggression is an innate social behavior that helps individuals to defend their territory against competitors and increases the probability of successful mating. However, extreme aggression can have devastating consequences on society. Recent studies implicate brain reward structures, such as the ventral striatum (vStr), in mediating the motivational and rewarding aspects of aggressive behavior in mice. DeltaFosB is a truncated splice variant of the *FosB* gene that plays a key role in both natural and drug reward, which we hypothesized to be involved in mediating aggression reward. Here we show that DeltaFosB is selectively induced in dopamine receptor 1-expressing GABAergic medium spiny neurons (D1-MSNs) of the vSTR in aggressive

mice after confrontation with a subordinate intruder mouse. Viral overexpression of deltaFosB in the D1 or D2-MSN populations of the vStr of aggressive mice differentially modulates aggressive bahavior and aggression reward. These findings support the notion that deltaFosB in vStr modulates aggressive behavior in a cell-specific manner. Considering the role of deltaFosB in reward-related behaviors, such as drug addiction, sexual pleasure and alcohol drinking, our data point to a new role of deltaFosB in regulating rewarding aspects aggressive behavior in mice.

Disclosures: **H. Aleyasin:** A. Employment/Salary (full or part-time): Icahn School of Medicine at Mount Sinai. **S. Golden:** None. **A. Takahashi:** None. **M. Flanigan:** None. **M. Pfau:** None. **C. Menard:** None. **G. Hodes:** None. **M. Heshmati:** None. **J. Multer:** None. **L. Bicks:** None. **J. Tai:** None. **E. Heller:** None. **S. Russo:** None.

Poster

067. Motivation and Reward Mechanisms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 67.06/RR15

Topic: G.02. Motivation

Support: Michigan State University Foundation

Title: Sex differences in behavioral and neural responses to palatable food reward

Authors: ***E. B. SINCLAIR**[1], K. L. KLUMP[2], C. L. SISK[1];
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Abstract: The sex difference in eating disorder prevalence is the most significant in all of psychiatry. Women are significantly more likely than men to suffer from eating disorders, particularly those associated with the core, maladaptive symptom of binge eating, defined as the consumption of a large amount of food, typically palatable food (PF), in a short period of time. To study binge eating, our lab uses an individual differences rat model of binge eating, which defines binge eating prone (BEP) and binge eating resistant (BER) rats based on consistently high and low consumption of intermittently-presented PF intake, respectively. Using this model, we have demonstrated that 1) females consume significantly more PF than males, and 2) females are more often classified as BEP as compared with males. One potential explanation for this sex difference is that PF is more rewarding to females, leading to more frequent bouts of binge eating on PF. Here, we here tested the hypothesis that females have more robust behavioral and neural responses to PF reward than do males. To investigate behavioral responses to PF, adult male (N=18) and female (N=17) Sprague-Dawley rats were exposed to the Conditioned Place

Preference paradigm using PF (~15g Betty Crocker™ vanilla frosting) as the unconditioned stimulus, paired with the initially non-preferred chamber. CPP consisted of a pre-conditioning test day, 8 total pairings alternating stimulus-paired and non-stimulus-paired days, and a post-conditioning test day. To investigate neural responses to PF, 24 hours after the CPP post-test day, male (N=13) and female (N=12) rats were exposed to PF for one hour prior to sacrifice to induce the expression of the neural activation marker Fos. Thereafter, brains were harvested and processed for quantification of Fos immunoreactivity in the medial prefrontal cortex, the nucleus accumbens, the central and basolateral amygdala, the arcuate nucleus, and the lateral hypothalamus. In the CPP paradigm, females displayed a more robust preference for the chamber paired with PF as compared with males. In addition, Fos expression was significantly higher in the infralimbic cortex of the medial prefrontal cortex and in the nucleus accumbens shell of females as compared with males. These data suggest that PF may be more rewarding to females than to males, possibly due to heightened responsiveness of neural substrates that mediate the hedonic and motivational responses to PF, which may in part explain sex differences in binge eating proneness.

Disclosures: **E.B. Sinclair:** None. **K.L. Klump:** None. **C.L. Sisk:** None.

Poster

067. Motivation and Reward Mechanisms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 67.07/RR16

Topic: G.02. Motivation

Title: A permissive role of serotonergic neurons on hedonic behavior

Authors: *A. GIORGI[1,2], G. MADDALONI[1], S. MIGLIARINI[1], M. GRITTI[3], R. TONINI[3], A. GOZZI[2], M. PASQUALETTI[1,2];
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Abstract: By densely innervating multiple forebrain targets, serotonin-producing neurons affect complex physiological and behavioral responses, an effect that may involve cross-talk with additional neurotransmitter systems. To selectively manipulate endogenous serotonergic activity in the living brain and identify the brainwide substrates modulated by serotonin (5-HT) neurotransmission, we generated two novel DREADD (Designed Receptors Exclusively Activated by Designed Drugs) conditional knock-in mouse models (*hMD3q* and *hM4Di*) that, crossed with Pet1-*Cre* transgenic mice, allowed us to activate (*hM3Dq*) or inhibit (*hM4Di*) 5-HT

neurons firing in a time-controlled manner by systemic administration of Clozapine-N-Oxide (CNO). By using functional magnetic resonance imaging (fMRI) and *c-Fos* immunostaining, we demonstrate that chemogenetically evoked 5-HT results in region-specific activation of cortical and subcortical brain substrates involved in emotional responses, plus a specific and robust activation of focal terminals of the dopamine (DA) reward-systems, suggesting a possible 5-HT-mediated modulation of reward processing. To probe this hypothesis, we chemogenetically inhibited 5-HT raphe neurons during a sucrose preference test and show that serotonergic neurons exerts a permissive role for the expression of this hedonic behavior. We also demonstrate that the same inhibition does not induce depressive-like behaviors, arguing against contribution of confounding hypo-hedonic states. Collectively, our results document a previously unreported permissive role of 5-HT neurons on hedonic behavior and corroborate the emerging view of a central role of 5-HT neurotransmission in reward processing.

Disclosures: **A. Giorgi:** None. **G. Maddaloni:** None. **S. Migliarini:** None. **M. Gritti:** None. **R. Tonini:** None. **A. Gozzi:** None. **M. Pasqualetti:** None.

Poster

067. Motivation and Reward Mechanisms

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Program#/Poster#: 67.08/RR17

Topic: G.02. Motivation

Title: No evidence of compulsive feeding in mice exposed to a junk food diet

Authors: *T. LONDON, K. H. LEBLANC, A. KRAVITZ;
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Abstract: An abundance of studies have implicated a major role for striatal dopamine neurons in compulsive food consumption and obesity (Johnson & Kenny, 2010, Wang et al., 2001, Davis et al., 2009, Geiger et al, 2009). However, the evidence is still conflicting on which subregion of the striatum – the dorsomedial striatum (DMS), dorsolateral striatum (DLS) or nucleus accumbens (NAcc) – is primarily involved in compulsive food consumption. To assess this, a cohort of mice were given 5 weeks of ad libitum access or restricted access(2h/day) to a junk food diet followed by measurements of compulsive feeding. We assayed compulsive feeding in two ways: we exposed the mice to a shock-paired tone while measuring their consumption of a freely- available junk food diet and secondly during an operant nose-poking task for chocolate pellets. We hypothesized that the ad libitum and restricted access group would be more compulsive as a result of the diet and have also increased striatal neural activation. While a subset of mice engaged in compulsive feeding behaviors in both assays, these mice were not

necessarily from the junk food diet groups. Counter to our hypothesis, similar numbers of mice in all three groups engaged in compulsive feeding. Fos immunohistochemistry in the dorsal striatum also did not reveal differences between compulsive and non-compulsive mice. Our results suggest that regions outside of the striatum may mediate compulsive behavior, at least under the conditions of our study. We are following up by examining Fos immunostaining in other related brain regions. Determining the physiological and behavioral factors that underlie compulsive food intake, as well as identifying which brain regions are involved could facilitate treatments for obesity.

Disclosures: T. London: None. **K.H. LeBlanc:** None. **A. Kravitz:** None.

Poster

067. Motivation and Reward Mechanisms

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Program#/Poster#: 67.09/RR18

Topic: G.02. Motivation

Support: NSF IOS-1121273

Title: High-running mice have reduced incentive salience for a sweet-taste reward.

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Abstract: Animals tend to seek activities that are rewarding, either psychologically or physically, and avoid those that are not. Although much is known about the reward system in mammals, questions remain, especially concerning the comparison of behavioral rewards. To what extent can one rewarding behavior substitute for another? How does the strength of a reward influence its ability to substitute for another reward? In humans and rodents, some rewarding substances have been shown to partially substitute for other rewarding substances or behaviors, but this substitution is not always reciprocal. To explore whether physical activity can be subject to "reward substitution," female mice from a selective breeding experiment for high voluntary wheel-running (HR mice) and their control counterparts (C) were allowed access to wheels along with sweet solutions as a potentially competing reward (saccharin, Sweet 'N Low, Equal, Splenda, sucrose). Fluid consumption and wheel running were measured daily. Home-cage activity (without access to wheels) was measured for a different generation of mice that had

access to the same sweeteners. Wheel running was not significantly affected by exposure to the artificial sweeteners. Sucrose significantly elevated wheel running in C but not HR mice. Fluid consumption increased in a dose-dependent manner with saccharin, sucrose, Sweet 'N Low, Equal, and Splenda. HR mice had a significantly smaller increase in fluid consumption with the artificial sweetener blends as compared with C mice, despite running ~2.6-fold more than C. These results suggest that HR mice have a reduced incentive salience for artificial sweeteners and this is likely attributable to the stronger competing reward of wheel running that has evolved in these lines. Supported by NSF IOS-1121273 to T. Garland Jr.

Disclosures: Z. Thompson: None. **E.M. Kolb:** None. **L. Hiramatsu:** None. **M. Cadney:** None. **T. Garland:** None.

Poster

067. Motivation and Reward Mechanisms

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Topic: G.02. Motivation

Support: NSERC 315827

Title: The effect of active or passive chocolate delivery on cue-induced responding following periods of forced abstinence and associated changes in DeltaFosB labeling

Authors: *E. W. TUPLIN, M. R. HOLAHAN;
Neurosci., Carleton Univ., Ottawa, ON, Canada

Abstract: Recent evidence following cocaine administration has revealed that the onset of craving is delayed and tends to amplify during the abstinence period. The craving associated with addictive-like substances is mediated, in part, by changes in dopamine (DA) concentrations in the Nucleus accumbens (NAc) and has been associated with changes in DeltaFosB expression and changes in spine density. The craving response for palatable food may be similar to the pattern of craving for drugs of abuse and may show similar changes in neural substrates. The present work examined whether active (through operant conditioning) or passive (through classical conditioning) delivery of chocolate flavored pellets resulted in a delayed and amplified craving response during various periods of forced abstinence from chocolate and associated changes in DeltaFosB labeling and spine density within the NAc. Male rats were trained for 1 hr./day/10 days using either classical (VI 30s chocolate delivery) or operant (FR2 lever press for chocolate delivery) conditioning followed by one extinction session at 24 hr., 7D, 14D, or 28D where lever pressing resulted in presentation of the cues associated with training but no

chocolate pellet. Overall, rats trained in the operant procedure showed 4x greater bar pressing responses than the rats in the classical conditioning procedure during all extinction sessions. Nose poke rates during the extinction sessions were similar between the two training procedures but rats trained with the classical conditioning procedure showed a peak in nose poke behavior 7 days following the end of training with a gradual decline over time; the operant conditioning groups showed similar levels of nose pokes at all time points. Locomotor activity was slightly higher in the operant-trained groups and showed a peak at the 14-day test interval. DeltaFosB assessment revealed 2 - 3 times more labeled cells in the NAc in the operant-trained groups than the classically-conditioned groups with both training conditions associated with peak levels at the 7 day test. This coincided with increased spine densities at the 7 day test in both training conditions. The results indicate that cues may gain longer-term motivational significance when paired with active, rather than passive, delivery of chocolate pellets, which is also associated with increased DeltaFosB labeling.

Disclosures: E.W. Tuplin: None. **M.R. Holahan:** None.

Poster

067. Motivation and Reward Mechanisms

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Topic: G.02. Motivation

Support: NIH grant DA022340 to JFC

Title: Endocannabinoids control the neural substrates of interval timing in the nucleus accumbens

Authors: *J. F. CHEER[1], M. ANAYA[2], N. ZLEBNIK[2];
[1]Anat. and Neurobio., [2]Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Cannabinoids disrupt time estimation by interfering with dedicated brain circuits. The ability to perceive and respond to temporally relevant information in the environment is critical for adaptive survival, and corticostriatal circuits play a central role in timing behavior. Our previous work demonstrated that phasic dopamine release in the nucleus accumbens (NAc) encodes interval timing and that CB1 receptor activation accelerates the perception of time and shifts temporally-engendered patterns of phasic dopamine release. In the present study, we examined the causal role of NAc dopamine release in interval timing and explored how endocannabinoid signaling orchestrates timing-mediated NAc network dynamics. We demonstrate that optogenetic stimulation of ventral tegmental area dopamine neurons during the

timing interval interfered with timing mechanisms and accelerated time estimation. Additionally, interval timing was encoded by progressive increases in accumbal gamma frequency power of the local field potential. Augmenting levels of the endocannabinoid 2-AG resulted in a leftward shift in the estimate of interval duration and disrupted interval encoding by attenuating gamma frequency oscillations in a CB1 receptor-dependent manner. These results reveal a significant role for the interplay between dopamine and endocannabinoids in accumbal network dynamics that guide timing behavior and may have important implications for the use of pharmacotherapies targeting the endocannabinoid system and for the recreational use of plant-based and synthetic cannabinoids.

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Poster

067. Motivation and Reward Mechanisms

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Topic: G.02. Motivation

Support: Intramural Program of NIMH and NINDS

Title: Temporal modulation of tonically active neurons (TANs) in monkey ventral striatum related to reward size and delay to obtain it.

Authors: *R. FALCONE[1], D. WEINTRAUB[3], B. RICHMOND, 20814[2];
[2]Lab. of Neuropsycology, [1]NIMH, Bethesda, MD; [3]Neural Surgery Br., NINDS, Bethesda, MD

Abstract: Tonically active neurons (TANs) are cholinergic striatal inter-neurons that are thought to play a role in reward-related and motivational processes. In primates these neurons are frequently described as having a pattern of responses with a period of inhibition followed by excitation. Are these two phases of response representing the same information? We recorded neuronal responses from 63 TANs in two monkeys while they performed a task in which 9 combinations of reward were offered by mixing 3 sizes (2, 4 or 6 drops of water) and 3 delays (1, 5 or 10s). A visual cue predicting the combination being offered was presented throughout the trial. The monkeys were required to respond in one of two periods represented by the appearance of a yellow or a purple dot. On the appearance of yellow dot the monkeys could refuse the offer by releasing a bar immediately or accept by releasing when a purple dot appeared. If a purple dot appeared, the monkeys could accept by releasing immediately or refuse by waiting for the yellow dot. To capture response feature that might represent the information we used principal component (PC) analysis of the responses elicited by the visual cue. The principal components

from all conditions regardless of cue were extracted and the scores of those were examined. We focused on the period immediately following the onset of the visual cue, but before any action were required. We found that for basically all of the TANs the cue-elicited responses were related to the two factors, reward size and delay through the spike count and/or principal components as seen through ANOVAs with reward size and delay as factors and spike count or coefficient for PC1 as dependent variable. The TANs could be divided in two sets of 27 and 36 cells respectively, characterized by a strong ($R2 = 0.82$) and weak ($R2 = 0.11$) coefficient of correlation between the percentage of variance explained of PC1 and the percentage of variance explained of spike count from 2-way ANOVAs. Thus, there appear to be two groups of TANs, the first for which the modulation is represented by the number of spikes, and the second for which the modulation is greatly underestimated by the spike count, and can only be represented as a temporal modulation of the response. That is, for the group having basically no correlation between the first PC and the spike count, there is a temporally modulated code carrying information about reward size and delay to reward.

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Poster

067. Motivation and Reward Mechanisms

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Beijing Municipal Goverment

Title: Learning and context shape the reward response patterns of serotonin and dopamine neurons

Authors: *W. ZHONG[1,2], Y. LI[1], M. LUO[1];
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Abstract: Learning and context influence our response to rewarding stimuli. After learning that a sensory cue predicts reward, we prepare ourselves to gain the reward. On the other hand, we become more hesitant if the cue is paired with an aversive stimulus. Rewards activate both dopamine neurons in the ventral tegmental area (VTA) and serotonin neurons in the dorsal raphe nucleus (DRN). It is well established that learning produces phasic responses of dopamine (dopamine) neurons to the reward-predicting cues and decreases the responses to the reward itself. However, it remains unclear how learning affects the responses of serotonin neurons. Here, we show that serotonin neurons and dopamine neurons are differentially shaped by learning. Moreover, aversive contexts suppress the responses of both dopamine neurons and serotonin neurons to rewarding stimuli. We trained mice using the paradigm of classical conditioning, which repetitively presents a previously neutral auditory tone (conditioned stimulus; CS) before sucrose (unconditioned stimulus; US). Throughout the entire classical conditioning process, we monitored the activity of DRN serotonin neurons using cell type-specific fiber photometry of Ca^{2+} signals. As a comparison, we also recorded the activity of dopamine neurons from the VTA. During the initial phase of conditioning, both serotonin neurons and dopamine neurons showed similar activation pattern to primary reward (US). As learning develops, the activity of serotonin neurons gradually ramped up following the CS onset and peaked upon the US delivery. In contrast, the dopamine neurons became strongly activated by the CS and only mildly activated by the US. We also tested the effect of aversive stimuli. For both serotonin neurons and dopamine neurons, the responses to rewards decrease when a mouse was head-restrained or placed in a fearful context that was previously associated with footshock. Therefore, both learning and context shape the anticipatory and consummatory responses of serotonin neurons and dopamine neurons to rewards. Moreover, our results indicate that the activity of DRN serotonin neurons positively encode “beneficialness” - the net confidence level of an organism in the probability of getting rewarded at the current state.

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Poster

067. Motivation and Reward Mechanisms

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Topic: G.02. Motivation

Support: Klarman Foundation

Title: Mu opioid receptor signaling in the nucleus accumbens drives cued approach to palatable food via excitation of NAc neurons only in the absence of hunger

Authors: *K. CAREF, S. M. NICOLA;
Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: The nucleus accumbens (NAc) has long been implicated as a key node in the circuitry that regulates food intake, but its specific contribution has remained elusive. Activation of NAc mu-opioid receptors (MORs) by local agonist injection selectively augments the consumption of palatable food, suggesting that opioids within the NAc regulate consumption. However, *endogenous* NAc opioid ligands may not play this role because blockade of MORs in the NAc does not consistently reduce consumption. Therefore, we set out to test an alternative hypothesis: that the NAc's endogenous opioids promote approach to food-associated stimuli rather than consumption. Because it is likely that the neural circuitry maintaining approach behavior differs depending on an animal's homeostatic state, we trained both sated and food-restricted rats on a cued approach task in which an auditory conditioned stimulus (CS) predicted availability of a heavy cream reward. In response to the CS, animals approached a receptacle in which cream was delivered. To establish a common neural substrate for cued approach in both sated and food-restricted rats, we first performed neural recordings in the NAc while rats performed the task. In both groups, many neurons were excited by the CS, and the peak magnitudes of these excitations were similar. However, food-restricted rats exhibited both a higher proportion of CS-excited neurons and a longer-duration response, suggesting that in a state of hunger more neurons are recruited to participate in the signal that drives approach. Next, we examined the contribution of endogenous MOR activation to both behavioral performance and CS-evoked neural activity. Rats trained on the cued approach task were surgically implanted with bilateral cannulated microelectrode arrays that allowed for simultaneous unit recordings and drug injections within the NAc. After obtaining a behavioral and neural recording baseline of ~30 min, the MOR antagonist CTAP was infused bilaterally into the NAc, allowing a pre- vs. post-injection comparison of behavior and neural activity. In sated rats, both CS-evoked receptacle approach and the magnitude of NAc neurons' CS-evoked excitations were attenuated following CTAP injection. Strikingly, in food-restricted animals, CTAP caused neither a reduction in cued approach behavior nor a reduction in the magnitude of CS-evoked excitations. Together, these results suggest that the NAc's endogenous opioids act at MORs to promote food seeking in the absence of a drive for caloric intake derived from energy deficit, a mechanism that could conceivably contribute to the development of obesity.

Disclosures: K. Caref: None. **S.M. Nicola:** None.

Poster

067. Motivation and Reward Mechanisms

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Topic: G.02. Motivation

Support: MRC PhD Studentship

Title: Touchscreen tests of motivation reveal selective effects of reinforcer value, developmental and chemogenetic manipulations

Authors: *B. PHILLIPS[1,2], C. J. HEATH[2,3], N. C. PENFOLD[4], J. APERGIS-SCHOUTE[5], T. AITTA-AHO[5,6], J. ALSIÖ[2], S. E. OZANNE[4], T. J. BUSSEY[2], L. M. SAKSIDA[2];
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Abstract: Ratio tasks are widely used in studies of operant and motivational processes in behavioural neuroscience. Here we demonstrate the application of the recently developed mouse touchscreen fixed (FR) and progressive ratio (PR) tasks in the investigation of 3 distinct manipulations. We show that within-session analysis of response rates can substantially extend the interpretative value of these simple operant schedules.
First, the influence of liquid reinforcer type on performance was investigated. This revealed distinct schedule-dependent effects on task performance. Specifically, whilst PR breakpoint did not differ, response rate was sensitive to reinforcer type. In contrast, consistent reinforcer type-dependent differences in total trials completed and response rate were detected on the FR schedule. These results demonstrate the sensitivity of touchscreen FR and PR to reinforcer type and highlight a disparity between the schedules.
Second, we investigated the effects of diet-induced maternal obesity on FR/PR behaviour. The adult offspring of mouse dams maintained on a high-fat/high simple sugar diet (HFD) during pregnancy and lactation achieved significantly higher PR breakpoints under the most challenging inter-reinforcer response increment. HFD mice also completed a higher number of FR trials. Response rate analysis indicated a corresponding increased pattern of responding in HFD animals. These results indicate that HFD offspring exhibit both higher motivation and an

increased capacity for operant responding for a palatable food reinforcer relative to controls. Finally, we demonstrate that mouse FR operant output can be bi-directionally modulated by chemogenetic manipulation of accumbal cholinergic interneuron activity. Mice expressing $G_{\alpha q}$ Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) in these neurons completed significantly fewer FR trials at an increasingly slower rate than animals expressing G_i DREADDs which maintained a higher response rate for longer. PR performance was unaffected by these manipulations. These results indicate a specific role for cholinergic interneuron signalling in the mediation of satiety processes that influence operant output.
In conclusion, these experiments demonstrate the versatility of ratio schedules in the investigation of diverse developmental and neural manipulations. The analysis of within-session response rate data is also shown to yield further insight into the behavioural profile of animals performing ratio schedules.

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Poster

067. Motivation and Reward Mechanisms

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Topic: G.02. Motivation

Support: JSPS KAKENHI 15K04200

JSPS KAKENHI 15H04262

AMED 15652297

Title: Social reward signals in primate lateral hypothalamic neurons: comparison with prefrontal and midbrain dopamine neurons

Authors: *A. NORITAKE[1], M. ISODA[2,3];
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Abstract: Other individuals are one of the primary factors that influence one's fitness and survival. In a finite resource environment, others can be direct competitors and therefore one would devalue a context in which they acquire a reward (e.g., food, money, or territory) very

often, even though one's own reward may eventually be unchanged. It remains elusive what neural substrates underlie such subjective reward valuation via taking others' reward into account. To address this issue, we devised a social Pavlovian conditioning procedure using two monkeys facing each other (SfN2014; 364.09). In the procedure, a visual conditioned stimulus (CS) differently signaled each monkey's reward probability. We have demonstrated that the monkeys lower their own reward value as their opponent's reward probability increases (SfN2014; 364.09). We have also shown that neurons in the dorsomedial prefrontal cortex (DMPFC) code a variety of social reward information (SfN2014; 364.10), while presumed dopamine (DA) neurons in the midbrain code a single piece of information expressed in a subjective value scale (SfN2015; 615.11). Here, we studied activity of lateral hypothalamic (LH) neurons to gain insight into how differences in neural coding between DMPFC and DA neurons might be generated. The LH area has anatomical connections with both the DMPFC and the midbrain containing DA neurons. Moreover, LH neurons play a role in motivational behavior (Noritake and Nakamura, SfN2010-2013). Furthermore, a recent neuroimaging study reveals a possible functional link between the hypothalamus and social behavior (Noonan et al., 2014). We found that in response to the CS, 137 of the 384 neurons we recorded initially (0.15-0.45 s following CS onset) showed monotonically increasing or decreasing activity with increasing self-reward probability and about one fourth of them (n = 31) additionally showed monotonically decreasing or increasing activity with increasing other-reward probability, respectively. A further test revealed that the response profile of LH neurons as a population was consistent with subjective reward coding, similar to DA neurons. In a later phase (0.7-1 s), however, LH neurons now exhibited multiple reward signals, like DMPFC neurons, such as self-reward probability, other-reward probability, both-reward probability, and subjective reward value. Our findings delineate distinct and shared coding of social reward signals in cortico-subcortical networks and suggest that LH neurons may play a unique role in social foraging behavior by representing a multitude of reward information in different temporal domains.

Disclosures: **A. Noritake:** None. **M. Isoda:** None.

Poster

067. Motivation and Reward Mechanisms

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Topic: G.02. Motivation

Support: Ph.D. grant from the South-Eastern Norway Regional Health Authority (2013053).

Title: Intact reward sensitivity in mothers undergoing long-term opioid maintenance treatment

Authors: *M. EIKEMO[1,2,4], M. L. PEDERSEN[3,1], P. LOBMAIER[2,1], N. KUNØE[2,1], M. SARFI[2,1], S. LEKNES[3,1];
[2]Norwegian Ctr. for Addiction Res., [3]Dept. of Psychology, [1]Univ. of Oslo, Oslo, Norway; [4]Div. of Mental Hlth. and Addiction, Oslo Univ. Hosp., Oslo, Norway

Abstract: Reduced sensitivity to non-drug rewards (anhedonia) is common across a range of drug use disorders. In opioid dependent individuals, anhedonia often persist in opioid maintenance treatment (OMT) with methadone or buprenorphine. It remains unclear whether the reduced reward sensitivity is caused by the opioid medication per se or by associated lifestyle factors (unemployment, recurring relapse to drug use etc.). We measured reward sensitivity and experience in a unique group of mothers in long-term OMT (n = 23) who have maintained the stable lifestyle required to retain custody of their children for > 7 years. Twenty-seven healthy comparison mothers were also tested. Reward responsiveness was measured using a simple decision making task with skewed rewards. Accuracy and reaction time data were fitted using a drift-diffusion model (DDM) of decision making to investigate sub-processes of the choice behavior. The DDM enabled simultaneous assessment of participants' (1) a priori reward preference (starting point), (2) drift rate (evidence accumulation efficiency), (3) boundary separation (speed-accuracy trade-off) and (4) non-decision time. Self-report of predicted hedonic capacity, quality of life, trait hedonia and health was also measured. Both groups of women showed a clear behavioral bias toward the high-reward probability option, comparable to that seen in healthy controls (meta-analysis, n = 653) in previous studies using this task. Further, no group differences were seen in the sub-processes of decision making as assessed by the DDM. In line with the modeling results, self-reported hedonic capacity (state or trait) did not significantly differ between the groups. In summary, mothers in stable long-term OMT display normal reward responsiveness. Therefore, the anhedonia reported by previous studies of opioid maintenance treatment are more likely to result from non-opioid factors such as an unstable lifestyle or male gender.

Disclosures: **M. Eikemo:** None. **M.L. Pedersen:** None. **P. Lobmaier:** None. **N. Kunøe:** None. **M. Sarfi:** None. **S. Leknes:** None.

Poster

068. Motivation: Risky Behaviors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 68.01/SS5

Topic: G.02. Motivation

Support: Stanford Neuroscience Institute Neurochoice Initiative

Stanford Center for Cognitive and Neurobiological Imaging Innovation Grant

Title: Dynamic activity in affective circuits predicts choices to invest in risky ventures

Authors: *L. TONG, B. KNUTSON;
Stanford Univ., Stanford, CA

Abstract: Venture capitalists make significant financial decisions on risky propositions that may unfold over long time horizons based on minimal and incomplete prior information -- often presented in the form of a brief "pitch." While research has begun to explore behavioral factors that influence these decisions, little is known about the neural mechanisms involved. Based on the recently proposed Affect-Integration-Motivation (AIM) framework, affective neural responses related to anticipation of gain and loss may motivate approach and avoidance decisions, even about uncertain and risky propositions. This framework has been implicated in a number of domains, including risky gambles and consumer behavior. In this study, we test the applicability of the AIM model in predicting choices to invest in venture capital.
Healthy young adults (n=20) viewed 16 videos of pitches for startup companies while undergoing functional magnetic resonance imaging (fMRI). The videos were actual startup pitches that had been presented to venture capitalists, and shared similar features including visual context, presentation structure, timing, and personnel. After watching each video, subjects rated whether they would invest in each company, as well as their affective response (in terms of valence and arousal) to each pitch. Neural activity acquired during video presentation was used to predict subjects' investment choices. At the whole brain level, bilateral putamen activation at the end (but not the beginning) of each video, was parametrically related to subsequent choices to invest. Furthermore, a volume of interest analysis was conducted to establish whether temporal dynamics of nucleus accumbens (NAcc) activity during video presentation predicted subsequent investment decisions. Both the level and linear trend of NAcc activity across the second half of each video positively predicted subsequent choices to invest ($ps<0.05$), but neither of these variables reliably correlated with investment choices during the first half of each video. These findings provide preliminary evidence that the dynamics of affective neural responses may motivate investment in uncertain and risky ventures, and identify initial targets for further characterization of affect dynamics.

Disclosures: L. Tong: None. **B. Knutson:** None.

Poster

068. Motivation: Risky Behaviors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 68.02/SS6

Topic: G.02. Motivation

Support: CIHR Operating Grant

Title: Acute inactivation of the nucleus accumbens shell leads to long-lasting impairments in cue-paired choice behaviour

Authors: *M. M. BARRUS, M. TREMBLAY, V6T 1Z4, C. A. WINSTANLEY;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The mechanisms by which salient cues can invigorate maladaptive decision making are an important and understudied component of both gambling and substance use disorders. The cued rat gambling task (CrGT) was designed to address the impact that cues may have in promoting maladaptive choice. In the CrGT, animals choose between four options associated with different magnitudes and frequencies of reward and punishing time-out periods. Favoring options associated with smaller per-trial rewards but smaller losses and avoiding the tempting "high-risk, high-reward" options maximizes profits. Rats' choice of the disadvantageous risky options was significantly greater when these options were paired with salient-win related cues. Furthermore, dopamine D3 receptor agonism increased choice of the disadvantageous options, whereas D3 antagonism had the opposite effect; this effect did not hold in the absence of the win-paired cues.
The precise regional contributions to this behavior have not been elucidated, but the nucleus accumbens is likely involved given the evidence for its role in reward-related behaviors and the relative abundance of D3 receptors. Accordingly, work was performed in order to investigate its potential contributions to behaviour on the CrGT. Animals were given infusions of a GABA agonist cocktail into either the shell (n=24) or core (n=12) of the nucleus accumbens during the first six sessions of the CrGT, and then tested drug free for an additional ~25 sessions. Infusions into the shell during task acquisition resulted in lasting impairments in choice behavior that lasted over the animal's lifespan. These animals adopted a disadvantageous preference for the risky options, and maintained that preference even after drug was no longer on board. These results suggest the shell plays a critical role in establishing optimal patterns of choice behavior, and that interfering with this process results in long-lasting perturbations in decision-making behaviour.

Disclosures: **M.M. Barrus:** None. **M. Tremblay:** None. **C.A. Winstanley:** None.

Poster

068. Motivation: Risky Behaviors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 68.03/SS7

Topic: G.02. Motivation

Title: Engaging with salient win-paired cues blunts locomotor responding for cocaine: implications for the reward deficiency hypothesis of addiction.

Authors: *J.-M. N. FERLAND[1], D. LINDENBACH[2], C. VONDER HAAR[3], C. D. HOUNJET[3], A. G. PHILLIPS[2], C. A. WINSTANLEY[3];
[1]Djavad Mowafaghian Ctr. for Brain Hlth., [2]Psychiatry, [3]Psychology, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Drug addiction is a psychiatric disorder characterized by persistent drug-seeking and relapse. Studies in clinical populations have shown that active and abstinent addicts demonstrate impaired decision-making on the Iowa Gambling Task (IGT), a validated measure of cost/benefit decision-making. Furthermore, poor IGT performance in these subjects was associated with greater drug craving and relapse, suggesting poor decision-making may play a pivotal role in the development and maintenance of addiction. Using rodent analogues of the IGT, the rat gambling task (rGT) and cued rat gambling task (cRGT), disadvantageous decision-making was found to be exacerbated in risk-preferring animals after cocaine self-administration. However, while risk-preferring rGT rats showed greater responding for cocaine, all cRGT animals showed comparable drug-seeking. These findings indicate that risk-preference is uniquely and adversely affected by cocaine self-administration, but suggest that win-paired cues may enhance sensitivity to cocaine. Recent literature shows exposure to uncertain rewards can sensitize the locomotor response to acute amphetamine, raising the interesting possibility that engaging with risk or uncertainty itself may enhance responding to psychostimulants. The current study sought to determine whether a more cognitively engaging task like the rGT or cRGT would produce a similar result in response to cocaine and whether this effect extended to responding for win-paired cues. Thirty two male Long-Evans rats were tested for cocaine-induced locomotor activity prior to and after rGT and cRGT training. Animals were also subjected to a conditioned reinforcement paradigm after behavioural training to determine how task experience may affect responding to reward- paired cues. Results showed that prior to any behavioural training, risk-preferring animals demonstrated a blunted locomotor response to cocaine. After training, rGT rats demonstrated a significant increase in cocaine-induced locomotor response. However, cRGT rats did not show this potentiation, indicating that repeated exposure to win-paired cues may blunt the response to cocaine, possibly accounting for greater cocaine self-administration seen in the previous study. Interestingly, conditioned reinforcement data show no significant differences by task or by behavioural phenotype. In-vivo microdialysis data suggest basal and cocaine-mediated dopamine efflux may account for the behavioural differences observed. Collectively these data demonstrate that experience with salient cues with uncertainty may affect susceptibility to abuse drugs like cocaine.

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Poster

068. Motivation: Risky Behaviors

Location: Halls B-H

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Program#/Poster#: 68.04/SS8

Topic: G.02. Motivation

Title: Deep-brain stimulation of the subthalamic nucleus leads to selective decreases in risky choice in risk preferring rats on a rodent analogue of the Iowa Gambling Task

Authors: *P. J. COCKER[1], W. K. ADAMS[1], O. VONDER HARR[1], M. TREMBLAY[1], C. BAUNEZ[2], C. A. WINSTANLEY[1,2];
[1]Psychology, Univ. of British Columbia, Vancouver, BC, Canada; [2]Neurosci., Institue de neurosciences, Marseille, France

Abstract: Intro One major concern with dopaminergic treatments for Parkinson's disease (PD) is the development of side effects such as increased impulsivity and poor decision-making. These can even present as *de novo* psychiatric diseases such as gambling disorder or drug addiction. Deep brain stimulation (DBS) has been used as a treatment for PD for many years with great success. Interestingly, DBS of the subthalamic nucleus (STN) has been reported to reduce the incidence rate of decision-making deficits in human subjects. Methods The current study sought to assess the effects of DBS in the STN on decision-making behavior in otherwise intact rats. Rats were trained on a rodent analog of the Iowa Gambling Task until stable baseline behavior was obtained (30 sessions). Electrodes were then implanted into the STN bilaterally. After recovering, the effects of stimulation with the electrodes were assessed using an A-B design in which half of the rats served as control while the other half underwent stimulation. Stimulation continued for 10 sessions in a row. Two weeks after stimulation, brains were examined for electrode placement. Results Overall, stimulation of the STN had no effect on choice behavior. However, when rats were divided by risk-preference, and the data re-examined, choice was significantly affected in the risk-preferring rats. Specifically, choice shifted from risky options that delivered high rewards, but also had high periods of time out, towards safer options that delivered fewer pellets on any given trial, but had higher overall payouts. This effect even persisted slightly beyond the stimulation, but eventually returned to baseline levels by ten sessions post-stimulation. Discussion This study demonstrates that stimulation of the STN can lead to considerable shifts in behavior for otherwise healthy animals. Of particular interest is the relatively selective and beneficial effect on animals that exhibit a preference towards riskier choices. These data suggest that STN-DBS may be useful in reversing or attenuating impulse control disorders that may arise through dopamine agonist therapy.

Disclosures: P.J. Cocker: None. **W.K. Adams:** None. **O. Vonder Harr:** None. **M. Tremblay:** None. **C. Baunez:** None. **C.A. Winstanley:** None.

Poster

068. Motivation: Risky Behaviors

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Program#/Poster#: 68.05/SS9

Topic: G.02. Motivation

Support: CIHR MOP-89700

Title: Risky choice on a cued gambling task in rats is not associated with elevated responding for conditioned reinforcement.

Authors: *C. A. WINSTANLEY, M. TREMBLAY;
Psychology, Univ. British Columbia, Vancouver, BC, Canada

Abstract: Drug-paired cues are believed to play an important role in maintaining the addicted state, and in triggering relapse. Salient stimuli are also omnipresent in casinos, and may likewise sustain or exacerbate gambling behaviours. Cues that are predictive of a reward increase the release of brain dopamine, and this dopamine efflux is maximal when the reward is probabilistic. Therefore, it has been suggested that both repeated choice of uncertain options, and repeated exposure to cues that predict reward with maximal uncertainty, may sensitise the dopamine system and thereby predispose subjects to the development of an addiction disorder. Previous studies show that the addition of reward-concurrent audiovisual cues that increase in complexity with the size of the reward can increase rats' choice of disadvantageous "high-risk, high-reward" options in a rat model of the Iowa Gambling Task (IGT), known as the rat gambling task (cued rGT). We therefore wanted to test the hypotheses that a) increased risky choice on the cued rGT reflects greater sensitivity at baseline to conditioned reinforcement (CRf) i.e. the degree to which a reward-paired cue comes to act as a reinforcer for which the animal will work, and b) repeated choice of risky options may itself precipitate greater sensitivity to the ability of reward-paired cues to act as conditioned reinforcers. Subjects were 32 female rats. Responding for CRf was tested either before (n = 16) or after (n = 16) acquisition of the cued rGT. In the cued rGT, rats chose between four options associated with distinct magnitudes and probabilities of reward (1-4 sugar pellets) or time-out penalties (5-40s). The optimal strategy was to favour options associated with smaller per trial gains, but shorter penalties, similar to that in the IGT. As expected, a majority of rats performing the cued rGT developed a preference for the disadvantageous risky options. However, contrary to our hypotheses, higher rates of responding for CRf prior to training on the cued rGT was associated with a better choice strategy, less motor impulsivity, and more trials completed on task. Furthermore, cued rGT training did not increase rats' motivation to respond for CRf. Instead, there was no evidence of any association between responding for CRf and risky choice when the CRf test occurred after cued rGT training. These

results suggest that rats' performance of simple cue-driven behaviours does not readily inform our understanding of how cues influence more complex tests of cost/benefit decision making.

Disclosures: C.A. Winstanley: None. **M. Tremblay:** None.

Poster

068. Motivation: Risky Behaviors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

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Topic: G.02. Motivation

Support: CIHR Fellowship to MV Cherkasova

Title: Reward-concurrent audiovisual cues increase risky decision-making in Iowa and Vancouver gambling tasks

Authors: *M. V. CHERKASOVA[1], J. J. S. BARTON[2], L. CLARK[3], A. J. STOESSL[1], C. A. WINSTANLEY[3];
[1]Neurol., [3]Psychology, [2]Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Reward cues can potently influence behaviour. In addicted individuals, exposure to contextual cues - e.g. drug paraphernalia - is believed to trigger cravings, drug use and relapse. Yet, mechanisms through which cues influence such outcomes remain poorly understood. As one mechanism, cues may influence decision-making processes. This hypothesis is supported by our work in rats, but remains largely untested in humans. Here, we examined the effects of reward-paired audio-visual cues (money images and casino-like jingles) on decision-making in healthy human volunteers. Decision-making was assessed using two paradigms: 1) the Iowa Gambling Task (IGT), which is an ecologically valid model of real life maladaptive risky choice; 2) the Vancouver Gambling Task (VGT), which evaluates the degree of risk preference and allows to model choice parameters under Prospect Theory. One group of participants completed the IGT with reward-paired cues present and the VGT without the cues; the other group completed the VGT with the cues and the IGT without the cues. Cues increased in perceptual saliency with increasing magnitude of rewards. On the IGT, there was a trend-level cues x gender interaction ($p = .07$): cues had no effect in females but tended to bias choice towards the maladaptive risky options in males. On the VGT, cues resulted in riskier choice in both males and females at intermediate reward probabilities ($p = .009$). There was also a trend for a cues x gender interaction on the value function parameter ($p = .09$), which describes subjective perception rewards' value as their magnitude increases. In males, cues tended to enhance the perceived difference in value between smaller and larger reward; in females, cues tended to decrease that

perceived difference. There was also a significant effect of cues on the probability function parameter describing subjective perception of reward probabilities ($p = .02$): cues augmented the typical distortion in probability perception, where low probabilities are overestimated, high probabilities are underestimated, and intermediate probabilities are poorly discriminated. Though the interaction with gender was not significant, the effect was likely driven by females, who showed a significant effect of cues ($p=.02$), while males did not ($p = .60$). Together, the results suggest that salient reward-paired sensory cues do result in riskier choice, albeit through different mechanisms in males and females. In males, the cues appear to increase the perceived value of larger rewards, presumably making them more attractive and worthy of risk. In females, cues appear to promote risk through enhancing distortions in probability perception.

Disclosures: M.V. Cherkasova: None. **J.J.S. Barton:** None. **L. Clark:** None. **A.J. Stoessl:** None. **C.A. Winstanley:** None.

Poster

068. Motivation: Risky Behaviors

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Topic: G.02. Motivation

Support: NSERC Discovery grant

NSERC doctoral award

CIHR doctoral award

Title: Dissociable contributions of dorsal and ventral regions of the striatum on a rodent cost/benefit decision-making task requiring cognitive effort

Authors: *M. SILVEIRA, M. TREMBLAY, C. A. WINSTANLEY;
Univ. OF British Columbia, Vancouver, BC, Canada

Abstract: Successful decision-making requires evaluating a given option's benefits in light of its associated costs, and notably perturbations in such cost/benefit decision-making are a feature of almost every mental illness. Our lab has validated a rodent Cognitive Effort Task (rCET), a variation of the 5-choice serial reaction time task, wherein animals can choose to expend greater visuospatial attention to obtain larger sucrose rewards. Previous work indicates that the choice to apply cognitive effort is neurochemically dissociable from attentional ability, and that a corticolimbic circuit including medial prefrontal and anterior cingulate cortices, in addition to the basolateral amygdala, mediates such effortful decisions. Outputs from these key areas converge

in the striatum, which can be functionally divided into dorsal and ventral components. Notably, while dorsal striatal contributions to decision-making have not been directly investigated, it has been proposed that the ventral striatum (i.e. nucleus accumbens) functions as a "corticolimbic interface" that integrates signals from these areas, and that the resulting signal outflow may bias the direction and intensity of behavior. To address the role of these striatal areas in cognitively effortful decision-making, 26 male Long-Evans rats were trained on the rCET and following confirmation of a stable behavioural baseline, the dorsomedial striatum (DMST) and later the core region of the nucleus accumbens were temporarily inactivated via a cocktail of baclofen-muscimol. Temporary inactivation of the DMST decreased all animals' choice of the high-effort, high-reward option, impaired attentional accuracy, and robustly increased premature responding without impairing general indices of motor ability. This profile suggests the DMST may set the behavioural program required for optimal task performance, and when this process is impaired, animals can no longer successfully complete high reward/high effort trials. In stark contrast, following temporary inactivation of the ventral striatum, subjects were fundamentally unable to perform the task, as reflected by a drastic decrease in the number of trials completed and an increase in omitted responses once a trial was initiated. Together, these data suggest the striatal subregions have dissociable roles in cognitively effortful decision-making, and that the striatum is part of a larger cortico-limbic-striatal network whose function is to optimize decisions requiring cognitive effort costs.

Disclosures: **M. Silveira:** None. **M. Tremblay:** None. **C.A. Winstanley:** None.

Poster

068. Motivation: Risky Behaviors

Location: Halls B-H

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Topic: G.02. Motivation

Support: NRF-2013M3C7A1056734

Title: A modified version of Lima-Dill model predicts approach-avoidance behavior of rats facing a predator-like robot

Authors: ***S. KIM**, H.-S. JIE, J.-S. CHOI;
Biopsy Lab, Korea Univ., Seoul, Korea, Republic of

Abstract: An ecological model by Lima and Dill (1990) (LDM) describes risky decision-making of animals under predatory threat in natural situation. Widely referenced, yet such model was not simulated or tested in laboratory conditions. The current study modified the LDM (m-LDM) to

analyze animal's subjectively perceived danger and aimed to simulate a dynamic predatory situation where animal has to evaluate not only the risk but also the gain. Previously in our laboratory, we developed an approach-avoidance conflict (AAC) paradigm which utilizes a bait (food pellet), a robot predator (Jawbot) and food-deprived rats. Whenever the animal approached the pellet under Jawbot's guard, it was attacked by snapping jaws, which elicited immediate withdrawal reflex. Most rats failed to procure food bait, and all showed pattern of approach which peaked initially and then diminished, leading to more avoidance in the later phase. We have also shown that such pattern is modulated by the value of the bait and the defensive neural circuit in the brain (e.g. amygdala). In the current study, a computer simulation of m-LDM was performed to compare with behavioral data acquired from the previous study in our lab. In the simulation, m-LDM incorporates the cumulatively experienced predation risk over number of prey-predator encounters, which takes a form of cumulative distribution function of geometrical distribution. The key parameter, d* (perceived net risk), determines how fast the perceived predation risk will increase to asymptote and reflects perceived probability of death adjusted by perceived probability of gain on a given single encounter. The simulated m-LDM predictions with varying d* values for each animal were fitted to the normalized data of each animal's cumulatively experienced attack duration, and have shown acceptable goodness of fit (all adjusted $R^2 > .85$). The d* showed negative correlations with approach and preference for risky area, while showing positive correlations with preference for safer area. Also, the variables shown to modulate AAC in the previous study (food/amygdala lesion) were found to have consistent effect on d* as exclusion of food increased d* and amygdala lesions reduced d* to a very small number. Together, the current results indicate that m-LDM is a succinct yet efficient framework that can describe modulation of animal's risk-taking behavior in AAC situation at the neurobiological as well as the conceptual level.

Disclosures: S. Kim: None. **H. Jie:** None. **J. Choi:** None.

Poster

068. Motivation: Risky Behaviors

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 68.09/SS13

Topic: G.02. Motivation

Support: Finnish Foundation for Alcohol Studies

Title: Behavioral differences between alcohol preferring AA rats and Wistar rats in rodent gambling task, focus in opioidergic mechanisms

Authors: *V. OINIO, M. SUNDSTRÖM, A. RAASMAJA, P. PIEPPONEN;
Univ. of Helsinki, Fac. of Pharm., Helsinki, Finland

Abstract: Co-morbidity with gambling disorder (DSM-V) and alcohol dependence is well reported and it is possible that these two share common neuronal pathway that is not present with non-alcohol gamblers. Opioid antagonist naltrexone is used in the treatment of gambling disorder and it has been shown to have best effect with gambling disorder patients with familial history of alcoholism. We have previously found that administration of opioid agonist morphine induces risk aversive behavior in AA rats and opioid antagonist naltrexone does not have any effect to this behavior. Now we have studied whether the same effect occurs also in "normal" rat strains. The aim of the study was collect data from different aspects of decision making behavior that included rational decision making, risk aversive behavior and effect of opioidergic drugs to the risk aversion. Wistar rats were trained to perform operant behavior task of reward guided decision making consisting total of 50 sessions. The rats executed operant self-administration tasks where there was free choice of two different value sucrose rewards by pressing levers. SS (Small - Sure) lever always delivered one pellet and LL (Large - Lucky) lever delivered three pellets at different probabilities. After adapting rational decision making behavior (LL lever probability 100%), probability of gaining three pellet reward from LL lever was decreased over time (50%, 33%, 25%). We found that Wistar rats expressed similar decision making pattern as AA rats in rational choice and probability discounting. On the other hand, naltrexone induced favoring of large but uncertain rewards, thus making wistar rats behave less risk aversive. Results obtained from this study suggest that there are neurobiological differences in risky decision making of gamblers that are genetically prone to alcohol dependence compared to "normal" population.

Disclosures: **V. Oinio:** None. **M. Sundström:** None. **A. Raasmaja:** None. **P. Piepponen:** None.

Poster

068. Motivation: Risky Behaviors

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Program#/Poster#: 68.10/SS14

Topic: G.02. Motivation

Support: NIH Grant T32 DA007288

Title: A dual role for the rostromedial tegmental nucleus in processing aversive costs

Authors: *P. J. VENTO, T. C. JHOU;
Med. Univ. of South Carolina, Charleston, SC

Abstract: The proper grading of the relative cost versus the benefit of engaging in a particular action is a critical component of daily life, and impairments in cost-benefit decision-making underlie several neuropsychiatric disorders, including mania and addiction. The rostromedial tegmental nucleus (RMTg) encodes negative reward prediction errors and sends robust inhibitory projections to dopamine neurons in the ventral tegmental area (VTA), but the functional role of the RMTg in mediating decision-making in response to negative stimuli remains unknown. Accordingly, the present experiments tested the hypothesis that the RMTg is required to properly inhibit behaviors that lead to negative consequences, and that this relies on inputs to the VTA. Using a progressive shock task in rats where lever pressing for food reward is punished by footshock of gradually increasing intensity, we found that lesions of the RMTg caused a robust increase in shock breakpoint, or the maximum shock rats tolerated to receive reward. This punishment resistance observed in RMTg-lesioned rats was not the result of several non-specific factors, but instead likely arises from an inability to properly encode aversive stimuli or impairments in behavioral inhibition. To more directly test this, we used the inhibitory light-sensitive proton pump archaerhodopsin to inhibit RMTg neurons at restricted time points in the progressive shock task. We found that inhibition of VTA-projecting RMTg neurons caused a significant increase in shock breakpoint when optical inhibition was solely restricted to the period of time that overlapped with footshock exposure. Interestingly, we observed a similar increase in shock breakpoint when RMTg inhibition occurred in the window leading up to the lever-press response when rats were presumably choosing between whether to press the lever or not. These data suggest that the RMTg plays a crucial role in the encoding of aversive consequences, but the RMTg also appears to be important in modulating the decision to engage in behaviors that ultimately lead to these same negative events.

Disclosures: P.J. Vento: None. **T.C. Jhou:** None.

Poster

068. Motivation: Risky Behaviors

Location: Halls B-H

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Topic: F.04. Stress and the Brain

Support: NIH Grant MH104603-01

NIH Fellowship F31NS093939

University of Kansas Strategic Initiatives Grant

Title: 5alpha-reductase type 2 contributes to emotion and mood regulation

Authors: *M. BORTOLATO[1], L. J. MOSHER[1,2], S. C. GODAR[1], K. M. MCFARLIN[1,2];
[1]Pharmacol. and Toxicology, Univ. of Utah, Salt Lake City, UT; [2]Pharmacol. and Toxicology, Univ. of Kansas, Lawrence, KS

Abstract: The enzyme 5α reductase 2 (5αR2) catalyzes the conversion of progesterone and testosterone into potent neuroactive metabolites. We recently showed that 5αR2 is present in key brain areas for emotional regulation; the functions of this enzyme in behavioral regulation, however, remain poorly understood. Based on these premises, we hypothesized that 5αR2 may be implicated in the regulation of emotion and mood. To test for this possibility, we tested the behavioral reactivity of 5αR2 knockout (KO) and heterozygous (HZ) mice, as compared with their wild-type (WT) littermates. Our results evidenced that 5αR2 KO male mice displayed reduced preference for natural rewards (saccharin and sex), as well as reduced proclivity to novelty-seeking and risk-taking responses, as signified by their reduced exploration of novel objects and the open arms of an elevated plus maze. Finally, 5αR2 KO male mice displayed a reduction in dominance status. No significant differences were found in sensory and motor changes, as well as object-recognition memory. Taken together, these results provide the first-ever demonstration that 5αR2 is directly involved in behavioral regulation, and strongly support a pivotal role of this enzyme in motivation.

Disclosures: M. Bortolato: None. **L.J. Mosher:** None. **S.C. Godar:** None. **K.M. McFarlin:** None.

Poster

069. Human Social Communication and Behavior

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 69.01/SS16

Topic: G.02. Motivation

Title: Effects of experiencing softness on empathy and memory.

Authors: *T. TAKANO[1], K. MOGI[2];
[1]Tokyo Inst. of Technol., Yokohama, Japan; [2]Sony Computer Sci. Labs., Tokyo, Japan

Abstract: Recent studies have shown that somatosensory stimuli affect our social emotion. For example, physical warmth (e.g. holding a cup of hot coffee) promotes good reputation, while touching soft materials leads to flexible impressions of others (LE Williams, JA Bargh, 2008).

Haptic stimuli can change social judgment related to others. Those phenomena have been suggested to be related to affectional bond (attachment). Attachment is built up through physical communications with a caregiver in childhood, where the warmth from physical proximity provides a sense of safety (J Bowlby, 1958). Appropriate haptic stimuli are thought to nurture attachment, which is constructed in a subject's childhood. There are few adult attachment studies focusing on physical warmth. Adult attachment does not explicitly depend on physical proximity as much as child attachment, while attachment behavior in adulthood is difficult to observe. However, it is possible that physical warmth would have some effects on one's sense of safety, not only in childhood but also in adulthood. Subjects are likely to see those with a good reputation as having a trustworthy personality, inducing reciprocity (L Mui, et al., 2002), and leading to empathizing with more a familiar person (in-group) than a stranger (out-group) (X Xu, et al., 2009). Positive emotions attuned to in-group members would be stronger than those attuned to out-group ones.
Emotion is significantly related to memory. It is relatively easy to recall where we were and what we were doing in situations where some emotions were aroused. There are studies suggesting that long-term memories are intensified by emotional arousal (MM Bradley, et al., 1992).
Here we investigated two hypotheses. We studied whether tactile stimuli of soft materials promote a subject's empathy, using a hard chair or a soft chair. We also studied the effect of a sense of safety on a subject's memory. A recent study showed that experiencing roughness (using hand wash or touching an object) increases subjects' empathy (C Wang, et al., 2015). The present study illustrated the effects on empathy of haptic senses in a more general spectrum than hands. Our findings suggest the importance of feeling a sense of safety in promoting social abilities.

Disclosures: **T. Takano:** None. **K. Mogi:** None.

Poster

069. Human Social Communication and Behavior

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 69.02/SS17

Topic: G.02. Motivation

Support: JSPS grant 15J09321

Title: Anger produces an opposite effect on positive and negative memory previously associated with a transgressor: An evidence from facial electromyogram

Authors: ***T. MINAMOTO**, M. HARUNO;
Nat'l Inst. of Info and Comm Technol., Suita, Japan

Abstract: Anger-related event enhances vengeful and avoidance motivation toward a transgressor, while it reduces approaching motivation. Although such an event is known to affect the following cognition and behavior toward the transgressor, a retroactive effect on acquired memory and related physiological features are less known. Using an associated memory paradigm and measurement of facial electromyogram (EMG), we investigated how an anger-provoking social interaction affected pre-learned emotional memory and sought physiological hallmarks associated with the retroactive effect. A total of twenty-two experimental groups joined the present study. Each group included five participants, where one of them was the test subject and the others were confederates. At the study phase, the test participant was instructed to pair three kinds of emotional adjectives that describes personality trait (positive, negative, and neutral) with four confederates. The phase was repeated until memory performance went beyond 50% accuracy. Anger was elicited by administering the social rejection task and dictator game where the transgressor acted in selfish manner as a dictator. In the social rejection task, one confederate always gave denial comments on participant's hobbies or favorites. In the dictator game, the same confederate repeatedly (14 times out of 15 trials) selected unfair monetary distribution between him/her and the test subject to maximize their own profit. Facial EMG was measured at the left corrugator region, while the test subject was engaged in the two tasks. Paired memory was tested, where the test subject selected a confederate's face in response to an adjective word presented on a monitor. The memory performance showed that positive adjectives associated with the control confederates are more recognized than those with the transgressor, indicating that anger experience suppresses retrieval of positive memory previously associated with the transgressor, possibly due to reduction of approaching motivation. Analysis of the facial EMG showed greater amplitude in the unfair distribution condition than the fair condition: $F\,(1, 21) = 4.79$, $p < .05$. Importantly, the EMG amplitude was positively correlated with memory performance of negative personality adjectives associated the transgressor: $r\,(18) = 0.68$, $p < .01$. The result indicates that the physiological response in the left corrugator region may reflect vengeful and avoidance motivation, which results in greater retrieval of negative aspects of the transgressor.

Disclosures: **T. Minamoto:** None. **M. Haruno:** None.

Poster

069. Human Social Communication and Behavior

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Topic: G.02. Motivation

Support: JSPS KAKENHI grant 16H02839

JSPS KAKENHI grant 15H01671

Title: Vicarious reward enhances the mirror neuron system activity: An EEG study

Authors: *T. INOMATA, T. ZAMA, Y. INOUE, S. SHIMADA;
Meiji Univ., KANAGAWA, Japan

Abstract: Mirror neuron system (MNS) is the brain regions that show activity as if an individual is acting when observing the behavior of others. A previous fMRI study has shown that the magnitude of MNS activity correlates with the reward system activity evoked by vicarious reward that was received by others whom the individual had cheered for [Shimada, S., Matsumoto, M., Takahashi, H., Yomogida, Y., & Matsumoto, K. (2016). Coordinated activation of premotor and ventromedial prefrontal cortices during vicarious reward. Social Cognitive and Affective Neuroscience, 11(3), 508-512.]. In the previous electroencephalography (EEG) studies, MNS activity was indexed by mu-suppression which is the attenuation in mu-band (8-13 Hz) power related with the motor activity. We investigated whether the magnitude of mu-suppression during observation of other's action was modulated before and after the vicarious reward was received by the other. The Experiment consisted of four sessions in the following order: i) 1st Observation session (ob1), ii) Cheering session, iii) 2nd Observation session (ob2), iv) Action session. In the ob1 and ob2 sessions, the subjects observed the movie, in which one (blue or yellow) right hand performed a gesture of Rock-Paper-Scissors game (RPS). In the cheering session, the subjects were instructed to cheer for a particular (blue or yellow) player in the two-player RPS game. In the Action session, subjects were instructed to perform a RPS gesture by themselves with his/her right hand. The magnitude of mu-suppression in ob1 and ob2 session was submitted to the 2 (before and after the cheering session) × 2 (player) repeated-measure ANOVA. The result showed that there was a statistically significant interaction between session and player in the left motor area (C3: $F(1, 10) = 8.44$, $p < 0.05$). There was no main effect in two factors. Subsequent analyses (Tukey's honestly significant difference) revealed that the magnitude of mu-suppression for cheered-for player became larger after cheering than before, while that for non-cheered player was smaller after cheering than before ($p < 0.05$). The result suggests that the vicarious reward enhances the MNS activity, which is likely to underlie the sense of unity with the cheered-for others.

Disclosures: **T. Inomata:** A. Employment/Salary (full or part-time): Honda Racing Corporation. **T. Zama:** None. **Y. Inoue:** None. **S. Shimada:** None.

Poster

069. Human Social Communication and Behavior

Location: Halls B-H

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Program#/Poster#: 69.04/SS19

Topic: G.02. Motivation

Title: Asymmetries of facial expressions and facial perception: An indication of Macchiavellian intelligence or of "grin and bear it" survival of the nicest

Authors: *B. PREILOWSKI;
Tuebingen Univ., Ravensburg, Germany

Abstract: Experimental data about asymmetries of facial expressions and facial perception will be presented and discussed as a possible indication of Macchiavellian intelligence or of a "grin and bear it" survival of the nicest.
Lateral asymmetries of facial emotional expressions reflect differences between unconscious and consciously controlled motor functions of the face. Facial perception also shows lateral asymmetries, especially with regard to the recognition of emotions. Interestingly, judging facial emotions is preferentially based on the expressions of the facial half, which can be consciously controlled. Thus there is the possibility to be deceived in face-to-face interactions with regard to an important aspect of communication.
Comparative studies have shown a correlation between cerebral cortical development and the capacity for deceptive behavior, which has been described as an indication of Macchiavellian intelligence. However, it can be argued that such a Darwinian trait is of doubtful value in a human society of individuals with a wide range of sensory, motor and cognitive developmental capacities. Human communities thrive on cooperation of highly diversified specialists. This cooperative interdependence in turn often requires the ability to suppress the momentary actually felt emotion and attempting to put a positive face even on a negative situation. In addition to avoiding negative interactions, the motivational and reward values of positive emotional expressions go both ways: Positive emotional expressions - even when deliberately produced - are rewarding also for those who have to "grin and bear it."

Disclosures: B. Preilowski: None.

Poster

069. Human Social Communication and Behavior

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 69.05/SS20

Topic: G.02. Motivation

Title: Social conditioned place preference in young children

Authors: *B. L. THOMPSON, M. CHOU, D. BARON, S. TAKATA;
Div. of Occup. Sci. and Occup. Therapy, USC, Los Angeles, CA

Abstract: Neurodevelopmental disorders disrupt neural circuitry involved in complex mental processes including cognition and affect. Affective processing is a more difficult construct to test in young children as it often depends on intact language skills. Key experiments have revealed important components of emotional development in children but less understood are the internal states determining affect, which directly impact, and possibly even drive specific behavioral output. Though challenging to assess, skill sets in domains influenced by affective processing are crucial for establishing capacity in developing executive function. Strategies that probe more complex internal responses, such as feelings, drives, and motivations independent from language, become necessary for populations of children with language impairments and even typically developing (TD) children. In this study we built upon our previously established paradigm of conditioned place preference (CPP) for use in young TD children by adapting the task for a social unconditioned stimulus. To our knowledge, this was the first study to differentiate rewarding, non-rewarding, and aversive characteristics of social stimuli in children. The paradigm utilized straightforward Pavlovian conditioning methods for TD children aged 30-60 months of age in a custom-built child friendly arena. During the training trials a novel social experimenter was present in one room and interacted with the child in a prescribed manner. When tested, children exhibited a conditioned preference for the social interaction room, thereby establishing that social stimuli are sufficiently salient and reinforcing for conditioning. The social CPP paradigm provides a continuous measure for describing associative learning, and social behavior phenotypes generating a dynamic range of possible behaviors. Our future studies will use this paradigm to determine what drives social deficits in specific neurodevelopmental disorders, such as ASD. Social CPP provides an exceptional opportunity to determine whether the social interaction phenotype in children with ASD is due to an aversion to social interactions or a lack of reward from social interactions. It is unique in that it represents the first assessment of social motivation in the absence of the social stimulus. This distinction is necessary for facilitating individualized intervention strategies that seek to improve social interactions in children. Translational studies like this are necessary for understanding the biological underpinnings of human behavior and social interaction disorders.

Disclosures: **B.L. Thompson:** None. **M. Chou:** None. **D. Baron:** None. **S. Takata:** None.

Poster

069. Human Social Communication and Behavior

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 69.06/SS21

Topic: G.02. Motivation

Title: Neurologic nudges to purchase health insurance

Authors: ***P. J. ZAK**[1], J. BARRAZA[2], E. TERRIS[2];
[1]Claremont Grad Univ., Claremont, CA; [2]Ctr. for Neuroeconomics Studies, Claremont Grad. Univ., Claremont, CA

Abstract: In 2016 the U.S. government began assessing penalties on people who do not have health insurance, but many people have not purchased insurance. We measured peripheral neural activity, EEG, and eye tracking as people who primarily spoke English or Spanish at home watched TV commercials about health insurance programs. Fully 23% of respondents wanted to be sent to the state health insurance website during a one-week follow-up But, ads failed to equally engage subsets of the target audience. Spanish-speaking participants were substantially under-engaged by the tested ads using multiple neurologic measures. Spanish-speaking viewers did not find the ads difficult to comprehend. Rather, our analysis shows that Spanish-speaking participants had significantly less emotional resonance with ads. Our research has shown that emotional resonance is necessary for an action to occur after an ad. Our results indicate that ads specifically designed to emotionally engage Spanish-speaking individuals are likely to be more effective.

Disclosures: **P.J. Zak:** None. **J. Barraza:** None. **E. Terris:** None.

Poster

069. Human Social Communication and Behavior

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 69.07/SS22

Topic: G.02. Motivation

Title: Burnout and mental health in a sample of university students

Authors: C. CAMILLERI, *S. SAMMUT;
Psychology, Franciscan Univ. of Steubenville, Steubenville, OH

Abstract: Background: Depression, anxiety, stress and burnout rates are an increasingly significant problem among university students nationwide. These problems can prove to be a significant negative influence on students' chances to be academically successful during their undergraduate years and effective in the post-graduation years.
Aim: The purpose of this study was to assess levels of burnout and its correlations to depression, anxiety and stress among college students, as well as their relationship with various factors (e.g. number of majors, extracurricular activities, studying hours, etc.) that could potentially influence the overall well-being of the student.
Methods: Prior to administration of the survey, approval from the IRB was obtained (IRB Approval #2015-9). Students were sent an invitation to participate in the survey via their student emails. Surveys were administered through SurveyMonkey®. The survey consisted of various demographic questions, the 21-item Maslach Burnout Inventory-Student Survey (MBI-SS), and the 21-item Depression Anxiety Stress Scale (DASS-21). The final analyzed sample consisted of 494 college students attending Franciscan University of Steubenville.
Results: The results indicated that professional efficacy (PE) was significantly negatively correlated with depression, anxiety, and stress. Exhaustion (EX) and cynicism (CY) were both positively correlated with negative mental health scores and negatively correlated with PE. PE scores were significantly positively correlated with increased hours of study, while on the contrary, CY scores were significantly negatively correlated. There was no significant difference in EX scores in relation to study hours. PE was also positively related to students' confidence in their post-graduation plans. The correlations between burnout and both paid employment and extracurricular activities were unclear potentially due to ambiguity in interpretation.
Conclusion: A key role of the undergraduate years is to prepare the students for the workforce. In part, the success of this endeavor is dependent on the direct motivations and experiences of the students, as well as their mental health. These factors collectively contribute to the accomplishments of the students, their satisfaction with their past and present achievements, as well as their continued effectiveness as they move beyond the undergraduate years. Thus, given the relationship between mental health and student success, we propose that broader strategies that actively address both professional/academic success and mental health together may provide more effective outcomes in addressing student well-being.

Disclosures: C. Camilleri: None. **S. Sammut:** None.

Poster

069. Human Social Communication and Behavior

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Topic: G.02. Motivation

Support: Emil Aaltonen Foundation Young Researcher Grant (to J.T.S.)

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European Research Council Starting Grant 313000 (to L.N.)

Finnish Cultural Foundation (to V.R.)

Title: Relationship-specific encoding of social touch in the somatosensory corticessomatosensory cortices

Authors: J. T. SUVILEHTO[1], *V. RENVALL[1], L. NUMMENMAA[1,2];
[1]Dept. of Neurosci. and Biomed. Engin., Aalto Univ. Sch. of Sci., AALTO, Espoo, Finland;
[2]Turku PET Ctr. and Dept. of Psychology, Univ. of Turku, Turku, Finland

Abstract: Background:
Social touch is an important form of affective communication. Patterns of social touch depend on the relationship between toucher and the person being touched. However, it remains unresolved how the brain represents the relationship-specific aspects of social touching.
Methods:
We studied 5 heterosexual couples (10 subjects) using functional magnetic resonance imaging (fMRI). During the fMRI scan the subject's partner and two researchers, one female and one male, took turns in 1) touching the subject's upper thigh, or 2) bringing their hand to close or 3) moderate proximity from the subject's thigh. Stimulation was performed in 6-second blocks. Data were analyzed with multi-voxel pattern analysis (MVPA). In addition to whole-brain cortical mask, regions of interest were drawn in the primary (SI) and secondary (SII) somatosensory cortices, anterior cingulate cortex (ACC), insula, and orbitofrontal cortex (OFC). A linear support vector machine classifier was trained to distinguish the individual touching the subject (partner vs. male researcher vs. female researcher; naïve chance level 33%), and the different combinations between touchers and their actions (touching vs. close proximity vs. moderate proximity, naïve chance level 11.1%).
Results
When classifying the individual touching the subject (irrespective of the action type), accuracies were significantly above the chance level in whole-brain mask (46.3%), SI (39.3%), SII (36.9%), ACC (37.1%), OFC (35.7%), and insula (35.4%), $p < 0.05$. In whole-brain cortical mask

classification, accuracy for distinguishing between the nine action types was 28.3% ($p < 0.001$). Significantly above-chance-level accuracies were also observed in SI (21.4 %), SII (18.2), ACC (16.7%), OFC (15.9%), and insula (15.4%); $p < 0.01$. Confusions occurred most often within an action category, across different actors.
Discussion:
These findings suggest that many cortical areas are involved in processing the social features of interpersonal touch. Even though whole-brain classification was by far the most robust, already primary somatosensory cortex (SI) encodes relationship-specific aspects of social touch and touch anticipation.

Disclosures: J.T. Suvilehto: None. **V. Renvall:** None. **L. Nummenmaa:** None.

Poster

069. Human Social Communication and Behavior

Location: Halls B-H

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Topic: G.02. Motivation

Support: NIMH R01 MH087721

Title: Vasopressin effects on human social responses are sex, dose, and context dependent

Authors: *R. R. THOMPSON[1], J. RILLING[2], D. PRICE[3];
[1]Dept Psychol / Neurosci, Bowdoin Col., Brunswick, ME; [2]Anthrop., Emory Univ., Atlanta, GA; [3]Psychiatry, Maine Med. Ctr., Portland, ME

Abstract: Arginine vasopressin (AVP) and its homologues have complex effects on social behavior in non-human vertebrates, some of which are sexually dimorphic, as well as dose- and context-dependent. We previously demonstrated that a single dose of AVP can induce antisocial responses to same-sex faces in men, but pro-social responses in women. Here we follow that work up by showing that AVP's behavioral effects are not only stimulus- and dose- dependent, but also modified by the individual's relationship status. In single men, intranasal delivery of 20IU AVP, but not 40IU, reduced positive ratings of the faces of other men, but not in men involved in romantic relationships. In women, the higher dose of AVP selectively increased positive ratings of the faces of other women. Additionally, there may be long term effects of single doses of AVP; on a follow up test 3-21 days after drug administration, single men given 20IU rated all faces, including those previously seen on the day when placebo had been given, less positively than men given 40IU, whereas men in relationships given 20IU rated all female faces less positively than did men given 40IU. In women, female faces previously seen after

40IU tended to be rated more positively than those seen after placebo, whereas male faces tended to be rated less positively. On the other hand, male faces previously seen after 40IU tended to be rated more positively in women who were in a romantic relationship, whereas female faces previously seen after 40IU were rated less positively. Together, these results suggest that AVP produces complex effects on human social responsiveness that are sex- and dose-dependent, modified by relationship status, and could be long-lasting.

Disclosures: R.R. Thompson: None. **J. Rilling:** None. **D. Price:** None.

Poster

069. Human Social Communication and Behavior

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Topic: G.02. Motivation

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Title: Performance decrement induced by increasing social incentive

Authors: *A. SUGIURA[1,2], Y. YOMOGIDA[3], K. IIJIMA[2,3], T. HASEGAWA[1], K. MATSUMOTO[3];
[1]Dept. of Life Sciences, GSAS, Univ. of Tokyo, Tokyo, Japan; [2]Japan Society for the Promotion of Sci., Tokyo, Japan; [3]Brain Sci. Institute, Tamagawa Univ., Tokyo, Japan

Abstract: When we are promised with a large amount of money as a result of successfully doing a task, we are likely to perform well compared to when we are promised a small amount. However, increasing incentive sometimes result in a paradoxical decrease of performance, which is called as choking under pressure. Similar to increasing money, increasing social incentive, such as decreasing indebtedness, may result in choking. In the present study, we examined how an increase in indebtedness will affect performance. In the experiment, a participant was asked to earn money for the three partners who had previously given effort to earn money for the participant. Since the participant felt indebtedness to pay back for the partner, her/his social incentive should increase according to the amount of money that was received from each partner,

resulting in the increase of performance, if the subjects did not choke on the pressure to pay back. The participant was instructed that the maximal amount of money that could be received from one partner was 5000 yen (approximately corresponding to $50), and the three partners had earned 4000 yen (Big), 2100 yen (Medium) and 300 yen (Small) for the participant. After receiving the money from each partner, the participant went into the scanner to earn back for each partner in an achievement task, where they had to stop a stopwatch in the range of 5 ± 0.05 sec after it started moving automatically. The amount of money that can be earned back in one trial was either 1000 yen or 100 yen (as control). The control condition was introduced to create a condition where there are no incentives for the subject to succeed since the amount that can be paid back is low, and to control for the effect caused by the simple increase in the amount of money. Forty trials (20 trials each for 1000 yen and 100 yen) were done for each partner and the participants were told that the actual earnings that would be given back to one partner were determined by the result of 5 randomly chosen trials done for that partner, which meant that the optimal strategy is to engage every trial with their best effort. Preliminary data (N = 7) have shown that participants' performance increased for the Medium partner compared to Small partner ($t = 2.00$, $p = .092$), but decreased for the Big partner compared to the Medium partner ($t = 1.98$, $p = .093$), although the incentive to succeed had monotonically increased. This trend was not present in the control (100 yen) condition. The behavioral results suggest that in the Big condition, participants choked on the pressure to pay back to the partner. Subsequent analysis will test for brain regions that will show the effect of performance decrement although there is a rise in incentive.

Disclosures: **A. Sugiura:** None. **Y. Yomogida:** None. **K. Iijima:** None. **T. Hasegawa:** None. **K. Matsumoto:** None.

Poster

069. Human Social Communication and Behavior

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 69.11/SS26

Topic: G.02. Motivation

Support: Lebanon Valley College, Arnold Grant

Title: The influence of personality and genetic factors on peer pressure susceptibility

Authors: ***M. NICULESCU**, E. A. AGUILAR, C. R. PARSONS, P. V. CARPER, R. S. BOYLE, J. K. KUTCH, V. J. TRANCHITELLA;
Lebanon Valley Coll, Annville, PA

Abstract: Our purpose was to develop a peer pressure model and identify personality influences and genetic correlates to susceptibility. We hypothesized participants would be persuaded by confederates to approve or disapprove of risky behavior. Furthermore, we expected that personality traits would affect how participants viewed risky behavior and the degree to which they were influenced by their peers. Finally, we postulated an interaction between specific personality traits (reward dependence, novelty seeking, and harm avoidance) and type of peer pressure (risk-promotion vs. risk-averse) on susceptibility. Participants completed personality questionnaires and viewed a movie clip portraying a college-aged man engaging in risky behavior. During a guided discussion, two confederates expressed approval (risk-promotion group) or disapproval (risk-averse group) of questions about risk-taking (i.e. studying abroad as a requirement, how integral partying is to college, and if the drinking age should be lowered), followed by participants rating their feelings aloud on a rating scale from 1 (absolutely not) to 10 (absolutely). Finally, saliva samples were collected for DNA analysis. Participants in the risk-promotion group answered all questions regarding risky behavior more positively (M = 24.00, SD = 3.89) than participants in the risk-averse group (M = 7.49, SD = 3.61), $p < .001$. Further, those who scored high in reward dependence were more likely to agree with the group (M = 8.07, SD = 2.33) about lowering the drinking age than those who scored low in reward dependence (M = 6.58, SD = 2.70), regardless of condition, $p = .035$. Also, those that scored high in novelty seeking were more likely to agree with the risk-promotion group that partying is an integral part to college life (M =8.83, SD = 1.11) than those that scored low in novelty seeking (M = 7.62, SD = 0.96), while in the risk-averse group, those that scored low in novelty seeking agreed with the group (M = 8.87, SD = 1.20) more than those that scored high in novelty seeking (M = 8.56, SD = 1.03), $p = .013$. Conversely, those that scored high in harm avoidance agreed with the risk-averse group significantly more (M = 9.07, SD = 1.10) than in the risk-promotion group (M = 7.07, SD = 1.62), with those that scored low in harm avoidance scoring in between in both groups (promotion: M = 8.09, SD = 1.10 and averse: M = 8.71, SD = 0.77) when answering if study abroad should be a college requirement, $p = .042$. In conclusion, peer pressure susceptibility is influenced by personality characteristics, and genetic correlates to novelty seeking (dopamine), harm avoidance (cannabinoid), and reward dependence (opioid) are currently being investigated.

Disclosures: **M. Niculescu:** None. **E.A. Aguilar:** None. **C.R. Parsons:** None. **P.V. Carper:** None. **R.S. Boyle:** None. **J.K. Kutch:** None. **V.J. Tranchitella:** None.

Poster

069. Human Social Communication and Behavior

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Topic: G.02. Motivation

Support: Swiss National Science Foundation (PBSKP3-124730)

G. Harold & Leila Y. Mathers Foundation (09212007)

Wellcome Trust (WT088977MF)

Title: Observational learning computations in neurones of the human anterior cingulate cortex

Authors: *M. R. HILL[1,2,3,5], E. D. BOORMAN[3,4,7], I. FRIED[6,8];
[1]EPFL, Ctr. For Neuroprosthetics, Geneve, Switzerland; [2]Div. of Biol. and Biol. Engin., [3]Computation and Neural Systems, [4]Dept. of Humanities and Social Sci., Caltech, Pasadena, CA; [5]Div. of Neurosurg. and Neuropsychiatric Inst., Univ. of California Los Angeles, Los Angeles, CA; [6]Div. of Neurosurg. and Neuropsychiatric Inst., Univ. of California Los Angeles, Los Anglese, CA; [7]Ctr. for Functional Magnetic Resonance Imaging of the Brain, Univ. of Oxford, Oxford, United Kingdom; [8]Functional Neurosurg. Unit, Tel-Aviv Med. Ctr. and Sackler Fac. of Med., Tel-Aviv Univ., Tel-Aviv, Israel

Abstract: When learning from direct experience, neurones in the primate brain have been shown to encode a teaching signal used by algorithms in artificial intelligence: the reward prediction error (PE) — the difference between how rewarding an event is, and how rewarding it was expected to be. However, in humans and other species learning often takes place by observing other individuals and to date the single-cell mechanisms that underpin such observational learning remain largely unknown. Here we show that, when subjects observed other players in a card game, neurones in their rostral anterior cingulate cortex (rACC) encoded both the expected value of an observed choice before the outcome of that choice was revealed, and the PE after the outcome was revealed. Notably, during the same task neurones recorded in the amygdala and the rostromedial prefrontal cortex did not exhibit this type of encoding. Our results suggest that humans learn by observing others, at least in part through the encoding of observational PEs in single neurones in the rACC. These findings provide the first single-cell evidence of the nature of the computation at work during observational learning in humans and have broad implications for our understanding of the neuronal circuitry underlying these processes.

Disclosures: **M.R. Hill:** None. **E.D. Boorman:** None. **I. Fried:** None.

Poster

069. Human Social Communication and Behavior

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Topic: G.02. Motivation

Support: NSF Grant BCS-1324461

Title: Cultural values moderate neural predictors of giving.

Authors: *B. PARK, J. L. TSAI, E. BLEVINS, B. KNUTSON;
Psychology, Stanford, Stanford, CA

Abstract: Can brain activity predict choices to share resources with others, and is this moderated by culture? We predicted that while neural activity in circuits associated with anticipatory affect (i.e., Nucleus Accumbens or NAcc) would predict choices to give to others. However, these effects might also depend on cultural variables, such as the match between givers' interpersonal values (e.g., desire to influence others vs. adjust to others) and targets' emotional expressions (e.g., excited versus calm). Based on previous findings that influencing others is associated with excitement whereas adjusting to others is associated with calm (Tsai et al., 2007), we predicted that givers who value influence (vs. adjustment) would offer more money to excited (vs. calm) targets, and that this cultural modulation would be mediated by enhanced activity of NAcc in response to excited (vs. calm) targets. To test this prediction, we asked European American (n=18) and Korean (n=18) subjects to play a modified dictator game as they underwent functional magnetic resonance imaging (FMRI). On each task trial, subjects chose whether or not to give a specific proportion of their endowment (i.e., $12) to targets whose avatars varied with respect to positive expression (excited versus calm), ethnicity (Caucasian versus Asian), and sex (male versus female). One randomly selected trial was actualized at the end of the experiment. Consistent with predictions, behavioral results indicated that subjects who valued influencing others (vs. adjusting to others) offered more to excited than calm targets, regardless of target avatars' ethnicity and sex (Interpersonal goals X receiver expression $B = .01$, $t = 3.20$, $p = .001$). Neurally, NAcc activity generally predicted choices to give overall ($B = .04$, $t = 2.74$, $p = .006$). However, NAcc activity also varied such that subjects who valued influence more and adjustment less showed enhanced NAcc activity towards excited targets versus calm targets (Interpersonal goals X receiver expression $B = .01$, $t = 2.19$, $p = .029$). Together, these results indicate that while NAcc activity predicts willingness to give to others, but this effect can be moderated by cultural variables related to interpersonal values. These findings may have implications for the cultural specificity of appeals for charitable giving and policy related to the distribution of resources.

Disclosures: **B. Park:** None. **J.L. Tsai:** None. **E. Blevins:** None. **B. Knutson:** None.

Poster

069. Human Social Communication and Behavior

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 69.14/TT3

Topic: G.02. Motivation

Support: NIH Emotion and CHoice Grant

Title: Generalization learning mechanisms support adaptive decisions to trust

Authors: *O. FELDMANHALL[1], J. DUNSMOOR[2], L. HUNTER[3], S. LACKOVIC[2], A. TODOROV[4], E. A. PHELPS, 10003[2];
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Abstract: Over the course of repeated encounters an individual can learn to trust through trial-and-error. However, in our everyday lives we constantly encounter new individuals where judgments of trustworthiness are blind to reputation. In these cases, what mechanism drives an individual to trust? The ability to generalize across stimuli and situations is an essential feature of survival that has robustly shown to be deployed under numerous cognitive domains. In two experiments, we test whether reputations of previously encountered individuals generalize to new individuals as a function of perceptual resemblance. In a learning task, subjects played an iterative trust game with three partners, one who was very trustworthy, one who was somewhat trustworthy, and one who was not trustworthy. After learning which partners could be trusted, in a generalization task, subjects could choose their partners for a future trust game. On each trial, subjects were presented with a new player who was morphed with one of the original three partners. Results reveal subjects successfully learned to invest more with the trustworthy partner. Subsequent behavior in the generalization task revealed that responses generalized to related stimuli: as perceptual resemblance became increasingly similar to the original trustworthy partner, subjects were significantly more likely to choose the novel player, whereas the opposite effect was observed for novel players perceptually resembling the untrustworthy player. Dovetailing with previous research dissociating the role of the dorsomedial PFC and amygdala in trustworthy and untrustworthy behavior respectively, morphs of the trustworthy partner was parametrically modulated by activity in the dorsomedial PFC, while morphs of the untrustworthy partner recruited greater BOLD activity in the amygdala. Moreover, untrustworthy morphs were systematically less likely to be selected than the trustworthy morphs, demonstrating an asymmetric stimulus generalization. Together, these data not only demonstrate how and where stimulus generalization mechanisms support complex social choice, but also that social stimuli bearing even the slightest perceptual resemblance to negatively experienced stimuli disproportionately elicit aversive responses. In other words, it is better to be safe than sorry.

Disclosures: **O. Feldmanhall:** None. **J. Dunsmoor:** None. **L. Hunter:** None. **S. Lackovic:** None. **A. Todorov:** None. **E.A. Phelps:** None.

Poster

070. Positive and Negative Emotional States

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 70.01/TT4

Topic: G.03. Emotion

Support: NIMH IRP

Title: Dorsal raphe regulation of aggression via the medial orbital frontal cortex

Authors: ***J. NORDMAN**, Z. LI;
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Abstract: Violence and aggression are serious concerns for modern society. Current therapeutic strategies are limited due to a lack of understanding about the neurological mechanisms underlying aggression and the environmental triggers that cause it. A number of studies have demonstrated that serotonin is a critical regulator of social behaviors like aggression. In this study we show that serotonin inhibits aggression via the dorsal raphe/medial orbital frontal cortex pathway. We performed circuit mapping analysis which showed that dorsal raphe neurons project onto a number of aggression loci within the mouse brain (e.g. medial orbital frontal cortex, medial amygdala, and ventromedial hypothalamus). c-Fos labeling revealed that optogenetic activation of the dorsal raphe increases activity in those brain regions. To determine which synaptic targets of the serotonin system regulate aggression, we optogenetically silenced neurons of the dorsal raphe as well as their projections onto loci of the aggression circuit. Silencing of the dorsal raphe and it's projections onto the medial orbital frontal cortex inhibited aggression during a social interaction test, while silencing the projections to other downstream targets (medial amygdala and ventromedial hypothalamus) did not. Finally we present a novel FRET based serotonin sensor for use *in vivo* to discern serotonin induced activity related to aggression. This study will help elucidate the role of serotonin in regulating predatory aggression and provide possible therapeutic targets in the fight against pathological aggression and violence.

Disclosures: **J. Nordman:** None. **Z. Li:** None.

Poster

070. Positive and Negative Emotional States

Location: Halls B-H

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Topic: G.03. Emotion

Support: NIH Grant AA013517

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NIH Grant AA011852

NIH Grant AA07471

NIH Grant DA032457

Title: Playback of 50-55 kHz ultrasonic vocalizations reduces alcohol intake and negative-affect in rats selectively bred for high- and low-alcohol consumption

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Abstract: Rats selectively bred to drink alcohol to intoxicating levels (P and HAD-1) have been shown to *spontaneously* emit a significant proportion of negative-affect associated ultrasonic vocalizations (USVs), with approximately equal proportions in the negative-affect range (22-28 kHz) and positive-affect range (50-55 kHz) (Reno et al, 2015; Thakore et al, 2016). In addition, the proportion of negative-affect USV emissions is sensitive to alcohol intake (Reno et al, 2015), and increases in the proportion of negative-affect USVs are associated with increases in alcohol consumption (Thakore, et al 2016). These findings are unique to these selectively bred alcohol rats, as all other rats emit close to 100% 50-55 kHz USVs. 50-55 kHz USV emissions are reliably associated with stimulant drugs (Ahrens et al 2009, 2013, Maier et al 2010, 2012) and alcohol anticipation in alcohol-dependent rats (Buck et al 2014) in common rats. However, USVs in the 50-55 kHz range are not importantly associated with alcohol intake or motivation in rat lines selectively bred for alcohol consumption (Reno et al, 2015; Thakore et al, 2016). The high proportion of spontaneously emitted negative-affect USVs in these high-alcohol drinking rats suggests a strong direct link between excessive alcohol consumption and negative affect. Playback of positive-affect USVs enhance socialization, approach behaviors and positive-affect in Wistar rats (Wohr and Schwarting, 2007, 2009, 2012, Sefer, et al 2014). Due to the

importance of negative-affect USVs and their large proportion in high alcohol drinking rat lines, it was of interest to determine whether playback of positive-affect USVs emissions might reduce subsequent alcohol intake and reduce negative-affect USVs in these animals. To explore this hypothesis, rats selectively bred for high (P and HAD-1) and low (NP and LAD-1) alcohol consumption were presented with positive-affect USV Playback or No Playback during 20 days of handling sessions (approx. 20 min/day for 20 days). 24 hrs after the final playback session, animals were given either: 1) 24-hour alcohol access on alternating days for 4 weeks, OR 2) were recorded for USVs over 4 weeks. Two findings are of interest. First, we found that rats exposed to 50-55 kHz USV playback showed: 1) suppressed alcohol intake, and 2) emitted fewer negative-affect USVs. Although these effects were transient (lasting 1-2 weeks), the effects were observed in all bi-directional lines tested. These results indicate USVs not only reveal the emotional phenotype of the sender, but also influence alcohol intake and emotional responses in the receiver.

Disclosures: C.L. Duvauchelle: None. **J.M. Reno:** None. **N. Thakore:** None. **W.T. Maddox:** None.

Poster

070. Positive and Negative Emotional States

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 70.03/TT6

Topic: G.03. Emotion

Support: DFG KR36915-1

Title: Fight or escape: A novel paradigm to study approach/avoidance behavior to provocation

Authors: *U. M. KRÄMER[1], M. BUADES-ROTGER[2], F. BEYER[3];
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Abstract: When confronted with a menace, one must decide between two possible responses: fight or flight. Whereas previous paradigms to study aggressive behavior provided only the option to retaliate but no “flight” option, we developed a novel paradigm that gives participants the option to (temporarily) escape the interpersonal conflict. The paradigm (Fight or Escape, FoE) is set up as competitive reaction time task which entitles the winner to punish the loser. Participants alternatively faced a highly or lowly provoking opponent, but were also given the chance to avoid the encounter before each trial. In two behavioural studies, we validated the paradigm by showing that participants avoid the highly provoking opponent more often but also

behave more aggressively when deciding to "fight" and by relating participants' behavior to established behavioral and personality measures of approach/avoidance behavior. Specifically, participants' behaviour in the FoE was found to correlate with the fear potentiation of the startle reflex. In an fMRI study with the FoE, we found increased activity in OFC, precuneus, and the motor network related to the decision to fight. When, on the contrary, participants decided to escape, we observed widespread activation in areas of the so-called mentalizing network, including temporal pole and superior temporal gyrus, as well as dorsal mediofrontal cortex. The results suggest that the decision to engage or not in a fight is governed by social-evaluative processes. By providing more behavioral options to participants, the paradigm has a higher ecological validity compared to previous aggression paradigm and can be useful to delineate the neural underpinnings of interindividual variability in responding to interpersonal provocation.

Disclosures: U.M. Krämer: None. **M. Buades-Rotger:** None. **F. Beyer:** None.

Poster

070. Positive and Negative Emotional States

Location: Halls B-H

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Program#/Poster#: 70.04/TT7

Topic: G.03. Emotion

Support: MOST 104-2320-B-006-009-MY3

MOST 104-2811-B-006-053

Title: Protein phosphatase 2A is required for rapid antidepressant responses of NMDA receptor blockade

Authors: *C.-H. CHANG[1], P.-W. GEAN[2];
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Abstract: NMDA receptor (NMDAR) blockade has a fast-acting antidepressant effect in patients with major depression and in chronic stress model of mice. Furthermore, our previous study has shown that MK801 treatment, a NMDAR antagonist, reduces acute stress-induced aggression and depression-like behavior in the post-weaning socially isolated mice. However, the underlying mechanism remains unclear. In this study, we reported that MK801 treatment decreased the level of phosphorylated eukaryotic elongation factor 2 (p-eEF2), an inactive form of eEF2, and increased hippocampal BDNF expression. Moreover, the *Bdnf* shRNA transfection in the hippocampus blocked the anti-aggressive and anti-depressant effects of MK801 treatment,

suggested the hippocampal BDNF expression was required for the MK801 effects. In addition, MK801 rapidly increased activity of protein phosphatase 2A (PP2A) and the intra-hippocampal infusion of okadaic acid, a PP2A inhibitor, blocked beneficial behavioral effects of MK801 and reversed eEF2 dephosphorylation and BDNF induction. Taken together, our data suggest that MK801 activates PP2A which dephosphorylates eEF2 and induces BDNF expression, resulting in antidepressant effects of NMDAR blockade.

Disclosures: C. Chang: None. **P. Gean:** None.

Poster

070. Positive and Negative Emotional States

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 70.05/TT8

Topic: G.03. Emotion

Title: Dopaminergic modulation of aggression and anxiety: A zebrafish model

Authors: E. HELMKE, Y. TAVERAS CRUZ, M. DOUMA, A. DE VENECIA, J. NEEDHAM, J. STEFANI, *S. SASZIK;
Psychology, Northeastern Illinois Univ., Chicago, IL

Abstract: Dopamine is a neurotransmitter that governs complex social behaviors and motor function. Using the Zebrafish (*Danio rerio*) model, our goal is to gain a better understanding of how variations in cortical dopamine levels effect behavior. Apomorphine (APO, dopamine agonist) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, dopaminergic neurotoxin) were used to increase and decrease dopamine, respectively. Adult zebrafish were randomly selected and assigned to 1 of 3 groups: [control, MPTP (150 μM), and APO (150 μM)], and dosed for two minutes. Within each treatment group, groups of 3 fish were recorded in 300 mL of water in a novel tank. Distance (cm) and velocity (cm/sec) were calculated for each fish as a way of assessing motor function and social behavior. Nearest neighbor distance (NND) (cm) and thigmotaxis (sec) were also calculated as measures of social behavior. After analysis of the data, there was no significant difference in distance (Control, M = 73.69 $\pm$ 2.9cm; MPTP, M = 70.88 $\pm$ 2.6cm; APO, M = 76.59 $\pm$ 3.8cm) or velocity (Control, M = 5.34 $\pm$ 0.2cm/sec; MPTP, M = 5.46 $\pm$ 0.2cm/sec; APO, M = 5.55 $\pm$ 0.2cm/sec) across the three groups. This indicates that the motor system was still functional. Both APO and MPTP fish maintained smaller nearest neighbor distances than control fish (Control, M = 5.44 $\pm$ 0.6cm; MPTP, M = 4.84 $\pm$ 0.7cm; APO, M = 5.20 $\pm$ 0.3cm). These results may have occurred due to increased chasing and striking in APO fish, characterizing an aggressive phenotype, and grouping together for protection because of fear and anxiety in MPTP fish. Thigmotaxis significantly increased in the MPTP group (Control,

M = 10.99 ± 0.7sec; MPTP, M = 11.04 ± 0.6sec; APO, M = 8.33 ± 0.7sec). These results suggest that although the motor system remains fully functional, there are detectable changes in social behavior. Further research is required to better understand the effect of pharmaceuticals that manipulate cortical dopamine levels on the regulation of complex social behaviors. Based on our results, manipulation of the dopaminergic system through administration of both APO and MPTP has the ability to alter social behavior, producing an aggressive phenotype in APO fish, and an anxiety phenotype in MPTP fish. The biological mechanisms of social behavior are complex. Alteration and manipulation of the dopaminergic pathway may help to better understand how behaviors such as aggression and anxiety are modulated.

Disclosures: E. Helmke: None. **Y. Taveras Cruz:** None. **M. Douma:** None. **A. De Venecia:** None. **J. Needham:** None. **J. Stefani:** None. **S. Saszik:** None.

Poster

070. Positive and Negative Emotional States

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Topic: G.03. Emotion

Support: NIH 1ZIAMH002784

Title: Role of hippocampal neurogenesis in behavioral responses to an operant model of frustrative nonreward

Authors: *M. C. TSUDA, R.-M. KARLSSON, H. A. CAMERON;
Section on Neuroplasticity, NIMH/NIH, Bethesda, MD

Abstract: A common characteristic of several psychiatric disorders is irritability, which is defined as aberrant responses to frustration, or the absence of an expected reward. The hippocampus has been implicated in response to frustration. The present study investigated the role of adult hippocampal neurogenesis in behavioral responses to frustration induced by loss of an expected reward. We used valganciclovir to inhibit adult neurogenesis in male mice expressing the herpes simplex virus thymidine kinase (TK) under a GFAP promoter. Both TK and wild-type (WT) littermate controls were mildly food restricted and trained in operant chambers to lever press for food tablets on fixed ratio (FR) schedule where a light cue above the lever was paired with the reward. A progressive ratio (PR) test followed FR, and mice that reached 150 lever presses in the PR either received (rewarded group) or did not receive (frustrated group) a reward. Mice in both groups were shown the light cue associated with reward. After the PR, mice remained in the operant box for an additional 10 min where lever

pressing had no scheduled consequence. Immediately after the 10 min period, mice were tested in the resident-intruder test of aggression. A negative control group spent time in the operant chamber but did not receive light cues or rewards during FR and PR. WT mice exposed to the frustration condition showed greater lever pressing and greater aggression relative to rewarded mice. Frustrated TK mice showed even greater lever pressing than WT mice in the same condition but showed baseline levels of aggression. These results suggest that adult hippocampal neurogenesis affects behavioral responses to frustration induced by the loss of an expected reward.

Disclosures: **M.C. Tsuda:** None. **R. Karlsson:** None. **H.A. Cameron:** None.

Poster

070. Positive and Negative Emotional States

Location: Halls B-H

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Topic: G.03. Emotion

Support: Grant-in-Aid for Scientific Research (A) 24243068

Title: Computational account of empathy toward human and computer: a study by reinforcement learning model.

Authors: ***N. SAITO**, K. KATAHIRA, H. OHIRA;
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Abstract: There are two components in empathy: affective empathy (AE) and cognitive empathy (CE). A theoretical model regarding empathy has proposed that AE and CE are mutually associated (Decety & Jackson, 2004). Although empirical studies have found that CE was impact on AE (Lamm et al., 2010), it is unclear how AE and CE interact and produce empathetic behaviors. It is necessary for learning through observing others to produce empathy (Behrens et al., 2008) Thus, examining learning processes in empathy sheds light on revealing how empathy is produced. Reinforcement learning (RL) model is widely used to examine learning processes that how one's action is modulated and then represented, and it applies for the observational situations (Suzuki et al., 2012). It is necessary to update the presumed action values for another person to yield appropriate empathy, and the RL model can explain it. In the present study, we assessed both AE and CE at the same time while taking the learning aspects of empathy into consideration. We aimed to establish a computational account of empathy using the RL model. To this end, we set a task in which participants were required to predict other's choice through observation of the stochastic avoidance learning. Participants performed three types of 2-armed

bandit tasks. In the Self task, participants were instructed to avoid an aversive sound, whereas in the Other task and the Computer (COM) task, they were required to predict other's choice on the basis of feedbacks. The COM task was set as a non-emotional condition to compare with the Other task. The parameters of the simulated reinforcement-learning model were divided into direct recruitment of one's own valuation process (i.e., using the other's reward) as AE, and prediction of the other's choices using the other's action as CE. The learning rate for other's outcomes and actions were corresponded with the valuation and the prediction processes, respectively. These parameters were validated by correlation analyses with peripheral physiological responses and the dispositional empathy scores. The results showed that learning rate for the other's outcomes was correlated with participant's attention to the other's outcomes measured by heart rate deceleration, whereas learning rate for the computer's outcomes was not correlated with heart rate deceleration. Moreover, learning rate for the other's action was correlated with the CE trait, but that for the computer's action was not correlated with any empathic traits. Results of this study suggest that people use their own physiological responses to learn another's action value on the state from another's behavior, but it can apply for only human.

Disclosures: **N. Saito:** None. **K. Katahira:** None. **H. Ohira:** None.

Poster

070. Positive and Negative Emotional States

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Hope for Depression Research Foundation

University of Michigan Depression Center

Title: Sepsis causes long-term changes in emotional behavior and HPA axis reactivity in the mouse.

Authors: *J. L. SPENCER-SEGAL, K. SOMAYAJI, M. NEWSTEAD, S. J. WATSON, T. J. STANDIFORD, H. AKIL;
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Abstract: There are millions of sepsis survivors in the United States alone. More than one third of these patients suffer from long-term psychiatric morbidity including post-traumatic stress disorder, anxiety, and depression, which are often comorbid. A better understanding of the mechanisms of psychiatric pathology in this patient population will hopefully lead to effective strategies for prevention and treatment. To investigate this in the mouse, we measured sickness behavior, hypothalamic-pituitary-adrenal (HPA) axis reactivity, and emotional behavior in a naturalistic mouse model of sepsis, cecal ligation and puncture (CLP). Male and female C57/Bl6 mice underwent CLP, sham laparotomy, or no surgery, followed by open field test at 14 days. A separate group of mice underwent locomotion testing at 1, 5, and 14 days after CLP, followed by forced swim stress and euthanasia with blood collection. CLP, but not control or sham mice showed weight loss and decreased locomotion that recovered between 5 and 14 days after surgery. At 14 days, despite physiologic recovery, CLP mice showed increased anxiety-like behavior in the open field test, and increased corticosterone response to forced swimming. These results demonstrate lasting changes in emotional behavior and HPA axis reactivity in mice after recovery from sepsis, suggesting long-term CNS effects of this acute illness similar to those seen in humans.

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Poster

070. Positive and Negative Emotional States

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Title: Negative affect-associated USV acoustic characteristics predict future excessive alcohol drinking and alcohol avoidance in P and NP male rats

Authors: *J. M. RENO, JR[1], N. THAKORE[2], L. K. CORMACK[3], T. SCHALLERT[3], R. BELL[4], W. T. MADDOX[3], C. L. DUVAUCHELLE[2];
[1]Univ. of Texas At Austin, Austin, TX; [2]Pharmacol. & Toxicology, [3]Psychology, Univ. of Texas, Austin, TX; [4]Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Negative emotional status plays an important role in alcohol use disorders, such that individuals experiencing greater levels of adverse emotional events are more vulnerable to developing alcohol use disorders (AUDs). Indeed, negative life events (e.g. abuse, trauma, discrimination) contribute to negative emotional status and to the risk of developing AUDs. Ultrasonic vocalizations (USVs) emitted by rats serves as a well-established model of emotional status that can reflect positive and/or negative affective responses in real-time. When applied to rats selectively bred for high- and low-alcohol consumption, USVs can provide an emotional phenotype of alcohol vulnerable and alcohol-resistant individuals. Though most USV studies are limited to assessing only USV counts, each individual USV is a multi-dimensional data point consisting of several acoustic characteristics with the potential to provide further insight into the neurocircuitry underlying emotional response. To determine whether acoustic characteristics can be used to differentiate between rats selectively bred for high- and low-alcohol consumption, we recorded USVs emitted from alcohol-naïve and alcohol-experienced P and NP rats and utilized advanced statistical analyses (linear mixed models-LMM and linear discriminant analyses-LDA) to analyze USV acoustic characteristics (e.g., frequency in kHz, USV duration, power and bandwidth) of negative (22-28 kHz) and positive (50-55 kHz) affect –related USVs between rat lines (e.g., high versus low drinkers). Using these analyses, we were able to develop a model to determine rat line membership purely on the acoustic characteristics of their 22-28 kHz USV emissions. In other words, with the use of advanced statistical techniques and negative USV emissions, we can confidently identify the future drinking levels of individual animals most likely to be vulnerable to excessive alcohol drinking and those most resistant to alcohol abuse. The differences in USV characteristics may indicate differences in affect-related motivation to consume alcohol and associated neural pathways mediating emotional response. Characterizing these differences would allow for the targeting of dysregulated emotional states seen in human alcohol abusers.

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Poster

070. Positive and Negative Emotional States

Location: Halls B-H

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Topic: G.03. Emotion

Support: SSIS Development Award from California State University, Sacramento to S.C.F.

Funding from Yale University to T.H.B.

Title: How do rats learn to fear alarm calls?

Authors: *S. C. FURTAK[1], C. CALUB[1], T. H. BROWN[2];
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Abstract: Auditory emotional communication is thought to play an essential role in the survival of many species of birds and mammals. Auditory communication of negative affective states has been extensively studied in rats and other rodents. Rats emit 22 kHz ultrasonic vocalizations (USVs) in association with pain, fear, anxiety, or distress. As social alarm or distress signals, these 22 kHz USVs are believed to play an essential role in group survival. Whereas the capacity to produce these USVs is innate, appropriate reactivity to them requires experience. Specifically, 22 kHz USVs fail to elicit freezing behavior (the classical fear index) in naïve laboratory rats. This fact raises the question: How do rats learn to react fearfully or defensively to these ethologically-important social signals? A possible clue came from the discovery that laboratory rats actually *do* react fearfully to alarm calls (evidenced by USV-elicited freezing behavior) if they experienced foot shocks on the previous day. Several lines of evidence lead to the hypothesis that acquired fear of 22 kHz USVs is based on "autoconditioning"—an emotional learning process in which self-generated 22 kHz USVs serve as Pavlovian cues that become associated either with foot shocks or with an internal fear state. The present experiment tested this hypothesis by attempting to devocalize rats in the Experimental group through a unilateral transection of the recurrent laryngeal nerve. Animals in the Control group underwent a nerve-sparing sham operation. Both groups received unsignaled foot shocks and were subsequently tested, in a novel context, for freezing levels in response to the playback of a pre-recorded 22 kHz USV from a conspecific. Recurrent laryngeal nerve transection failed to prevent or even diminish USV-elicited freezing. Both groups showed significant and comparable USV-elicited freezing. It turned out that some Control animals failed to vocalize during conditioning and some Experimental animals did vocalize during conditioning. The animals were next re-grouped into Vocalizers versus Non-Vocalizers and reanalyzed based on whether they vocalized during conditioning. Again, there were no significant group differences in USV-elicited freezing. These results are obviously incompatible with the autoconditioning hypothesis. We therefore consider

several alternative associative and non-associative mechanisms for acquired fear of USVs. Additional research is needed to understand the neuroethology of emotional communication in rodents and related mammals. The results of such research across multiple social species will help illuminate the evolution of auditory emotional communication.

Disclosures: **S.C. Furtak:** None. **C. Calub:** None. **T.H. Brown:** None.

Poster

070. Positive and Negative Emotional States

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Deutsche Forschungsgemeinschaft (DFG) Sonderforschungsbereich Transregio (SFB TRR) 58/A1 and A5

Title: Brain serotonin and the mediation of early life stress

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Abstract: The epigenetic mediation of events that occur early in development is a widely discussed phenomenon. However, the underlying molecular mechanisms remain somewhat diffuse. In recent years, the programming of the hypothalamic-pituitary-adrenal (HPA) axis via glucocorticoid receptors was identified as an important factor in this process. The serotonin (5-HT) system has been suggested as another vital factor in this respect. Moreover, various studies show an effect of corticosteroids on number and functionality of 5-HT receptors. This goes together with various findings linking changes in the 5-HT system to early life stress exposure. To further elucidate the role of 5-HT in developmental programming by early life stress, we employed a B6.Tph2 mouse line. Mice of this line were bred towards a constitutive knock out of the *tryptophan hydroxylase 2* (*Tph2*) gene. Tph2 is the rate-limiting enzyme of the serotonin synthesis in the brain. Tph2 -/- animals of this line have been shown to be completely depleted of 5-HT in the brain. For our experiments, we exposed animals of this line to maternal separation, a well-established model of early life stress. Using this paradigm, all pups of a litter, regardless to sex and genotype, were separated from their mother for 3 hours per day. This separation is

repeated from postnatal day 2 to postnatal day 15. After this period of stress exposure, the animals were allowed to grow up under normal conditions. From postnatal day 60 onwards, male and female offspring were tested in diverse behavioural tasks to assess motor activity (open field), anxiety-like behaviours (dark-light-box, open field, elevated plus maze) as well as aggressive behaviour (resident-intruder test). Following the behavioural screening, brains were harvested for molecular analyses. We found several effects of a genotype-by-environment interaction on the display of anxiety-like behaviours and aggression. In particular, in Tph2 -/- animals we observed a different level of coping following maternal separation. These findings seen in the light of the observed molecular changes provide more insight into the role of brain 5-HT in the mediation of early life stress.

Disclosures: **M.T. Weidner:** None. **C. Auth:** None. **J. Waider:** None. **A.G. Schmitt:** None. **D.L.A. van den Hove:** None. **K. Lesch:** None.

Poster

070. Positive and Negative Emotional States

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 70.12/UU1

Topic: G.03. Emotion

Support: R01 MH090264

KAKEN 15K12773

Title: The role of central interleukin 1 in aggressive behavior of male mice

Authors: ***A. TAKAHASHI**[1,2,5], H. ALEYASIN[2], M. E. FLANIGAN[2], A. BRANCATO[2], C. MENARD[2], M. L. PFAU[2], V. KANA[3], J. WANG[4], G. E. HODES[2], B. S. MCEWEN[5], S. J. RUSSO[2];
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Abstract: Human studies have shown that there are correlations between peripheral cytokines and aggression trait. Like humans, outbred CD-1 mice show individual differences in aggressive behavior with approximately 70% of mice exhibiting a spectrum of aggressive behavior (termed Aggressors: AGG) and 30% showing no aggressive behavior (termed non-aggressors; NON). In this study, we examined neuroimunnological mechanisms of individual differences in aggression

in male CD-1 mice. Aggressive behavior of CD-1 males toward BALB/c intruder males were first characterized by resident intruder test for 3 days to define aggressors and non-aggressors. Peripheral and central cytokine/chemokine levels were measured by using ELISA following the resident intruder test. We found that aggressive encounter caused a phasic increase of interleukin 1 beta (IL-1β) in the blood in both AGG and NON; however, there was no difference between groups. There were also no pre-existing difference in IL-1β between AGG and NON nor were there any differences in the number of leukocytes or release of IL-1β following incubation with lipopolysaccharide. By contrast, we found significant higher levels of central IL-1β in the midbrain dorsal raphe nucleus (DRN) in NON versus AGG. To examine the role of IL-1β in the DRN, we injected a IL-1 receptor antagonist into either lateral ventricle or directly into the DRN. Our result showed that both i.c.v. injection and intra-DRN microinjection of IL-1 receptor antagonist increased aggressive behavior. Therefore, IL-1 receptor mediated pathway in the DRN has an inhibitory role on aggressive behavior.

Disclosures: **A. Takahashi:** None. **H. Aleyasin:** None. **M.E. Flanigan:** None. **A. Brancato:** None. **C. Menard:** None. **M.L. Pfau:** None. **V. Kana:** None. **J. Wang:** None. **G.E. Hodes:** None. **B.S. McEwen:** None. **S.J. Russo:** None.

Poster

070. Positive and Negative Emotional States

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 70.13/UU2

Topic: G.03. Emotion

Title: Profiles of animals exposed to an incentive downshift

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Abstract: Consummatory Successive Negative Contrast (cSNC) is one of the phenomenon most commonly observed when animals are confronted to an incentive downshift. It occurs when animals having an exposure to an unexpected downshift, from a high palatable reward (e.g., 32% sucrose solution) to a less preferred one (e.g., 4% sucrose solution), show an abrupt and transient suppression of the consummatory response. Control animals that have always access to the small reward do not show this suppression. Previous studies show that cSNC is a consequence of an aversive emotional state experienced when a negative discrepancy between the expected and the obtained reward occurs. Some researchers call this discrepancy frustration and pose that it is closely related to fear and anxiety. Consistent with this view, the administration of the

benzodiazepine chlordiazepoxide reduces the size of cSNC, increased hypothalamic-pituitary-adrenal activation levels happen after animals have exposure to downshifting situations, and lesions in the lateral amygdala attenuate the cSNC while lesions in the corticomedial and central nuclei eliminate it. Most jobs have addressed this phenomenon based on the analysis of mean-level responses of all experimental animals. However, there is a range of individual differences in the animals' responses to a reward devaluation event that suggests that experimental animals do not have a homogenous response; selective breeding studies also support this idea. Here we used a data analytic method for identifying heterogeneous samples to analyze divergent behavior patterns in cSNC in rats. Specifically, we analyzed the data of 53 animals with latent class growth analysis (LCGA). This particular technique is highly relevant for longitudinal data with relatively small samples. We found two profiles: animals showing negative contrast and no recovery, and animals expressing both negative contrast and recovery of consummatory response. Our results are consistent with previous literature who imply that individual differences do exist when animals have exposure to an incentive downshift.

Disclosures: I.D. Annicchiarico Iseda: None. **L. Cuenya:** None.

Poster

070. Positive and Negative Emotional States

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 70.14/UU3

Topic: G.03. Emotion

Title: Central amygdala circuits controlling feeding and appetitive behaviours

Authors: *A. DOUGLASS[1,2], H. KÜCÜKDERELI[1,2,3], M. PONSERRE[1], M. MARKOVIC[4], J. GRÜNDEMANN[4], P. ALCALA MORALES[1], C. STROBEL[1], A. LÜTHI[4], R. KLEIN[1]; [1]Max Planck Inst. For Neurobio., Martinsried, Germany; [2]Grad. Sch. of Systemic Neurosciences (LMU), Munich, Germany; [3]Intl. Max Planck Res. Sch. for Mol. and Cell. Life Sci., Munich, Germany; [4]Friedrich Miescher Inst. for Biomed. Res., Basel, Switzerland

Abstract: The central nucleus of the amygdala (CeA) has long been described as regulating feeding behaviour. However, the cellular heterogeneity of this region has rendered the precise role of the CeA in food intake a challenge to investigate by conventional methods. Neurons within the mouse CeA that express protein kinase C-δ (PKCδ) are known to mediate the anorexigenic effect of malaise and satiety signals, likely via local inhibitory connections within the CeA. With the aim of identifying the neural players in the CeA that promote food intake, we have identified a population of PKCδ-negative neurons that express Htr2a. Using deep-brain calcium imaging, in which GCaMP6s was expressed in the CeA of Htr2a-cre expressing mice,

we found that CeA^{Htr2a} neurons display distinct activity patterns during food consumption. In line with these results we found that chemogenetic and optogenetic-mediated activation of CeA^{Htr2a} neurons increased feeding in satiated animals, promoted reward-related behaviours and rescued the appetite suppressing effect of malaise-inducing drugs. Virus-mediated ablation of these neurons further substantiated the role of these cells in food intake and motivated behaviour. We further probed that local interactions between $CeA^{PKC\delta}$ and CeA^{Htr2a} neurons as well as the efferent projections of CeA^{Htr2a} neurons to brain regions with described roles in food intake and energy homeostasis and found that the functions of CeA^{Htr2a} can be segregated based on projection target. Together, these data suggest that the CeA influences feeding behaviour through both local inhibitory connections. Our study has demonstrated that in addition to the classical role of the CeA in mediating fear and appetite suppression, CeA^{Htr2a} neurons within this region encode appetitive and reward-related behaviours.

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Poster

070. Positive and Negative Emotional States

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Topic: G.03. Emotion

Support: Temporal Dynamics of Learning Center NSF SMA #1041755

NSF BRAIN EAGER SMA #1451221

Kavli Institute for Brain and Mind

Title: iRat as a tool for social neuroscience

Authors: L. F. SCHUSTER[1], N. W. BUTLER[1], D. BALL[2], S. HEATH[2], J. TAUFATOFUA[2], *L. K. QUINN[3,1], J. WILES[2], A. A. CHIBA[1];
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Abstract: The accretion of tools for measuring and analyzing neural properties is accelerating our knowledge of the numerous ways in which the brain represents the environment. Yet, the bulk of this knowledge is accrued through laboratory studies that are typically limited to investigating an individual animal and the singular capacity of an animal's brain to process

stimuli. Thus, despite the sophisticated techniques for measuring brain function and even for controlling one brain with the neural activity of another, techniques for studying direct neural responses to the environment in the presence of another relevant social companion have not been developed. For the most part, this is due to the inability to adequately control the relevant behavioral variables in neural recording experiments involving multiple animals.
Here, the design affordances offered by synthetic agents present an opportunity to develop a socially relevant research companion for the rat while maintaining adequate control over experimental variables. We endowed the iRat (intelligent rat animat technology) navigating robotic rat, with ecologically sound social behaviors in order to use it as a tool for studies in cognitive and neuroscientific research. We determined the minimum characteristics necessary for a rat to view iRat as a socially relevant companion.
To do this we undertook extensive social interaction studies with pairs of rodents in order to hypothesize the most relevant features for developing a social companion for the laboratory rat. Preliminary investigations indicate that temporal coordination of pack following behavior, mutual play, and submissive social retreat, may be sufficient. In order to test whether the iRat garnered social attention from the laboratory rat, we undertook a replication of the "jail-break" experiment in which a rat unlocks the cage of a trapped conspecific. In this case, the experiment was conducted with real rats and with trapped iRats. If iRat is sufficiently relevant to the laboratory rat, we expected the laboratory rat to release iRat from his trappings. We found that an iRat that showed the above social behaviors, including releasing the rat from his enclosure, was released significantly more often than an iRat that did not show these behaviors. By successfully developing a socially relevant companion for the laboratory rat, the basic neuroscientific questions that can be addressed with this tool are boundless.

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Poster

070. Positive and Negative Emotional States

Location: Halls B-H

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Program#/Poster#: 70.16/UU5

Topic: G.03. Emotion

Title: The role of estrogen receptors in response to positive emotional stimuli in rats observed in a seminatural environment

Authors: *O. LE MOENE, A. ÅGMO;
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Abstract: We studied the differential role of estrogen receptors (ER) α and β in socio-sexual interactions among rats exposed to two positive stimuli. Ten groups of 7 rats (4 females and 3 males) were housed for 8 days in a semi-natural environment (SNE) consisting of an open area and a complex burrow system. The introduction of the rats into the SNE was set as experimental day 0. Females had been ovariectomized about 2 weeks before the introduction. On day 5, each female was given a different treatment: Control females: 1 ml/kg peanut oil; estradiol females: 18 µg/kg 17β-estradiol benzoate; females α: 10 mg/kg propylpyrazoletriol (PPT); females β: 10 mg/kg diarylpropionitrile (DPN). On day 6, agonist treatment was repeated for females α and β while the other females received peanut oil. On day 7, all females received 1 mg/rat of progesterone. Four hours after the injection of progesterone, the rats were exposed to a natural anxiolytic stimulus (smell of lavender oil). Fifty min later they were given access to a palatable food (35 g of chocolate pellets). The rats were observed for 15 minutes without any stimulation to define a baseline, and for 15 minutes during each of the stimuli. The frequency and/or duration of all socio-sexual interactions were recorded. We found no difference in behaviors between females β and control females. Estradiol females presented the more salient profile by displaying more paracopulatory behaviors, mores lordoses and having a higher lordosis quotient than control females and females β. Females α also displayed more paracopulatory behaviors than control females and females β. Notably, females α displayed more lordoses than control females and females β only during the lavender phase. Regarding the stimulations, females displayed more paracopulatory behaviors during the lavender phase, and males emitted less nose-offs during that phase compared to the two others. The behavioral changes observed in both males and females confirm that the stimulations elicited different reactions from the rats. The lavender phase was marked by reduced aggressivity from the males and enhanced sexual behaviors in females. A possible explanation is that the novel olfactory stimulus increased females' arousal and thus stimulated sexual interactions. The effects of estrogens seemed to be limited to sexual behaviors and did not seem to regulate the reaction to positive stimuli. The fact that only females α showed a pattern similar to the one of estradiol females, and that females β were not different from the controls, suggest that the effects of estrogens on sexual behaviors depend on the ER α.

Disclosures: O. Le Moene: None. **A. Ågmo:** None.

Poster

070. Positive and Negative Emotional States

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Topic: G.03. Emotion

Support: Beca Doctorado Nacional Conicyt año convocatoria 2014

Title: Gender effect of humor on decision-making: a behavioral and electrophysiological report

Authors: *J. A. FLORES[1], I. RUBIO[2], G. CAMPOS[3], E. RODRIGUEZ[4];
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Abstract: Female show poorer decision-making performance when compared with male in the Iowa Gambling Task (IGT). Evidence suggests this could be related to a stronger emotional reaction during punishments, which negatively affect female's risky decision-making performance. Recent evidence shows a gender specific down-regulation of negative emotions (NE) in which women seems to down-regulate NE in a non-automatic fashion activating reward related regions. Because humor effectively increase reward related activity, we expected humor to down-regulate the women´s NE of punishment during IGT thus increasing decision-making performance. EEG was recorded from female & male participants exposed to either humorous or non-humorous short films before their decisions were made. IGT performance & feedback related negativity (fRN) were measured. Preliminary data show a statistical difference in IGT performance in male & females, obtaining more long-term advantageous decisions during the humorous condition when compared with non-humorous condition. Additionally, we observed a significant decrease in fRN amplitude in females during the humorous condition, which has been related in previous studies with changes in emotional valence.

Disclosures: J.A. Flores: None. **I. Rubio:** None. **G. Campos:** None. **E. Rodriguez:** None.

Poster

070. Positive and Negative Emotional States

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Topic: G.03. Emotion

Support: Wellcome Trust Doctoral Training Programme in Neural Dynamics 099699/Z/12/Z

Title: Investigating rapid versus delayed onset antidepressant action in rodents: a behavioural and computational approach.

Authors: *C. A. HALES[1], E. S. J. ROBINSON[2], C. J. HOUGHTON[3];
[2]Physiology, Pharmacol. and Neurosci., [3]Computer Sci., [1]Univ. of Bristol, Bristol, United Kingdom

Abstract: Major depressive disorder (MDD) is one of the most common psychiatric disorders. Until the recent discovery of the rapid onset antidepressant action of ketamine (an NMDA

receptor antagonist), pharmacological treatments for MDD were limited to drugs with delayed efficacy. In this study, acute and chronic treatments were used to investigate the effect of rapid vs delayed onset antidepressants in rats on: (1) judgement bias, a behavioural measure of affective state and (2) the decision making processes underlying bias using the diffusion model. Male Lister hooded rats were trained on a reward-based judgement bias task where correct responses to two distinct tones corresponded to obtaining high or low value reward. Judgement bias was measured by recording responses to a midpoint ambiguous tone following: (1) acute treatment with conventional antidepressants: fluoxetine (0.3, 1.0 mg/kg), reboxetine (0.3, 1.0 mg/kg), venlafaxine (1.0, 3.0 mg/kg) or vehicle; (2) chronic treatment with fluoxetine (0.0, 1.0 mg/kg); (3) acute treatment with ketamine (0.0, 0.3, 1.0, 3.0 mg/kg); and (4) acute treatment with phencyclidine (PCP; 0.0, 0.3, 1.0, 3.0 mg/kg), an NMDA receptor antagonist with no known antidepressant properties. Behavioural data were modelled using the diffusion model to investigate the changes in decision making processes that underlie biases induced by pharmacological treatments.
Acute treatment with conventional antidepressants had no effect on judgement of the ambiguous tone, however a positive bias was induced in rats chronically treated with fluoxetine (pairwise group difference: $p=0.021$). Acute treatment with ketamine (1.0 mg/kg) also caused a positive bias (one sample t-test: $p=0.012$), whilst PCP had no effect. Diffusion modelling revealed that a more positive drift rate underlies the bias following ketamine treatment (main effect of treatment: $p=0.047$, post-hoc test: $p=0.034$), whereas there was a more positive decision starting point across sessions where chronic fluoxetine induced a bias (session*group interaction: $p=0.016$, post-hoc test: $p=0.041$).
Positive biases were induced in this judgement bias task specifically by pharmacological treatments with known antidepressant properties, over time frames that match the rapid vs delayed antidepressant efficacy of these drugs. Diffusion modelling revealed that the drugs have different effects on decision making processes which may help explain their different rates of onset of efficacy. This combination of behaviour and computational modelling may provide a useful approach to further investigate the mechanisms underlying rapid antidepressant effect.

Disclosures: **C.A. Hales:** None. **E.S.J. Robinson:** None. **C.J. Houghton:** None.

Poster

070. Positive and Negative Emotional States

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Topic: G.03. Emotion

Support: McDonnell Foundation Collaborative Action Award #220020387

Title: When focal brain damage is a good thing: Neural correlates of improvements in personality and behavior following a neurological event

Authors: *M. KING, J. BRUSS, D. TRANEL;
The Univ. of Iowa, Iowa City, IA

Abstract: Research on changes in personality and behavior following brain damage has largely focused on negative outcomes. On occasion, however, it has been noted that there are patients who actually show improvements on various personality dimensions following lesion onset. In the current work, we investigated the neuroanatomical correlates involved in this phenomenon. Using the Iowa Scales of Personality Change (ISPC), an instrument developed to measure changes in real-world executive functioning, social behavior, emotionality, irascibility, and distress following neurological damage, we identified a group of patients rated by an informant (e.g., close family member or friend) as having improved on one or more dimensions of personality and behavior following a neurological event. Informants rated patients on various personality characteristics, assessing the person's personality characteristics and behavior prior to the onset of a lesion ("Before" rating) compared to the person's personality characteristics and behavior following the onset of the lesion ("After" rating). A personality dimension was defined as an acquired improved characteristic if the "After" score on the ISPC represented a decrease in personality disturbance over the longstanding rating of the characteristic ("After" < "Before"). Using the same method, we also identified a comparison group of patients rated by informants as having acquired personality disturbances on one or more dimensions of personality following lesion onset ("After" > "Before"). To examine the neuroanatomical correlates of the observed changes in personality following lesion onset, we conducted a lesion mapping analysis. We compared patients who showed personality improvements following lesion onset to those who showed personality disturbances following lesion onset, calculating voxelwise proportional differences in lesion locations between the groups. We found that improvements on personality dimensions were related to damage to the anterior temporal lobe bilaterally, extending into the parahippocampal gyrus on the right side. To a lesser extent, improvements on personality dimensions were related to damage to the left superior temporal gyrus and the right posterior-inferior parietal lobule. This work illustrates neuroanatomical correlates involved in improvement in personality dimensions following brain lesions. Future work investigating compensatory reorganization of neural networks following lesion onset (e.g., using resting state fMRI), may help further explain the neuroanatomical correlates involved in improvement following brain damage identified in the current study.

Disclosures: **M. King:** None. **J. Bruss:** None. **D. Tranel:** None.

Poster

070. Positive and Negative Emotional States

Location: Halls B-H

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Topic: G.03. Emotion

Title: The neural underpinnings of adolescent emotion regulation in the context of reward pursuit.

Authors: S. J. DEWITT[1], *F. FILBEY[2];
[1]The Univ. of Texas at Dallas, Dallas, TX; [2]Univ. of Texas At Dallas, Dallas, TX

Abstract: *Rationale:* The ability for adolescents to regulate reward anticipation is an important question given the propensity for risk-taking behavior observed during this period of development. The goals of the present study were to test the efficacy of directed emotion regulation in early adolescence (12-14) in the context of active reward pursuit and to identify the brain and behavioral profile associated with regulatory ability. *Methods:* 17 (8 males) healthy adolescents (mean age: 13.24) were consented (with parental consent as well) to participate in the study which included an fMRI scan and behavioral assessments. Participants were trained on a cognitive reappraisal task known as distancing, which they then used during an in-scanner modified monetary incentive delay task. Activation in a known regulatory region of the prefrontal cortex, the bilateral dorsolateral prefrontal cortex (DLPFC), as well as a putative reward region, the bilateral nucleus accumbens (Nac), was investigated using a region of interest approach. Subjective ratings of reward anticipation were collected following each trial of the task. Behavioral measures of Impulsivity and sensation seeking (IMPSS) and subdomains of the Junior Temperament and Character Inventory (JTCI, harm avoidance and novelty seeking) were collected to investigate a behavioral profile of regulatory success. *Results:* Task results indicate that when told to regulate, adolescents had slower decision reaction time ($F\ (1,16) = 14.95, p \leq 0.001$) and lower ratings of reward anticipation ($F\ (1,16) = 14.81, p \leq 0.001$). At the neural level, adolescents selectively recruited regions of the emotion regulation network, particularly right DLPFC ($F\ (1,16) = 13.30, p \leq 0.01$) and bilateral anterior cingulate gyrus (ACG) (*left:* $F\ (1, 16) = 6.42, p \leq 0.05$); right: $F\ (1,16) = 6.78, p \leq 0.05$) when asked to regulate reward anticipation. Bilateral Nac showed no attenuation in response to the regulatory strategy. Activation in this region demonstrated a positive correlation with impulsivity and sensation seeking summary scores ($r = 0.50, p \leq 0.05$) and an inverse correlation with harm avoidance summary scores ($r = -0.67, p \leq 0.05$). *Conclusion*: The current findings demonstrate the ability in early adolescence to regulate behavior during active reward pursuit. Regulation appears to be achieved through recruitment of specific regions of the emotion regulation network and is done within the context of persistent reward activation.

Disclosures: **S.J. DeWitt:** None. **F. Filbey:** None.

Poster

070. Positive and Negative Emotional States

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Topic: G.03. Emotion

Support: NIMH Intramural Research Program

Title: Autonomic responses during Pavlovian learning in monkeys with orbitofrontal cortex lesions

Authors: ***J. HWANG**, P. L. NOBLE, E. A. MURRAY;
Section on Neurobio. of Learning and Memory, Lab. of Neuropsychology, NIMH/NIH, Bethesda, MD

Abstract: Previous work has shown that the subgenual portion of the anterior cingulate cortex in macaques contributes to autonomic arousal during anticipation of positive (rewarding) events (Rudebeck et al., 2014, PNAS). Because orbitofrontal cortex (OFC) is involved in stimulus-based reward learning, we wondered whether this region, too, contributed to either the learning or maintenance of autonomic arousal associated with positive events. Accordingly, we evaluated autonomic responses in rhesus monkeys (Macaca mulatta) that had sustained bilateral excitotoxic lesions of OFC (n=4) and unoperated controls (n=2). Monkeys were trained on a task in which Pavlovian conditioning of stimulus-reward associations was superimposed on instrumental conditioning of active visual fixation. The Pavlovian conditioning procedure and the instrumental fixation task proceeded independently. Small fluid rewards were earned for maintaining gaze on a central fixation spot for 4 s. On some trials, one of two Pavlovian stimuli (CS+, CS-) was presented on the monitor screen for 1 s during the 4-s fixation period. The CS+ was always followed by a large fluid reward; the CS- led to no reward. If the subject broke fixation when a CS was presented during the fixation period, the fixation spot was extinguished but the Pavlovian trial continued unaffected by the oculomotor behavior. We recorded pupil size as a measure of autonomic response. Controls exhibited an increased pupil size to the CS+, compared to the response to the CS-, within a couple of training sessions (2 ± 1) and continued to show the conditioned pupil response in anticipation of reward across at least 4 consecutive sessions. By contrast, monkeys with OFC lesions required more sessions (20 ± 7 sessions) to acquire the conditioned autonomic response and three out of four failed to sustain it for 4 consecutive sessions even with an extended period of training (50 sessions). Training with a second set of stimuli yielded a similar result. Thus, in line with a previous finding that the

primate OFC is important for regulating cardiovascular arousal upon omission of reward (Reekie et al., 2008, PNAS), the present results suggest that OFC is involved in acquiring appropriate autonomic responses to cues predicting positive events.

Disclosures: J. Hwang: None. **P.L. Noble:** None. **E.A. Murray:** None.

Poster

070. Positive and Negative Emotional States

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Topic: G.03. Emotion

Support: NARSAD 17680

NIMH Grant KO1MH82818

Title: Neural mechanisms of mood induced modulation of reality monitoring in schizophrenia

Authors: *K. SUBRAMANIAM, D. MATHALON, S. NAGARAJAN, S. VINOGRADOV; UCSF, San Francisco, CA

Abstract: This study investigates the neural basis of how a positive mood can modulate cognition in schizophrenia, specifically, in reality monitoring (RM) abilities. RM is the ability to accurately distinguish the source of self-generated items from the source of externally-derived information. In a prior fMRI study, we found that healthy control subjects (HC) showed increased medial prefrontal cortex (mPFC) signal during accurate identification of self-generated items vs. externally-presented items, which correlated with self-generated information recall. We have also shown that HC in a positive mood show increased mPFC and posterior cingulate cortex (PCC) activation. Based on these findings, we predicted that both mPFC and PCC would reveal positive mood sensitive effects that enable better RM performance.
Participants completed a mood-induction RM fMRI task. In the MRI scanner, we induced positive, neutral and negative mood states through autobiographical recall. Subjects then completed the recall phase of the RM task. The RM task consisted of an encoding phase prior to scanning and a recall phase in the scanner. In the encoding phase, subjects were presented with "noun-verb-noun" sentences. The final noun was either presented by the experimenter, or left blank for subjects to generate themselves. During the recall phase, subjects viewed the noun pairs from the sentence list and had to indicate whether the second word was previously self-generated or externally-presented.
HC showed better RM source-memory performance overall than SZ, and showed an influence of

mood on overall RM accuracy. This accuracy difference was driven by HC performing better in the positive vs. neutral mood but not in the negative mood condition. Specifically, HC recalled more self-generated information and marginally more externally-presented information in the positive vs. neutral mood condition, showing increased mPFC and PCC signals, which predicted better self-generated word recall. In SZ, however, we found that both positive and negative mood states enhanced externally-presented information recall only. When compared to HC, SZ showed reduced mPFC and PCC activation during positive vs. neutral mood states and demonstrated poorer self-generated information recall during positive and negative mood states.
These data have several interesting implications: 1) SZ do not show the normal relationship between positive mood and better RM, suggesting impairments in how hedonic experiences influence neurocognitive systems that support self-referential processing; 2) They may also show more sensitivity to the effects of a negative mood, in terms of enhancing source-memory recall.

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Poster

071. Antidepressants in Animal Models of Depression

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 71.01/UU12

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Hope for Depression Research Foundation

The American Foundation for Suicide Prevention

Title: Sex differences and commonalities in the epigenetic modulation of mGlu2-dependent structural plasticity of key limbic brain regions by rapid and slow acting antidepressants

Authors: *C. NASCA[1], B. BIGIO[1], T. LAU[1], D. ZELLI[1], H. CATES[3], J. NI[2], L. BRICHTA[2], P. GREENGARD[2], E. J. NESTLER[3], B. S. MCEWEN[1];
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Abstract: Speed of action, treatment resistance and sex-dependent effects are major issues for treating depression, a global health problem with a prevalence twice as high in women than men and less than 50% of affected people respond to current treatments. Excitatory amino acids, principally glutamate, are key players and normalizing their activity, through the use of external drugs, like acetyl-L-carnitine (LAC) and ketamine, is important for reactivation of

neurochemical and structural plasticity. Such plasticity is impaired in val66met mice (hets) with a BDNF loss-of-function that are resistant to SSRIs. Here, using behavioral, molecular, structural, pharmacological and next-generation sequencing approaches, we find that both susceptible male and female hets screened at the light-dark test for light avoidance show depressive-like traits (e.g.: passive behavior) and, as shown by ChIP, have reduced acetylation of histone-3-lisyne-27 (H3K27ac) in the promoter gene Grm2 that leads to reduced expression of the metabotropic glutamate receptor 2 mGlu2 in the ventral dentate gyrus (vDG), a mood regulatory region. Such decrease in mGlu2, inhibitor of glutamate release, in the vDG is concomitant with dendritic atrophy of vDG neurons in both male and female hets. While such vDG alterations and depressive-like traits are found, and corrected in both sexes by a short term treatment with a novel rapid-acting antidepressant candidate LAC in both sexes (with different doses), we find meaningful sex differences in the male-specific structural atrophy of stellate neurons in the medial amygdala (MeA), a limbic brain region important for social behavior, which is a hallmark of depression in humans. Indeed, male, and not female, hets show retraction and decreased branching of MeA stellate neurons that are corrected by LAC while elevating mGlu2 in the vDG. Viral overexpression of mGlu2 in vDG of male hets rescues depressive-like traits, as does LAC (an mGlu2 modulator), and corrects atrophy of MeA stellate neurons. LAC is an energy-promoting agent and a novel antidepressant candidate with actions similar to HDAC inhibitors and faster than those of SSRIs, which we found also upregulate mGlu2 in the vDG. A RNAseq specifically targeted to the vDG and MeA is underway to reveal other mediators involved in LAC rapid antidepressant effects in both sexes. These findings prove mechanistic insights in the neurobiology underlying speed of action and treatment-resistance and reveal brain interconnectivity in higher level cognitive brain regions with implication for the development of drugs with better profiles of efficacy and tolerability and with emphasis on sex dependent treatment regimens.

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Poster

071. Antidepressants in Animal Models of Depression

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Program#/Poster#: 71.02/UU13

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIDA Grant T32DA007268

Title: Effects of the multimodal antidepressant vortioxetine on the psychostimulant effects of MK-801-induced changes in locomotor activity and attentional processes

Authors: *D. SMITH[1], T. M. HILLHOUSE[3,4], C. R. MERRITT[2], A. L. PEHRSON[5], C. SANCHEZ[5], J. H. PORTER[2];
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Abstract: Vortioxetine is a multimodal antidepressant approved for the treatment of major depressive disorder (MDD) and clinical studies have shown that cognitive functions such as attention, processing speed, executive function, and memory may be improved by vortioxetine in patients. While the exact mechanisms are unknown, vortioxetine's multimodal serotonergic activity may be responsible for these effects. In addition to inhibiting serotonin (5-HT) reuptake, vortioxetine is an antagonist at 5-HT_{1D}, 5-HT_3, and 5-HT_7 receptors, an agonist at 5-HT_{1A} receptors, and a partial agonist at 5-HT_{1B} receptors. This unique pharmacologic profile at serotonin sites has been hypothesized to selectively modulate glutamate transmission and may be an important factor in vortioxetine's effects on cognition (Pehrson & Sanchez 2014). The present study examined the possible interaction of vortioxetine with glutamatergic mechanisms in two behavioral tasks. The first aim examined the effects of vortioxetine on the psychostimulant effects of the noncompetitive glutamatergic NMDA antagonist MK-801 on locomotor activity, and the second aim examined attentional processes in a signal detection task in rats. MK-801(0.2 mg/kg) produced significant increases in locomotor activity, while 10 mg/kg vortioxetine did not affect locomotor activity. When given in combination, vortioxetine did not affect the MK-801-induced hyperlocomotion. In the signal detection task, 0.1 mg/kg MK-801 produced a significant decrease in percent hit (light intensity dependent) and correct rejections as compared to vehicle controls. When given alone, 10.0 mg/kg vortioxetine did not alter signal detection performance on any dependent measure; however, the combination of 10.0 mg/kg vortioxetine and 0.1 mg/kg MK-801 exacerbated the deficits produced by MK-801 alone on all dependent measures. Given recent demonstrations that acute vortioxetine enhances frontal cortex pyramidal neuron firing (Riga et al. 2016), vortioxetine may potentiate MK-801-induced signal detection impairments by enhancing membrane depolarization in glutamatergic cells, thereby increasing MK-801 occupancy at NMDA receptors. Thus, these data suggest that vortioxetine's serotonergic mechanisms may selectively enhance glutamate neurotransmission in systems that regulate attentional processes but not locomotor activity.

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Poster

071. Antidepressants in Animal Models of Depression

Location: Halls B-H

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NCN grant 2014/13/B/NZ7/02293 (Opus7)

Title: Evaluation of the role of transcription factor CREB in noradrenergic neurons in response to antidepressant treatment: study on novel transgenic mouse model

Authors: *G. KREINER, K. RAFA-ZABLOCKA, W. BUCZEK, M. BAGINSKA, I. NALEPA;
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Abstract: Decreased noradrenaline and serotonin levels in the central nervous system represent one of the crucial statements of the monoaminergic hypothesis underlying the pathophysiological basis of depression. Current treatments for depression are primarily based on the modulation of noradrenergic or/and serotonergic signal transduction pathways. There have been several attempts to identify a protein that could serve as a convergence point for antidepressant treatment, the prominent example being the cyclic AMP response element binding protein (CREB) transcription factor. The generally accepted statement is that chronically given antidepressants enhance CREB levels and activity, thus implicating its important function in the mechanism of antidepressant treatment. Nevertheless, the data are not conclusive and the feedback from the analysis of transgenic models was somehow surprising as most of these studies demonstrated that the inactivation of CREB contributes rather to antidepressant-like behavior. However, these loss-of-function studies possesses many caveats: mutation was targeting CREB not selectively in the brain and compensatory effects of cAMP response element modulator (CREM) were not taken into consideration.
The aim of this study was to evaluate the function of CREB in the mechanisms of antidepressant treatment by exploiting novel transgenic mouse model characterized by the selective and functional ablation of CREB based on the Cre/loxP system where the deletion of CREB was restricted only to the noradrenergic cells and maintained in CREM deficient background ($Creb^{DBHCre}$Crem-/- mice).
$Creb^{DBHCre}$Crem-/- mice did not show any abnormalities in their basal phenotype (weight gain, movement, anxiety- or depressive-like behavior). Also the mRNA levels of various neurotrophins, adrenergic receptors and cytokines investigated in selected brain structures were not changed in these mutants except for interleukin 10, an anti-inflammatory cytokine, which expression was profoundly diminished compared to control animals. Functional ablation of CREB resulted in a treatment-resistant phenotype after acute desipramine administration (20

mg/kg, i.p.) in tail suspension test, which effect was not observed in mice with only single CREB deletion. Our preliminary results provide further evidence for the important role of CREM as a compensatory factor and indicate that these mice may represent an unique tool to dissect the role of CREB in the mechanism of antidepressant drugs action.

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Poster

071. Antidepressants in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: FCT Grant SFRH/BD/77490/2011

Title: Epigenetic regulation of adult hippocampal structural neuroplasticity by chronic stress and antidepressants: is there a role for DNA 5hmC?

Authors: *A. MATEUS PINHEIRO[1,2], P. PATRICIO[1,2], N. ALVES[1,2], A. MACHADO-SANTOS[1,2], M. MORAIS[1,2], J. CORREIA[1,2], J. BESSA[1,2], J. MARQUES[1,2], M. BRANCO[3], N. SOUSA[1,2], L. PINTO[1,2];
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Abstract: The adult central nervous system (CNS) is endowed with considerable regenerative and neuroplastic potential. Neuroplasticity, in its different forms, is dynamically modulated by intrinsic genetic factors in conjugation with environmentally imposed factors of our everyday life. Nowadays, stress-exposure is a common environmental etiological factor leading to various pathological marks in neurochemical and neuroplastic phenomena, many of them underlying the so-called stress-related disorders. Moreover, several studies have shed light on how epigenetic mechanisms serve as mediators of the pathological effects of stress on brain homeostasis. Here, we aimed to study the effects of stress on the epigenetic landscapes of the dorsal and ventral hippocampal dentate gyrus (DG). Furthermore, we aimed to explore how chronic exposure to stress impacts on the DG epigenome and its repercussions to fundamental emotional and cognitive modalities. We have exposed young-adult Wistar-Han rats to a pre-validated unpredictable chronic mild stress (uCMS) protocol during 6 weeks and performed a battery of behavioral tests to analyze emotional and cognitive dimensions. After sacrifice, we have

analyzed cytogenesis and dendritic morphological rearrengements. In addition, we have conducted genome-wide analysis of methylation and hydroxymethylation in these areas.
So far, results demonstrate that stress exposure compromises different forms of hippocampal structural plasticity, producing a time-dependent manifestation of different anhedonic, anxious-like and cognitive deficits . Moreover, hydroxymethylation pathways are likely to be involved in the pathological effects of stress exposure, as they are differentially regulated in control and stress-exposed animals. Indeed, we have found a downregulation of TET3 enzyme in the dorsal hippocampal dentate gyrus (DG), correlated with an overall decrease in 5hmC levels in the same region, quantified by oxRRBS. Moreover, 5hmC alterations in the dorsal DG were reversed after chronic fluoxetine treatment.

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Poster

071. Antidepressants in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Hope for Depression Research Foundation

American Foundation for Suicide Prevention

Title: Signatures of central metabolic syndrome are associated with depressive-like phenotypes and rescued by an antidepressant candidate

Authors: *B. BIGIO[1,3], D. ZELLI[2], A. A. MATHE'[4], V. C. SOUSA[4], P. SVENNINGSSON[4], B. S. MCEWEN[2], C. NASCA[2];
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Abstract: The ventral dentate gyrus (vDG) has emerged as a target for resilience to stress and antidepressant action, including rapid actions. Previous research has shown that agents that influence energy metabolism, such as the epigenetic molecule acetyl-L-carnitine (LAC), exert rapid antidepressant-like effects. However, very little is know about the role that energy regulation plays in the occurrence of depressive-like phenotypes and responsiveness and/or

resistance to antidepressants. Here, we implemented a micro-dissection approach to screen global gene expression, using RNAseq, in the vDG of the SSRI-resistant BDNF Val66Met heterozygous male mice (hets) and in the Flinders-Sensitive-Line male rats that received oral administration of LAC or regular water. In hets, behavioral assessments were carried out using the forced swim (FS), light dark and social interaction tests after 1 day of treatment. In naïve FSLs and also in FSLs subjected to an acute stress to study if a stress episode could impact the responsiveness to a drug, behavioral assessment was done using the FS after 6 and 7-days of treatment. We report that LAC oral administration exerts rapid antidepressant-like effects by improving central energy regulation in the vDG of endogenously depressed hets and FSLs. RNAseq for the vDG identified the insulin (Insr), glucose receptors (Glut-4) and Cartpt, a regulator of appetite, as metabolic markers for predisposition to depression in hets and FSLs. These alterations are concomitant with activation of the mineralocorticoid receptors (MR) that boosts down-regulation of the metabotropic glutamate receptors 2, mGlu2, inhibitors of glutamate release. While correcting a MR-boosted mGlu2 decrease, LAC short term treatment corrects changes in the metabolic markers InsR, Glut-4 and Cartpt in the vDG of hets and FSLs. Also, in FSLs, after acute stress during LAC treatment, a subset of FSLs continued to respond to LAC (rFSL), whereas the other subset did not respond (nrFSL), suggesting that the acute stress episode altered the responsiveness to the drug. It is noteworthy that the occurrence of some resistance to LAC is associated with a unique gene expression profile in the vDG of nrFSLs with robust changes in metabolic regulators of fatty acid elongation, such as Evolv7 and Cybr5, and in markers of synaptic reorganization, such as NPAS4. These findings establish brain energy regulation as a factor to be considered for the development of better therapeutics and suggest that agents like LAC, by reducing a MR-boosted glutamate overflow, could rapidly ameliorate depressive disorders and could also be considered for treatment of insulin-resistance in depressed subjects.

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Poster

071. Antidepressants in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH MH103322

Title: Sex differences in antidepressant effects of kappa opioid receptor antagonists

Authors: *A. LAMAN-MAHARG[1], M. ZUFELT[1], T. COPELAND[1], R. SNYDER[2], T. FENNELL[2], F. I. CARROLL[2], B. C. TRAINOR[1];
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Abstract: Psychosocial stress leads to activation of kappa opioid receptors (KOR) which, in turn, facilitate depression-like behaviors. This has generated a strong interest in the development of KOR antagonists as a potential novel class of antidepressant. However, most studies showing stress-induced activation of KOR focus on more short-term effects of stress and only study males. New evidence suggests that long-term effects of stress (over weeks or months) have important implications for KOR function, and that there may be sex differences. We examined the effects of social defeat stress on KOR action using California mice (*Peromyscus californicus*), a monogamous species in which social defeat can be studied in both males and females. Behavioral observations were conducted two weeks after three episodes of social defeat or control conditions. A 10 mg/kg i.p. injection of KOR antagonist norBNI decreased immobility in a two day forced swim test (FST) in control male California mice, but norBNI had no effect on immobility in control or stressed female California mice at doses of 0.5, 1, 10, or 25 mg/kg. Similar results were observed in male and female C57Bl/6 mice. To examine possible sex differences in the pharmacokinetics of norBNI, we used liquid chromatography-mass spectrometry to quantitate norBNI in plasma and whole brain of male and female C57Bl/6 mice at 1 hr, 5 hrs, 48 hrs, and 3 weeks after i.p. injection with 10 mg/kg norBNI. There were no sex differences in the pharmacokinetics of norBNI. Interestingly, we have shown that AZ-MTAB, a shorter acting KOR antagonist, reduced immobility and had anxiolytic effects in female California mice. A 10 mg/kg i.p. injection of U50,488 significantly increased freezing in the elevated plus maze in control and stressed female California mice, and this effect was significantly decreased with a 10 mg/kg i.p. injection of AZ-MTAB. A 10 mg/kg treatment of AZ-MTAB also significantly decreased U50,488-induced increases in immobility in the FST in both control and stressed California mouse females. Ongoing studies are investigating potential mechanisms underlying sex differences in the effects of norBNI and AZ-MTAB. These data indicate that KOR antagonists may have novel antidepressant effects in the context of stress-induced psychiatric disorders, but that the mechanism of action may be different in males and females.

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Poster

071. Antidepressants in Animal Models of Depression

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Program#/Poster#: 71.07/VV4

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Changes in cortical astroglial cells in response to stress and antidepressant treatment

Authors: *S. SIMARD[1], G. COPPOLA[2], S. ABDIRASHID[1], R. THEDE[1], S. LAHAIE[1], S. HAYLEY[1], N. SALMASO[1,2];
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Abstract: An emerging body of work has suggested a potential role for astroglial cells in both the etiology and treatment of depression. Much of this work has focused on changes within the hippocampus and amygdala. However, recent evidence has shown decreases in expression of proteins associated with glial plasticity, such as glial fibrillary acidic protein, in cortical astroglial cells of depressed-suicide human patients that were examined post-mortem (Torres-Platas et al, 2011). This is supported by evidence from rodent models that have suggested that cortical astroglial cells may be involved in the pathophysiology of depression (Banasr et al, 2008). In the current study, we have employed a chronic unpredictable stress (CUS) paradigm in conjunction with chronic administration of a selective serotonin reuptake inhibitor, fluoxetine, as an antidepressant treatment in transgenic mice that express green fluorescent protein in astroglial cells in order to examine changes in cortical astroglial cells. Mice were exposed to CUS (or control conditions) for five weeks and injected with fluoxetine (or vehicle control) for the final three weeks of the study. Performance on behavioural tests associated with anxious and depressive behaviours were examined. As expected, CUS significantly increased anxiety and depressive-like behaviours on the forced swim test, sucrose preference test, open field and elevated plus maze. Interestingly, however, fluoxetine did not always reverse the effects of CUS and in some cases, exacerbated the effect of CUS. Fluorescence Activated Cell Sorting (FACS) was used to isolate GFP+ astrocytes for RNA extraction and sequencing. Protein and gene expression patterns were examined in cortical astroglial cells in response to CUS and fluoxetine and correlated with behavioural changes.

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Poster

071. Antidepressants in Animal Models of Depression

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Program#/Poster#: 71.08/VV5

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Antidepressant effects of AMPA receptor potentiation in a model of treatment-resistant depression

Authors: *S. W. WHITE[1], R. S. GADEPALLI[2], J. M. RIMOLDI[2,3], K. J. SUFKA[1,3]; [1]Dept. of Psychology, [2]Dept. of Biomolecular Sci., [3]Res. Inst. of Pharmaceut. Sci., Univ. of Mississippi, University, MS

Abstract: Recent research in this lab has identified a stress-vulnerable, treatment-resistant, and ketamine sensitive Black Australorp genetic line that displays homologies with the clinical presentation of treatment-resistant depression (TRD). Testing in pre-clinical rodent models of depression has shown that ketamine, an NMDA antagonist, exerts its antidepressant effects through AMPA receptor activation. Indeed, AMPA potentiators possess antidepressant effects in rodent models of depression. However, antidepressant effects of AMPA receptor activation have not been tested in a preclinical model of TRD. This research sought to determine if AMPA receptor activation produces antidepressant effects in the TRD Black Australorp genetic line. Separate groups of 5-6 day old male cockerels were given IP vehicle or one of three doses of the AMPA potentiator LY392098 (2.5, 5.0, and 10.0 mg/kg) 15 min prior to a 90 min isolation test period in which distress vocalizations (DVocs) were recorded. Vehicle treated animals displayed high DVoc rates in the anxiety phase (first 5 min) that declined to about half the initial rate during the depression phase (30-90 min), a pattern typical of other behavioral despair models. The AMPA receptor potentiator LY392098 significantly attenuated behavioral despair as indicated by increased DVoc rates in the depression phase at the 2.5 mg/kg dose. This AMPA-mediated antidepressant effect is consistent with findings in rodent models of depression and generalize to an avian model of TRD.

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Poster

071. Antidepressants in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: The Japan Agency for Medical Research and Development (AMED) grants

Title: A novel δ opioid receptor agonist NC-2800 produces the anxiolytic-like and antidepressant-like effects in animal models.

Authors: *A. SAITOH[1], E. NAKATA[2], L. GOTOH[1], M. HIROSE[2], J. SAKAI[2], T. KOMATSU[2], H. FUJII[3], M. YAMADA[1], H. NAGASE[4], T. YAMAKAWA[2];
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Abstract: δ Opioid receptor (DOR) agonists have been proposed to be attractive targets for the development of the novel antidepressants and/or anxiolytics. Recently, we succeeded in synthesizing a novel small molecule DOR agonist, named NC-2800, containing the morphinan structure. NC-2800 showed highly selective and effective DOR agonistic activity in functional cAMP assays using recombinant cells stably expressing opioid μ, δ or κ receptor, respectively. In this study, we investigated the anxiolytic-like and antidepressant-like effects of NC-2800 using animal models in rodents. In the rat elevated plus maze test, which is an animal model of innate anxiety, NC-2800 (1, 3 and 10 mg/kg, p.o.) significantly increased the time spent in the open arms 120 min after administration. The magnitude of the NC-2800-induced anxiolytic-like effects was similar to that produced by diazepam (3 mg/kg, p.o.), a benzodiazepine (BZD) anxiolytic. Interestingly, in contrast to diazepam, NC-2800 caused no significant performance changes in the Y-maze test, the ethanol-induced sleeping test and footprint test in rats. In the olfactory bulbectomized (OBX) rat, which is an animal model of depression, repeated administration of NC-2800 (1 mg/kg/day, s.c.) significantly decreased the total score of hyperemotional responses from day 1 to over the entire period on day 14. On the other hand, fluoxetine (10 mg/kg/day, s.c.), a selective serotonin reuptake inhibitor (SSRI) antidepressant, significantly reduced the total score on day 14 only. The inhibitory effects of NC-2800 were greater than those of fluoxetine in OBX rats. No significant changes in the body weight gain of OBX rats were observed between vehicle and NC-2800 treatment groups, while the fluoxetine treatment group significantly decreased the body weight gain. In conclusion, we suggest that NC-2800 is a novel orally available DOR agonist for anxiolytics/antidepressants with high efficacy and a rapid onset therapeutic effect without producing the adverse effects associated with BZDs and SSRIs. This study was supported by the Japan Agency for Medical Research and Development (AMED) grants.

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Poster

071. Antidepressants in Animal Models of Depression

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Title: Boosting glutamatergic tone in infralimbic -but not prelimbic- cortex evokes antidepressant-like effects in rats through AMPA receptor activation

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Abstract: Since the discovery of the antidepressant effects of ketamine, a growing number of clinical and preclinical studies have shown the capability of several drugs to induce rapid antidepressant responses. Ketamine is known to increase glutamate release in the medial

prefrontal cortex (mPFC) and an increased AMPA receptor (AMPA-R) signaling has been suggested to mediate the effects of several fast-acting antidepressant strategies. The dorsal (prelimbic, PrL) and ventral (infralimbic, IL) subdivisions of mPFC exert a differential control over emotional behavior. Thus, the main goal of the present study was to investigate the possible involvement of excitatory neurotransmission in the IL and PrL cortex on mood regulation. Hence, we examined the potential antidepressant responses of boosting glutamatergic function in these mPFC subdivisions and studied the involvement of AMPA receptors (AMPA-R). We characterized the rapid behavioral consequences evoked by the bilateral microinfusion of a depolarizing drug, veratridine (50 pmol); a selective inhibitor of the glutamate transporter 1 (GLT-1; mostly present in astrocytes and main responsible for synaptic glutamate reuptake), dihydrokainic acid (DHK; 0.15, 1.5 and 5 nmol); or an AMPA-R agonist, s-AMPA (50 pmol), either in the PrL or IL. Neurochemical effects of these drugs over glutamate and serotonin (5-HT) concentration were determined by *in vivo* microdialysis in freely-moving rats. Veratridine reduced immobility time on the forced-swim test (FST) when infused into IL but not PrL. Likewise, DHK (5 nmol) and s-AMPA reduced both immobility on the FST and latency to feed on the novelty-suppressed feeding test (NSFT) when infused into IL, but not PrL. Lower doses of DHK were without effect. Furthermore, the local blockade of AMPA-R with NBQX (10 nmol) previous to DHK microinfusion into IL prevented DHK-induced reduction in immobility, supporting the involvement of IL AMPA-R in mediating rapid antidepressant responses. Microdialysis studies showed that the inhibition of GLT-1 with DHK increased extracellular glutamate to the same extent in IL and PrL. However 5-HT output increased only after GLT-1 blockade in IL, suggesting a differential control of both mPFC areas on serotonergic activity. Accordingly, s-AMPA increased glutamate and serotonin concentration in IL. Overall, the present results indicate that an acute increase of glutamatergic neurotransmission in the IL mPFC triggers immediate antidepressant-like responses in rats, which are mediated by AMPA-R activation and likely involve a PFC-driven enhancement of serotonergic activity.

Disclosures: **J. Gasull-Camós:** None. **F. Artigas:** None. **A. Castañe:** None.

Poster

071. Antidepressants in Animal Models of Depression

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 71.11/VV8

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: CREST

KAKENHI 25242078

Title: Transcriptomic evidence for dematuration of the mouse hippocampus and frontal cortex by chronic antidepressant treatment

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Abstract: Fluoxetine (FLX), a serotonergic antidepressant drug, has been widely used to treat depression and anxiety disorders, but mechanisms underlying its antidepressant effect remain unclear. Previous studies that evaluated several molecular and/or electrophysiological features of the maturation stages of each neuron type have demonstrated that FLX treatment can reverse the established maturation of certain types of neurons in the hippocampus and frontal cortex (FC). However, this dematuration effect of FLX in the adult brain has not been assessed with regard to genome-wide gene expression patterns. In this study, we compared gene expression patterns in the FLX-treated FC and hippocampus of adult mice with those of the corresponding brain regions of normal infant mice. The gene expression patterns of FLX-treated mice significantly resembled those of normal infant mice in the FC and, to a large extent, in the hippocampus. In addition, time-course analyses of the ages of infant mice used in the comparisons with FLX-treated mice indicated that the gene expression patterns of FLX-treated mice were most similar to those of the youngest infants examined (1-week-old hippocampus and 2-week-old FC). Our results suggest that FLX treatment induces dematuration of the FC and hippocampus of adult mice with respect to genome-wide gene expression patterns. The dematuration of these brain regions might be involved in the therapeutic mechanism of FLX and/or some of its adverse effects.

Disclosures: **H. Hagihara:** None. **K. Ohira:** None. **T. Miyakawa:** None.

Poster

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Title: Uncoupling DAPK1 from NMDA receptor NR2B subunit exerts rapid antidepressant-like effects

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Abstract: Clinical and preclinical studies consistently demonstrate the rapid antidepressant effects of *N*-methyl-D-aspartate receptor (NMDAR) antagonists, although the underlying mechanisms are still unclear. Death-associated protein kinase 1 (DAPK1) couples NR2B subunits at extrasynaptic sites to regulate the NMDA receptor channel conductance. Here we found that chronic unpredictable stress (CUS) induces extracellular glutamate accumulation, accompanied by increased DAPK1-NMDA receptor interaction and high expression of DAPK1 and *p*-NR2B at Ser1303 and synaptic protein deficits in rat medial prefrontal cortex (mPFC). CUS also enhances NR2B-mediated NMDA currents and extrasynaptic responses induced by bursts of high-frequency stimulation, which may be associated with astrocyte loss and low expression of glutamate transporter-1 (GLT-1). Blockade of transporter GLT-1 in the mPFC is sufficient to induce depressive-like behaviors and causes similar molecular changes. Administration of selective NR2B antagonist, DAPK1 inhibitor or uncoupling DAPK1 from NMDA receptor NR2B subunit produced rapid antidepressant effects and reversed CUS-induced alterations in the mPFC. Moreover, selective NR2B antagonist did not have rewarding effect measured with conditioned place preference (CPP) paradigm. Altogether, our findings suggest that DAPK1 interaction with NMDA receptor NR2B subunit acts as a critical component in the pathophysiology of depression and is a potential target for new antidepressant treatments.

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Poster

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH Grant R00-MH092438

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Title: Sex differences in the antidepressant effect of scopolamine in rats

Authors: *B. WICKS, M. SHORE, S. KHANTSIS, A. CERETTI, S. COHEN, A. FRITZ, Y. KAWASUMI, D. BANGASSER;
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Abstract: Scopolamine, a non-selective muscarinic receptor antagonist, has recently been found to have rapid antidepressant effects, even in a subset of patients who are resistant to other treatments. Compellingly, women have a greater antidepressant response to scopolamine than men, an effect that suggests heightened female sensitivity to the drug. Most sex differences in drug efficacy are thought to be attributable to circulating ovarian hormones. To test whether scopolamine efficacy is regulated by ovarian hormones, we utilized rat models of antidepressant efficacy. First we found that in both the Forced Swim and the Novelty Suppressed Feeding (NSF) tests of antidepressant efficacy, scopolamine had a greater antidepressant effect in female rats than it had in male rats. Next using the NSF task we determined that the magnitude of scopolamine's efficacy changed across the estrous cycle. To further examine a role for circulating ovarian hormones we tested the effects of scopolamine in ovariectomized female rats utilizing NSF. Female sensitivity to scopolamine was reduced in ovariectomized compared to gonadally intact female rats. Collectively, these results indicate that circulating ovarian hormones contribute to the sex differences in scopolamine efficacy in rodents. Scopolamine has a narrow therapeutic index and can cause severe side effects (e.g. psychosis). These preclinical findings suggest that clinical studies into the efficacy and safety of scopolamine should consider hormonal status of women in case different dosing recommendations are needed for women during their reproductive years.

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Poster

071. Antidepressants in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Portuguese Science and Technology Foundation

Title: Cell cycle regulation of the hippocampal progenitor cells in depression and by antidepressants

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Abstract: Depression is a complex multidimensional disorder affecting around 20% of the world population. Nevertheless, its pathophysiology is still incompletely understood. Depression is thought to result from the interplay between genetic predisposition and environmental factors, such as stress. Plasticity impairments in the hippocampus, a brain region involved in memory and stress response, are involved in the pathophysiology of depression. Moreover, the hippocampus comprises one of the few brain regions in the adult brain where neurogenesis occurs, the dentate gyrus (hDG). Previous works showed that decreased cell genesis and dendritic morphology alterations are detected in the hippocampus of depressed individuals and animal models of depression, whereas antidepressant treatment prevents these changes.
We used a validated rat model of depression, the unpredictable chronic mild stress (uCMS), to explore the molecular underpinnings of neural plasticity in the hDG, in the context of depression and antidepressant treatment. The aim of this works was to assess the cell cycle mechanisms regulating hippocampal cell proliferation in the context of depression and antidepressant treatment. For that we used both *in vivo* - uCMS rat model -and *in vitro* - rat hippocampal-derived neurospheres - approaches. UCMS-exposed animals presented decreased proliferation and generation of newborn neurons that were reversed by fluoxetine. In our *in vivo* model, results suggest that uCMS-exposure produces a cell cycle arrest in the hDG progenitor cells that is accompanied by decreased levels of cyclins D1, E and A expression. Chronic fluoxetine treatment reversed the G1 phase arrest and increased cyclin E expression. Dexamethasone (DEX), used to mimic the elevation of glucocorticoids after chronic stress, decreased proliferation *in vitro,* whereas the administration of serotonin (5-HT), a neurotransmitter involved in the actions of fluoxetine, partly reversed this decrease. DEX also induced a G1-phase arrest, decreasing the expression of cyclins D1, cyclin D2 and Cdk6, while increasing p27 expression. 5-HT treatment partly reversed the G1-phase arrest and restored the expression of cyclin D1 and Cdk6. These results suggest that the anti-proliferative actions of chronic stress in the adult hDG result from a glucocorticoid-mediated G1-phase arrest in the progenitor cells that may be overcome by antidepressant treatment.
These findings contribute to the identification of novel molecular players and targets underlying neural plasticity phenomena in the hDG in the context of depression, thus opening new avenues for the development of novel antidepressant strategies.

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Poster

071. Antidepressants in Animal Models of Depression

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Program#/Poster#: 71.15/VV12

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Effect of the antidepressant agomelatine on the IL-6 pathway in rats exposed to chronic mild stress: role of suppressor of cytokine signaling 3 (SOCS3)

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Abstract: Major depression (MD) is a debilitating disorder whose treatment is being challenged by the high rate of failure and relapse of the pathology. Among the molecular systems thought to be involved in the MD etiology and in the mechanism of action of antidepressant drugs, inflammation has emerged as an important actor. In particular, increased levels of pro-inflammatory cytokines have been observed in the plasma and cerebrospinal fluid of depressed patients and, among these inflammatory mediators, interleukin (IL-) 6 has been recently proposed to play a crucial role (Fonseka et al., 2015). IL-6 triggers a peculiar pathway comprising the JAK/STAT signaling proteins and characterized by a specific negative feedback loop exerted by the cytoplasmic protein SOCS3 (Suppressor Of Cytokine Signalling-3). We have recently demonstrated that a seven weeks lasting chronic mild stress (CMS) paradigm, able to induce a depressive-like phenotype, up-regulates the expression of different pro-inflammatory cytokines in the rat brain. In this scenario, the pharmacological treatment with the antidepressant agomelatine during the last 5 weeks of stress (daily i.p., 40mg/kg) was able to normalize not only the pathologic phenotype but also the inflammatory state (Rossetti et al 2015). With these premises, the aim of the present work was to further investigate the mechanisms underpinning the anti-inflammatory activity of agomelatine by evaluating the impact of the drug on IL-6 pathway in the prefrontal cortex of rats exposed to CMS. As expected, stress was able to activate the IL-6 cascade, including SOCS3 gene and protein expression and JAK1/STAT3 phosphorylation, without any suppressive effect of SOCS3 on the feedback-loop inhibition. On the contrary, chronic treatment with agomelatine was able not only to normalize the stress-induced activation of IL-6 signaling, but also to induce SOCS3 transcription and translation under basal conditions. To better understand how agomelatine modulates IL-6 pathway, we deepened our analyses measuring the nuclear phosphorylation of STAT3 at Ser727, the activation of MAP-kinases, and STAT3-mediated gene expression of molecules involved in the control of apoptosis (i.e. Bcl-XL, Casp1, Casp3). The results show that in both non-stressed and stressed animals agomelatine is inducing SOCS3 expression by different mechanisms, but with a

common potential neuroprotective effect. Furthermore, given the potentiality of IL-6 signaling as target of antidepressant treatment and the key protective activity of agomelatine on this system, probably through SOCS3, this data suggest that SOCS3 modulation might be a valuable target for new drug development.

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Poster

071. Antidepressants in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NCCR Synapsy

Préfargier Foundation

KAUST

Title: Peripheral administration of Lactate produces antidepressant-like effects

Authors: A. CARRARD[1], M. ELSAYED[2], M. MARGINEANU[3], B. BOURY-JAMOT[1], L. FRAGNIÈRE[1], E. MEYLAN[1], J.-M. PETIT[1,2], H. FIUMELLI[3], P. J. MAGISTRETTI[3,2], *J.-L. MARTIN[1];
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Abstract: In addition to its role as metabolic substrate that can sustain neuronal function and viability, emerging evidence supports a role for L-lactate as an intercellular signaling molecule involved in synaptic plasticity. Clinical and basic research studies have shown that major depression and chronic stress are associated with alterations in structural and functional plasticity. These findings led us to investigate the role of L-lactate as a potential novel antidepressant. We found that peripheral administration of L-lactate produced antidepressant-like effects in the forced swim test. These antidepressant effects of L-lactate were not reproduced by

the enantiomer D-lactate and did not result from changes in locomotor activity and muscle strength. The antidepressant response induced by acute L-lactate administration was accompanied by increases in hippocampal L-lactate concentration and by changes in GSK3α/β and CREB phosphorylation levels as well as by alterations of Arc, COX-2 and NOS1 mRNA expression. Further investigation revealed that chronic administration of L-lactate induced antidepressant-like effects in two animal models that respond to chronic but not acute antidepressant treatment, including the open-space forced swim test and the corticosterone model of depression. In particular, we found that chronic administration of L-lactate partially restored mobility in the open-space forced swim test and completely reversed the corticosterone-induced anhedonia-like behavior. The antidepressant-like effects induced by chronic L-lactate administration were accompanied by changes in the expression of target genes implicated in serotonin receptor trafficking (p11), astrocyte functions (S100β), neurogenesis (Hes5), nitric oxide synthesis (NOS1 and NOS1AP) and cAMP signaling (PDE4D). Collectively, these studies identify a previously unrecognized action of L-lactate by which acute and chronic peripheral administration of L-lactate produces antidepressant-like behavioral responses. Further elucidation of the mechanisms underlying the antidepressant effects of L-lactate may help to identify novel therapeutic targets for the treatment of depression.

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Poster

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Rising Star Depression Research Award in Memory of George Largay (IMHRO)

NARSAD

T32MH020065

R01-MH099248

Title: Role of medial prefrontal cortical Brain-Derived Neurotrophic Factor in the rapid antidepressant effects of scopolamine in mice

Authors: *M. RAMAKER[1], M. ZHANG[2], K. VU[1], M. HAWKINS[2], S. L. THOMPSON[1], S. C. DULAWA[1];
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Abstract: Introduction: Classical antidepressants require 2-4 weeks of treatment to elicit a therapeutic response. On the other hand, recent clinical trials have shown fast-onset antidepressant effects within days of a single administration of scopolamine, a muscarinic acetylcholine antagonist. This rapid-onset of action has reignited interest in the neural changes that underlie this difference in time to onset between different classes of antidepressants. One common molecular marker of antidepressant onset is Brain Derived Neurotropic Factor (**BDNF**); virtually all known classical and fast-onset antidepressants increase BDNF levels in the hippocampus and medial prefrontal cortex (**mPFC**), concurrently with their behavioral effects. However, one potential deviation from this is scopolamine, which has been shown in multiple studies to decrease BDNF levels. The present study aimed to examine the necessity of BDNF induction in the mPFC for the fast-onset antidepressant effect of scopolamine. Methods: Adult male and female BDNF floxed mice on a BALB/cJ background were infused bilaterally into the mPFC with AAV-cre-GFP or AAV-GFP control. Four weeks later, mice were implanted with subcutaneous osmotic mini-pumps (0 or 20 mg/kg/day). A catheter was attached to the mini-pump allowing a 2-day saline delay prior to 24-hours of drug treatment. At the conclusion of treatment, mice were tested in the open field test (**OFT**), followed immediately by the chronic forced swim test (**cFST**). In the OFT, horizontal activity was collected via automated MedPC software. In the cFST, behavior was scored for time immobile, swimming, or climbing by an experimenter blind to condition. Results: There were no interactions with sex for any measures, so male and female data were collapsed. Scopolamine significantly decreased immobility and increased swimming in the cFST, but there were no virus by drug interactions for any measure. In the open field test, scopolamine significantly increased horizontal activity, but there were no drug by virus interactions. Additionally, horizontal activity in the OFT and immobility in the cFST were not correlated. Discussion: These data suggest that induction of BDNF in the mPFC is not necessary for the antidepressant effects of scopolamine as measured in the chronic forced swim test. While scopolamine did have a locomotor activating effect, consistent with previous reports, locomotor behavior did not predict immobility in the cFST. Future studies examining whether preventing mPFC BDNF induction prevents the antidepressant effect of other classes of antidepressants will enhance our understanding of this pathway.

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Poster

071. Antidepressants in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIDA Grant T32DA007268

Title: Antidepressant-like effects of the atypical antipsychotic amisulpride in the differential-reinforcement-of-low-rate (drl) 72 sec operant procedure and in the forced swim task

Authors: *M. KHAN[1], H. NANGUNURI[2], S. E. CARLAN[2], S. E. YOUNG[2], S. E. YOUNG[2], A. R. GRANT[2], D. SMITH[2], K. A. WEBSTER[2], T. M. HILLHOUSE[3,4], J. H. PORTER[2]; [1]Psychology, Virginia Commonwealth Univ., Glen Allen, VA; [2]Dept. of Psychology, Virginia Commonwealth Univ., Richmond, VA; [3]Dept. of Pharmacol., Univ. of Michigan, Ann Arbor, MI; [4]Psychology, Weber State Univ., Ogden, UT

Abstract: Amisulpride is an atypical antipsychotic antidepressant used in Europe that is clinically effective in the treatment of both positive and negative symptoms of schizophrenia with a low incidence of extrapyramidal motor side effects. At low doses, amisulpride is used for the treatment of depression (primarily dysthymia). Amisulpride has a high binding affinity at dopamine D_2 and D_3 and serotonin 5-HT_7 and 5-HT_{2B} receptors. More recent studies suggest that amisulpride's antidepressant activity is due to 5-HT_{7A} antagonism and not D2/D3 antagonism. However, the degree to which amisulpride produces antidepressant-like behavioral effects is poorly understood. The present study examined the antidepressant-like effects of amisulpride in a differential-reinforcement-of-low-rate (DRL) 72-sec task in mice and rats, which has been shown to effectively screen antidepressant drugs, and in a forced swim test in mice. Adult male C57BL/6 mice were tested in the DRL 72 sec task for sweet milk reward and were tested with the tricyclic antidepressant imipramine (3.2, 10.0, and 17.8 mg/kg) as a positive control and with the atypical antipsychotic amisulpride (1.0, 3.2, 10.0, 17.8, 32, and 56 mg/kg). Imipramine produced an antidepressant-like profile with a significant increase in number of reinforcers at 17.8 mg/kg and a significant decrease in responses at 17.8 mg/kg. Amisulpride produced significant increases in number of reinforcers at 17.8, 32, and 56 mg/kg; however, there were no significant changes in responses. In adult Sprague-Dawley rats tested in the DRL 72 sec task, imipramine (3 and 10 mg/kg) significantly increased reinforcers and decreased responses. Amisulpride also produced significant increases in reinforcers at 3 and 10 mg/kg doses; however, there were no significant changes in responses. Adult C57BL/6 mice also were tested in in a forced swim task. In contrast to results reported by Abbas et al. (2009), 0.1 mg/kg amisulpride did not significantly reduce immobility in the forced swim test. Future studies should examine higher doses of amisulpride to more fully characterize amisulpride's effects on immobility in the

forced swim task. Regardless, these findings suggest that amisulpride's antidepressant-like effects may have different efficacies in the DRL and forced swim task in mice.

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Poster

071. Antidepressants in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: KAKENHI 26860943

Title: Evaluation of home-cage social behaviors in a mouse model of mood disorders by chronic treatment with imipramine

Authors: *G. KURATOMI, Y. ARIME, S. SUZUKI, K. AKIYAMA;
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Abstract: Mutant mitochondrial DNA polymerase (*Polg1*) transgenic (Tg) mice that express mutant *POLG1* with defective 3'-5' exonuclease activity under the promoter of calcium/calmodulin dependent protein kinase II α have forebrain specific accumulation of multiple deleted mitochondrial DNA. Female Tg mice were reported to demonstrate mood disorder-like phenotypes and periodic wheel-running activity pattern associated with estrous cycle (Kasahara et al., 2006, 2016). In this study, we evaluated the long-term home-cage social behaviors and anhedonia-like behavior of Tg mice chronically treated with tricyclic antidepressant imipramine (IMI).
Twenty- to twenty-two-week-old female Tg mice and control C57BL/6J mice were housed on a 12 h light/dark cycle and used for experiments. For behavioral observation, mice were administered ether saline (SAL) or IMI (30 mg/kg/day) subcutaneously via implanted osmotic pumps for 28 days (n = 6-8 for each group). The IMI- or SAL-administered mouse was paired with a female wild-type sibling (cagemate) and bred in a polycarbonate cage with a transparent lid for 24 days. Home cages were consecutively recorded using digital video cameras which were placed above the cage. The video files obtained by recording home-cage behaviors during the first hour of dark period every other day were analyzed. The total duration of behaviors were blind scored to rate aggressive behaviors (chase, food competition, boxing posture), non-aggressive behaviors (huddle, allogrooming, body contact), and non-social behaviors (feeding,

self-grooming). Social interaction test with unfamiliar female mouse was conducted the day after the end of the 24-day observation (day 25). Sucrose preference was tested using two-bottle choice procedure between bottle containing a 1% sucrose and water-containing bottle for 2 days (day 27, 28). The whole study was formally approved by the animal care and use committee of Dokkyo Medical University School of Medicine.
The behavioral observation in home cage revealed that IMI-treated Tg and non-Tg mice showed significantly shorter duration of nose-to-body contact with their cagemates as compared in SAL-treated Tg ($p = 0.001$, one-way ANOVA). A comparison between early (< 12 days) and late periods during administration demonstrated that IMI-treated Tg mice only displayed increases in duration of passive nose-to-genital contact and inactive state by a two-way repeated measures ANOVA (group × period effect; $p = 0.005$ and 0.035, respectively). These results indicate mutant *Polg1* Tg mice continuously administrated with imipramine show altered manifestation of activity and social interactions in the home-cage environment.

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Poster

071. Antidepressants in Animal Models of Depression

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Chateaubriand Fellowship - Embassy of France in the USA

NARSAD

Title: Maternal-fetal transfer of the SSRI Citalopram: pharmacokinetics and effects on fetal brain development.

Authors: *J. C. VELASQUEZ[1,2,3], N. GOEDEN[1], L. GALINDO[1,4], S. HEROD[5], C. SIMASOTCHI[3], T. FOURNIER[2,3], S. GIL[2,3], A. BONNIN[1];
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Abstract: Selective Serotonin Reuptake Inhibitors (SSRIs) antidepressants are increasingly prescribed during pregnancy. As a result, prenatal exposures to SSRIs are becoming more

common despite an unclear safety profile for the fetus and conflicting findings from epidemiological studies regarding neonatal outcomes. While few studies focus on the consequences on fetal neurodevelopment, our understanding is also limited by not delineating the effects of maternal depression from those that are pharmacologically induced. These observations raise questions about the developmental effects of SSRIs during pregnancy and the factors that affect fetal drug exposures. Using human and mouse models and the widely-prescribed SSRI Citalopram (CIT), this project aims to assess maternal-fetal drug disposition throughout pregnancy and examine the developmental effects of CIT exposures on the fetal brain.
In this study, we evaluated the pharmacokinetics of CIT transfer across the mouse and human placentas. We implemented *ex vivo* placental perfusion systems to assess maternal-fetal drug transfer independently of maternal and fetal metabolism in addition to the effects on placental physiology. *In vivo* experiments were also conducted to assess fetal drug disposition profiles to CIT and its primary metabolite at different developmental stages. Results revealed a rapid maternal-fetal CIT transfer, significant placental tissue drug accumulation and a pregnancy stage-dependent exposure of the fetal brain to bioactive CIT metabolites. Enzymatic activity assays showed that fetal drug metabolic capacity develops in late gestation, resulting in elevated circulating and brain concentrations of DCIT at embryonic day (E)18. Furthermore, we measured the effects of CIT exposures on fetal brain serotonin at multiple developmental time points. Results show that prenatal exposure to CIT differentially impacts fetal brain neurochemistry and that the effects are modulated by duration and developmental stage of exposure. In particular, CIT exposure significantly altered fetal brain tissue concentrations of serotonin and other biogenic amines with critical roles in various neurodevelopmental processes. Immunohistochemical analyses of serotonergic and thalamocortical axon pathways formation revealed differential impacts on fetal brain circuit formation. Taken together, the results suggest that the pharmacological inhibition of serotonin reuptake during fetal development alters axonal circuit formation. The effects of maternal depression on these outcomes, independently and combined with SSRI exposure, are under investigation.

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Poster

071. Antidepressants in Animal Models of Depression

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 71.21/VV18

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: SC2 GM109811

Title: Altered sensitivity to the rewarding properties of cocaine in adult female c57bl/6 mice exposed to fluoxetine during adolescence

Authors: F. J. FLORES-RAMIREZ[1], D. O. SANCHEZ[1], I. GARCIA[1], C. GONZALEZ[1], A. R. ZAVALA[2], *S. D. INIGUEZ[1];
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Abstract: Accumulating preclinical evidence indicates that early-life exposure to psychotropic medications results in long-lasting altered behavioral responses to drugs of abuse - suggesting a risk of enhanced drug liability, later in life. However, to date, these preclinical experimental approaches have been conducted primarily using male subjects. This is surprising given that females, when compared to males, are more likely to be diagnosed with mood-related disorders, and thus, to be prescribed with psychotropic drugs, such as antidepressants. Therefore, to examine whether long-lasting alterations to the rewarding properties of drugs of abuse are exhibited as a result of juvenile antidepressant exposure, we exposed adolescent female mice to the selective serotonin reuptake inhibitor (SSRI) fluoxetine (FLX). We selected FLX given that it is the only SSRI approved by the US Food and Drug Administration for the treatment of pediatric depression. Specifically, female c57bl/6 mice were exposed to FLX in their drinking water (250 mg/L) during adolescence (postnatal days [PD] 35-49), and were later assessed in adulthood (PD 70+) on behavioral responsivity to cocaine (0, 2.5, 5, and 7.5 mg/kg) place conditioning (CPP). Our results show that adult female mice pretreated with FLX during adolescence displayed a decreased preference for environments previously paired with cocaine, when compared to saline-pretreated controls. Collectively, our data suggest that adolescent exposure to the antidepressant FLX causes behavioral adaptations that endure into adulthood, and that are indicative of a decreased sensitivity to the rewarding properties of cocaine in female mice.

Disclosures: F.J. Flores-Ramirez: None. **D.O. Sanchez:** None. **I. Garcia:** None. **C. Gonzalez:** None. **A.R. Zavala:** None. **S.D. Iniguez:** None.

Poster

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NC123240.1

Title: Effect of amygdaloid kindling and the fluoxetine in the depressive behavior and the susceptibility tonic-clonic seizures in rats

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Abstract: The fluoxetine (FLX) is a selective serotonin reuptake inhibitor used for the treatment of depression. Despite of the efficacy of FLX in depressive disorders, its effect on epilepsy remains controversial. Experimental models have shown that FLX affects excitability in short time scales which may lead to changes in epilepsy severity. Amygdaloid kindling (AK) is a model of epilepsy characterized by sustained increase in seizure susceptibility. Therefore, the goal of the present study was to examine AK epileptogenesis with behavioral correlates of depression in the rat forced swim test (FST) as well as the effects of FLX on seizure susceptibility and depressive behaviors. Male Wistar rats (280-320 g) were used. Tripolar electrodes were placed in the basolateral nucleus of left temporal lobe amygdala (P: 2.8, L: 5.0, H: 8.5) and both frontal cortices. AK was induced by daily electrical stimulation (1 s train, 1 ms pulses, 60 Hz, 250-500 μA) until AK stage V (tonic-clonic seizure) was achieved for three consecutive days. One hour after last seizure, FST sessions were conducted by placing rats in individual glass cylinders (46 cm height; 20 cm diameter). An initial FST of 15 min (termed pre-test) was performed and after 24 h, was performed a 5 min test. A 10 mg/kg of fluoxetine hydrochloride (2 ml/kg) dose was administered following a sub-acute schedule, three injections administered between pretest and test sessions (21 h, 5 h, and 1 h before test session). Rats were assigned to seven experimental groups: K-FLX (n = 7), in which AK, FST and FLX injections were applied; K-Vh (n = 7), AK, FST and vehicle (saline solution 0.9%); Sham-FLX (n = 7), FST and FLX; Sham-Vh (n = 7), FST and vehicle; Control-FST (C-FST) (n = 7) solely with FST and Control-K (C-K) (n = 7) solely with AK. Immobility time in FST and seizure susceptibility on test stimulations of post stage V were assessed. We found a decrement in immobility time in the FST on all groups compared with C-FST ($P < 0.001$) and between Sham-FLX and K-FLX ($P < 0.02$). Whereas also there were changes between initial kindling threshold and threshold postFST by susceptibility spike in the groups K-FLX ($P < 0.001$), K-Vh ($P < 0.017$) and C-K ($P < 0.01$), and only group K-FLX for susceptibility seizure tonic-clonic ($P < 0.005$). Our results suggest that the FLX has effects on seizure susceptibility, thus the neuronal plastic changes associated with limbic system, particularly with the amygdala, could be interfered by the development of depressive behavior.

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Poster

071. Antidepressants in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Danish Council for Independent Research 4092-00121B FSS

Lundbeck Foundation R171-2014-1092

Canadian Institutes for Health Research CIHR MOP 142308

Title: Behavioural and hippocampal changes across the pre- to postpartum transition within a postpartum depression model with and without the effects of a serotonin reuptake inhibitor in rats

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Abstract: Postpartum depression (PPD) affects ~13% of mothers, and maternal mood is coupled to peripartum changes in sex steroid hormones, potentially in a manner dependent on serotonergic dysfunctions and stress hormone biology.
AIMS: To determine behavioural and neurobiological changes across natural peripartum, as well as in a model of PPD.
METHODS: We examined changes in anhedonia- (sucrose preference), anxiety- (elevated plus maze (EPM) and open field test (OFT)), and coping behaviours (using the forced swim test (FST)) in nulliparous rats, pregnant rats, and early, mid, and late postpartum, and maternal behaviour of the dams. The effects of postpartum exposure to corticosterone (CORT; 40 mg/kg/d s.c; a model of postpartum stress/depression) with or without concurrent administration of the selective serotonin reuptake inhibitor (SSRI) paroxetine (5 mg/kg/d s.c.) were also evaluated.
RESULTS: We found decreased anxiety-like behaviour (OFT) in the early postpartum and increased anhedonia-like behaviour mid postpartum relative to nulliparous and pregnant rats. Both postpartum CORT and SSRI increased immobility in the FST. While postpartum CORT did not affect anxiety, SSRI treatment postpartum increased anxiety (OFT and EPM) in both CORT and control dams. Neither CORT nor SSRI affected anhedonia-like behaviour. Postpartum CORT increased the percent time spent off the nest and SSRI did not remediate this effect.
CONCLUSIONS: We find increased anxiety- and anhedonia-like behaviour, but unchanged coping behaviour during different times postpartum in control dams. In accordance with the

literature, our PPD model increased depression-like behaviour, decreased maternal care, and did not affect anxiety. SSRI treatment did not remediate the behavioural effects of PPD in this model, and triggered in itself anxiety-like behaviour and reduced coping. Hippocampal neurogenesis data measurements are ongoing. Taken together, our study will advance the understanding of the time course and nature of PPD and may provide a rationale for evaluating treatment strategies in the immediate postpartum period, which interestingly may benefit from including non-serotonergic targets.

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Poster

071. Antidepressants in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Depressive and anxiety-like behaviors in Akt3 KO mice are rescued by chronic lithium treatment

Authors: *Y. BERGERON, G. BUREAU, M.-É. LAURIER-LAURIN, E. ASSELIN, G. MASSICOTTE, M. CYR;
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Abstract: The protein kinase B (PKB/Akt) is found in three distinctive isoforms: PKBα/Akt1, PKBβ/Akt2, and PKBγ/Akt3. While Akt1 and Akt2 have been intensively investigated, less is known about the Akt3 isoform. Yet, Akt3 differs from Akt1 and Akt2, being the most highly expressed isoform in the brain and being critical for postnatal brain development. Then, we hypothesized that Akt3 could play a distinctive role in cerebral functions. To address this hypothesis, we have investigated the impact of Akt3 deletion on behaviors, synaptic plasticity and biochemical signaling. First, in the Morris water maze and the novel object recognition test, no difference was observed between WT and Akt3 KO mice, suggesting no effect of Akt3 deletion on cognitive functions. In addition, electrophysiology on Akt3 KO hippocampal slices showed that neither STP nor LTP levels were modified compared to WT hippocampal slices. However, Akt3 deletion induced several behavioral anomalies: reduced prepulse inhibition, social behavior deficits as well as high level of depressive and anxiety-like behaviors. Biochemical investigations revealed that levels of total Akt1 and Akt2 in the anterior cortex, striatum, hippocampus and cerebellum were not modulated by Akt3 deletion. However, levels of phosphorylated GSK3α/β at serine 21/9 were decreased in the anterior cortex, hippocampus,

striatum and cerebellum of Akt3 KO mice; no change in levels of total GSK3α/β was observed. To verify if the modulation of GSK3α/β levels could be related to the observed depressive and anxiety-like behaviors, a lithium diet was chronically administrated to WT and Akt3 KO mice. This treatment has increased levels of phosphorylated GSK3α/β and rescued the depressive and anxiety-like behaviors observed in Akt3 KO mice. Then, these results suggest that GSK3α/β may contribute to the depressive and anxiety-like behaviors found in the Akt3 KO mice and support that an isoform-specific mechanism may define the neuronal signaling of Akt in psychiatric illnesses-related behaviors.

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Poster

072. Neural Circuits in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: CNPq Grant 142451/2014-2

Title: Prefrontal cortex and amygdala oscillatory and single-unit activity during escapable and inescapable stress in the learned helplessness model

Authors: ***M. T. ROSSIGNOLI**, D. B. MARQUES, R. N. RUGGIERO, L. S. BUENO-JUNIOR, J. P. LEITE;
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Abstract: Both the learned helplessness (LH) model of induced depressive behavior and clinical major depression are associated with anatomical and functional impairments in the prefrontal cortex (PFC) and amygdala (AMY). To date, there are no electrophysiological data from these brain sites in the LH model, which motivated our study. Thus, we implanted chronic microwire bundles into the PFC and AMY of adult rats for the recording of local field potentials (LFP) and single-unit activity (SUA). Once recovered from surgery, rats were submitted to a single session (~100 min duration) with escapable shocks trials (ES), paired inescapable shock trials (IS) or none (1 min intertrial interval), as proposed in the triadic model of LH (Amat et al., 2005, Nat Neurosci, 8(3): 365-71). ES and IS-trials consisted of conditioned stimulus (light, 10 s) immediately followed by unconditioned stimulus (foot shock, 10 s, 0.6 mA, 40-60 s intertrial), however in ES trials foot shock can be avoided by crossing to the opposite side of the chamber during the unconditioned stimulus. On day two, the helplessness behavior was assessed using an

active avoidance task, similar to ES trials. Ongoing perievent analyses show that conditioned stimuli transiently decreased the PFC alpha band power (8-12 Hz) in all trial types, but this effect was stronger in IS compared to ES. In contrast, the same stimuli decreased and increased PFC theta power (4-8 Hz) in IS and ES trials, respectively. PFC neural activity was consistently modulated across trial events. Particularly, the post-shock period induced greater SUA changes in the PFC of helpless rats compared to escaping animals. Our preliminary findings suggest distinct effects of escapable and inescapable stress on the PFC activity. Analyses of AMY recordings will potentially provide additional insights on the amygdala-prefrontal interaction in the LH model.

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Poster

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: CAPES

CNPQ

Title: Taurine reduces oxidative stress and DNA damage in the frontal cortex and in the hippocampus of diabetic rats

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Abstract: Diabetes is a chronic metabolic disease and hyperglycemia is associated with unbalance between pro- and antioxidants parameters and DNA damage. Taurine is a non-essential amino acid, widespread in the brain and peripheral tissues, with antioxidant, osmoregulatory and neuromodulatory properties. It may postpone the tissue injury and DNA damage of diabetes. Our objective was to evaluate the effect of chronic taurine treatment on antioxidant parameters and DNA damage in the hippocampus and frontal cortex in diabetic rats. Forty male adult Wistar rats were divided into control (CTR) and streptozotocin-induced diabetic (STZ) group. Rats from each group were daily administered with saline or 100 mg/kg of taurine

(CTR0; CTR100; STZ0; STZ100, n = 10/group), i.p., for 28 days. After 1 hour from the last taurine/saline administration, rats were killed and the frontal cortex and hippocampus were dissected and stored to further DCFH-DA assay, SOD, and CAT activity analysis. DNA damage was analyzed with the alkaline comet assay. A two-way ANOVA showed that diabetes condition significantly increased the DCF values in both brain structures, evidencing a pro-oxidant effect of chronic hyperglycemia. STZ rats also increased SOD and CAT enzymes activity in both brain areas. Moreover, diabetes significantly increased DNA damage in hippocampus ($P = 0.003$) and frontal cortex ($P = 0.039$). Prolonged taurine treatment reduced the DCF oxidation with consequent reduction in the SOD and CAT enzymes activity in both brain areas of STZ100 rats. Additionally, taurine decreased DNA damage in both brain areas evaluated of the diabetic rats, showing a protective effects on genotoxic damage (Hippocampus = 0.022; frontal cortex: $P = 0.008$). Therefore, taurine reduces oxidative stress and DNA damage in the frontal cortex and in the hippocampus of STZ diabetic rats. Additional studies need to be conducted to better explore therapeutic properties of taurine in humans.

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Poster

072. Neural Circuits in Animal Models of Depression

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Program#/Poster#: 72.03/VV24

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Sex-dependent alterations in habenular endocannabinoid signaling following exposure to chronic unpredictable stress

Authors: ***A. L. BERGER**[1], A. M. HENRICKS[1], L. N. BAXTER-POTTER[2], J. M. LUGO[2], M. A. STITCH[3], M. N. HILL[3], R. J. MCLAUGHLIN[2];
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Abstract: Stress is a pervasive aspect of life and a significant risk factor for a multitude of mental, cardiovascular, metabolic, and immune diseases. As such, understanding the effects of stress on the brain may uncover novel therapeutic targets for the treatment of stress-related illnesses. The epithalamic habenular nuclei have recently been linked to the pathological development of stress-related neuropsychiatric disorders via modulation of downstream monoaminergic neurotransmission. Moreover, multiple lines of evidence from our laboratory and

others have implicated the endocannabinoid (eCB) system in the neuroendocrine and behavioral response to stress. However, the role of eCB signaling in the habenula, and more specifically, how this system may become perturbed following chronic stress exposure, has yet to be empirically evaluated. Thus, we sought to examine the extent to which chronic unpredictable stress (CUS) exposure alters eCB content and mRNA expression for the primary routes of eCB biosynthesis and metabolism in the habenula of male and female Sprague Dawley rats. Rats were exposed to six weeks of CUS and the habenula was rapidly dissected for determination of anandamide (AEA) and 2-arachidonoylglycerol (2-AG) concentrations and relative mRNA expression for biosynthetic (NAPE-PLD, DAGLα) and degradative (FAAH, MAGL) enzymes using LC/MS/MS and RT-qPCR, respectively. Preliminary results indicate that AEA content in habenular tissue was significantly higher in female rats compared to male rats under basal, non-stress conditions, with no significant differences in AEA content in either sex following CUS exposure. In contrast, 2-AG content was significantly elevated in the habenula only in female rats exposed to CUS compared to non-stressed female rats. Concentrations of AEA and 2-AG were also strongly correlated with adrenal gland weights and body weight gain. With respect to eCB mRNA in the habenula, we observed a trend (p=.06) for reduced NAPE-PLD expression in stress-exposed male rats compared to non-stressed controls while no other evaluated genes differed in male rats. Together, these results indicate that eCB signaling in the habenula is significantly altered by CUS exposure in a sex-dependent and ligand-specific manner. These findings contribute to the growing body of evidence suggesting an important role for the habenula in response to stress and argue that eCB signaling within the habenula may be fundamentally involved in stress-related perturbations within this nucleus, as well as its downstream targets, thereby increasing vulnerability for the development of stress-related psychopathology.

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Poster

072. Neural Circuits in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: RO1 MH101145 to RHM

Title: Interferon-gamma and galectin-9 interact to induce indoleamine-2,3-dioxgenase 1 and 2 expression in the mouse hippocampus

Authors: A. K. BROOKS[1,2,3], M. A. LAWSON[1,2,3], J. L. RYTYCH[1,3], K. C. YU[1], T. M. JANDA[1], A. J. STEELMAN[1,2,3,4], *R. H. MCCUSKER[1,2,3,5];
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Abstract: Increased kynurenine (Kyn) within the central nervous system is associated with symptomologies of depression and other neurological disorders such as schizophrenia, multiple sclerosis, Parkinson's and Alzheimer's disease. In particular, depression behaviors correlate with an elevation of pro-inflammatory cytokines responsible for the induction of rate-limiting enzymes that generate Kyn from tryptophan: indoleamine-2,3-dioxgenases (Ido1 and Ido2). Galectins (Gals) are well-established inflammatory modifiers which may play significant roles in the pathogenesis of the aforementioned disorders. However, there are no reports describing the ability of Gals to modulate the Kyn Pathway, thus linking their expression to major depression. Lipopolysaccharide (LPS) is frequently used to induce cytokine expression, Ido expression and brain Kyn culminating in depression-like behaviors of rodents. Here, we show that intraperitoneal injection of mice with LPS induces Ido1, Ido2, interferon-gamma (IFNγ), Gal-3 and Gal-9 expression in the mouse brain, and that IFNγ and Gal-9 play a critical role for Ido1 and Ido2 induction within the hippocampus. Organotypic hippocampal slice cultures (OHSCs) were used to determine if in vivo surges of IFNγ and Gal-9 are responsive for elevated Ido1 and Ido2 expression and if Ido expression is sensitive to antidepressant therapy. OHSCs were treated with IFNγ plus Gal-1, Gal-3 or Gal-9 with or without desipramine (Desip); mRNA levels were quantified by qPCR. IFNγ was not expressed by OHSCs, but the addition of IFNγ increased Gal-9 expression. IFNγ also increased expression of two distinct Ido1 transcripts (Ido1-FL and Ido1-v1), and remarkably, the antidepressant Desip blocked this induction. Although not active alone, Gal-9 interacted with IFNγ to synergistically upregulate Ido1-FL, an effect also blocked by Desip. IFNγ also increased expression of Ido2 transcripts (Ido2-v1, Ido2-v2 and Ido2-v3), and similarly, IFNγ-induced Ido2 expression was decreased by Desip. Gal-9 further accentuated IFNγ-induced Ido2-v1 expression; this interaction was blocked by Desip. These effects are Gal-9 specific as Gal-1 and Gal-3 did not affect Ido expression. These data are the first to show that both IFNγ and Gal-9 are up-regulated in the hippocampus during LPS-induced neuroinflammation. Since IFNγ is not expressed by the OHSCs, our data suggest that IFNγ (probably derived from infiltrating peripheral cells in vivo) induces central Gal-9 to augment IFNγ-dependent Ido expression. The exciting discovery that desipramine blocks inflammation-dependent Ido1 and Ido2 expression expands the mechanism-of-action for antidepressants.

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Poster

072. Neural Circuits in Animal Models of Depression

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Program#/Poster#: 72.05/VV26

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Amygdala Cholecystokinin-Nucleus accumbens circuit control depression-related behaviors

Authors: *S. CHENJIE[1], K.-X. LI[2], X.-D. YU[1], J.-Y. FU[1], H. WANG[1], K.-L. PENG[1], H.-Q. PAN[1], Y. ZHANG[1], Y. LI[1], H.-Y. GENG[1], S.-M. DUAN[1], X.-M. LI[1];
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Abstract: The Nucleus accumbens (NAc) has recently emerged as a key reward brain region in the symptomatology of depression, including reduced motivation and anhedonia. It has been proved that BDNF signaling from the VTA to the NAc exerts a pro-depression-like effect in the social defeat stress. Apart from VTA, the NAc also receives dense glutamatergic innervation from basolateral amygdala (BLA). Despite previous studies demonstrate excitatory transmission from the amygdala to NAc facilitates reward seeking, the individual role of amygdala-NAc circuit in aversion affective behaviors and their contribution to the depressive behavior are poorly understand. Recently, we found a class of BLA cholecystokinin neurons projecting to the NAc, which release excitatory transmitter "glutamate". Social defeat stress can rapidly activate amygdala cholecystokinin neurons and their terminal in NAc in susceptible, but not resilient mice. Activation or silence of NAc-projecting BLA CCK neurons can bidirectionally regulate depression-like behavior.

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Poster

072. Neural Circuits in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIMH P50 MH096890

Title: RNAseq on FACS-isolated medium spiny neurons reveals distinct roles for D1 and D2 cells at baseline and in response to chronic stress

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Abstract: Stress induces many pathological changes in the brain's reward circuit. In particular, stress exerts potent effects on the nucleus accumbens (NAc), a largely GABAergic nucleus receiving dopaminergic input from the ventral tegmental area. The NAc contains several neuronal populations of distinct identity, the most abundant of which is the population of dopamine-responsive, GABAergic medium spiny neurons (MSNs). MSNs are subdivided by their response to dopamine, with one class expressing predominantly the dopamine receptor type 1 (D1) and the other expressing dopamine receptor type 2 (D2). While D1- and D2-expressing MSNs utilize different G protein signaling cascades and have been shown to play different – and often opposing – roles in stress responses, only a handful of D1- and D2-enriched genes are known. The purpose of the present study was to define transcriptional signatures for D1 and D2 MSNs at baseline and in response to stress, and to assess the relative contribution of D1- and D2-enriched genes in stress action. To this end, we used a cutting edge combination of techniques, performing RNA sequencing on whole cells and on nuclei sorted from D1 and D2 transgenic reporter mice using Fluorescence-Associated Cell Sorting (FACS). Bioinformatic analysis of the RNAseq data included differential expression, network, and clustering analyses to evaluate the transcriptional profiles of D1 and D2 MSNs. Ingenuity Pathway Analysis (IPA) of D1- and D2-enriched gene lists indicated that each cell type was enriched for its known signaling pathway at baseline, and that stress induced cell type-specific patterns in mice susceptible vs. resilient to stress. The importance of specific transcripts was assessed by manipulation of key genes selectively in D1 and D2 cells using cell type-specific viral-mediated gene transfer. Furthermore, we found global patterns in D1 and D2 responses to stress, with D2 MSNs altering their transcriptional landscape to more closely resemble D1 MSNs. The improved resolution of cell type-specific over whole-tissue RNAseq in NAc uncovered transcriptional effects previously masked by population averaging, and generated novel targets for genetic manipulation to promote stress resilience.

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Poster

072. Neural Circuits in Animal Models of Depression

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 72.07/WW2

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: MH101477

P51OD011132

Title: Reversal of the durable consequences of adolescent social isolation

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Abstract: Adolescent-onset depression is associated with high rates of depression recurrence later in life, is frequently precipitated by social adversity, and is more prevalent in women. Therefore, identifying both the durable consequences of early-life social adversity in female populations and corrective interventions is of great importance. Here, female C57BL/6 mice were isolated or group-housed from postnatal days (P) 31-60. At P60, all mice were "re-socialized," with each cage containing 3-4 previously-isolated mice (n=6-8/pen). Expression levels of 2',3'-Cyclic-nucleotide 3'-phosphodiesterase (CNPase) were assessed throughout the prefrontal cortex (PFC), amygdala, and hippocampus. Circulating corticosterone (CORT) levels were analyzed via ELISA. Decision-making strategies were classified using response-outcome contingency degradation. Anhedonic-like behavior was assessed via the sucrose consumption test. Dendritic spines on deep-layer PFC neurons of *thy1*-YFP-expressing mice were imaged using confocal microscopy. Finally, CaMKIIa-controlled Gi-coupled Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) were expressed bilaterally in the ventromedial PFC (vmPFC), and activated by their ligand Clozapine-N-oxide via *i.p.* injection. We found that isolation during adolescence induced hypocortisolemia. Despite social re-integration and normalization of CORT, a history of social isolation also reduced CNPase expression, increased vmPFC spine densities, impaired goal-directed decision-making, and induced anhedonic-like behavior. Reversal of these behavioral outcomes via DREADD-mediated vmPFC inhibition provides a novel G protein-coupled receptor-based approach for mitigating neuropsychiatric outcomes related to social adversity in adolescence.

Disclosures: E.A. Hinton: None. **A.G. Allen:** None. **S.L. Gourley:** None.

Poster

072. Neural Circuits in Animal Models of Depression

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National Science and Technology Major Project (no. 2012ZX09103301-049)

Title: Perineuronal nets in the medial prefrontal cortex regulate vulnerability to stress

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Abstract: Introduction: Perineuronal nets (PNNs) are extracellular matrix structures enwrapping parvalbumin-positive γ-aminobutyric acid (GABA)-ergic interneurons. PNNs have recently been implicated in experience-dependent neuroplastic changes in central nervous system, but it is poorly understood that whether PNNs modulates the neural maladaptation after repeated exposure to stress.
Methods: First, we measured levels of PNNs and proteins constituting PNNs in the medial prefrontal cortex (mPFC) of adolescent and adult rats which exhibited different responses to 10-days chronic unpredictable mild stress (CUMS). Then we evaluated the effect of enzymatic degradation of PNNs in mPFC on stress vulnerability in adults, and the effect of elevating PNNs in adolescent rats through environment enrichment on CUMS-induced depressive and anxiety-like behaviors. And we also injected fluoxetine in adolescent rats and measured the stress vulnerability and PNNs levels. Finally we investigated the role of PNNs in regulating the expression of glutamic acid decarboxylase 67 (GAD 67) and frequency and amplitude of inhibitory postsynaptic current (IPSC) after CUMS in rat mPFC.
Results: We found that adolescent rats with lower level of PNNs, tenascin-R and aggrecan in the mPFC exhibited increased susceptibility to stress. Degradation of PNNs in mPFC promoted vulnerability to 10-days CUMS in adult rats. Elevating PNNs in the mPFC through environment enrichment prevented CUMS-induced depressive and anxiety-like behavior. Fluoxetine also reversed the stress vulnerability in adolescent rats. Lower level of PNNs rendered GABAergic neurons susceptible to CUMS, manifesting as decreases in expression of GAD 67 and frequency and amplitude of IPSC after CUMS.

Conclusion: The organization of PNNs coincided with the developmental switch in stress vulnerability to resilience. These findings indicate a role of PNNs in mPFC in predicting and modulating vulnerability to stress, and the effect may be produced though regulating GABAergic functions.

Disclosures: **J. Shi:** None. **N. Chen:** None. **D. Hu:** None. **Y. Han:** None. **L. Lu:** None.

Poster

072. Neural Circuits in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: R01MH082802

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R01MH100616

Title: Unfolded protein response activity in hippocampus of rodent models of depression

Authors: ***M. TIMBERLAKE**, Y. DWIVEDI;
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Abstract: Background: Major Depressive Disorder (MDD) is a debilitating mental disorder that is characterized by low mood, self-esteem dips, and loss of interest/pleasure in otherwise pleasurable activities that can affect a person's work, family, and other relationships as well as sleeping, eating and general health quite adversely. One of the symptoms of MDD is a decline in memory aptitude as well structural changes in hippocampus - the part of the brain responsible for converting short-term memories to long-term ones; the underlying cause of this decline has yet to be detailed. Multiple lines of evidence suggest that a process known collectively as the unfolded protein response (UPR) may be responsible for some of the adverse, underlying physiology of the MDD phenotype. **Methods:** To examine whether UPR system plays a role in depression, we examined the expression of genes that are part of the three different pathways for UPR activation, namely GRP78, GRP94, ATF6, XBP-1, ATF4 and CHOP in the hippocampus of two animal models of depression: learned helplessness and restraint. **Results:** We have demonstrated that rats subjected to the stress paradigms of learned helplessness and restraint, show a significant increase in UPR activity in the hippocampus. **Conclusion:** Our data show strong evidence of altered UPR system in depressed rats, which could be associated with development of depressive behavior. We hypothesized that the cellular stress response could be responsible

for the initiation of some apoptotic pathways in conjunction with immune system activity in the depressed brain.

Disclosures: M. Timberlake: None. **Y. Dwivedi:** None.

Poster

072. Neural Circuits in Animal Models of Depression

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Title: Cell type and projection specific roles of ventral pallidal neurons in depression

Authors: *D. KNOWLAND, V. LILASCHAROEN, C. PACIA, S. SHIN, B. LIM;
UCSD, San Diego, CA

Abstract: Although nearly 10% of adults are diagnosed with Major Depressive Disorder (MDD) during their lifetime, positive patient response to existing treatments are highly variable and often treat symptoms rather than address the underlying cause. A significant hurdle in treating MDD is the wide and often variable spectrum of symptoms patients exhibit such as anhedonia, social withdrawal, and a lack of motivation. Although the behavioral symptoms are well described in MDD, much less is understood about the precise changes in neural circuitry that accompany MDD and if specific changes underlie separate behavioral symptoms. In particular, are there certain circuit adaptations that mark the transition from a healthy patient to one with MDD? To address this question, we anatomically and functionally dissect the circuitry of the ventral pallidum (VP), a largely overlooked area in the reward and motivational circuit. Using a novel retrograde viral system our lab has developed to outline the cell-type and projection-specific circuit of VP PV neurons we find that VP PV neurons send projections to the ventral tegmental area (VTA) and lateral habenula (LHb), two areas that have been previously implicated in depression. Furthermore, these cells represent distinct subpopulations that predominantly send their projections specifically to the LHb or VTA, but not both. Using optogenetic-mediated axon terminal stimulation we also find that these subpopulations mediate

separate behaviors. At basal levels, we find that LHb-, but not VTA-projecting cells mediate rewarding effects. To assess contributions to depressive behaviors, we use the chronic social defeat stress (SDS) model of depression which has shown to elicit a broad spectrum of phenotypes that parallel those seen in humans with MDD. Interestingly, we find that different subpopulations underlie distinct symptoms of depression in mice. Only LHb-, but not VTA-projecting neurons modulate helplessness or behavioral despair. Conversely, VTA- but not LHb-projecting neurons mediates social avoidance behaviors induced by SDS. These results suggest that ventral pallidal PV neurons are critical in mediating SDS-induced depressive phenotypes. Furthermore, separate subpopulations of VP PV neurons distinguished by their projection target modulate distinct and separate depressive behaviors and exhibit different stress-induced physiological changes. This data points to an increased need in specificity in cell-type specific circuit studies and the importance of considering projection-specific subpopulations when studying behaviors related to mental disorders.

Disclosures: D. Knowland: None. **V. Lilascharoen:** None. **C. Pacia:** None. **S. Shin:** None. **B. Lim:** None.

Poster

073. New Molecular Targets in Animal Models of Depression

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 73.01/WW6

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Beta-hydroxybutyrate, endogenic NLRP3 inflammasome inhibitor, ameliorates neuro inflammation caused by stress.

Authors: *T. YAMANASHI[1], M. IWATA[1], K. TSUNETOMI[1], N. KAJITANI[1], A. MIURA[1], R. S. DUMAN[2], K. KANEKO[1];
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Abstract: Stress decreases neurogenesis and synaptogenesis in the adult hippocampus, contributing to depressive-like behaviors in rodent models, but the mechanisms by which stress causes neuronal damage remain unknown. It has been reported that interleukin-1beta (IL-1beta) decreases neurogenesis and causes depressive behavior. We had previously reported that stress increases ATP and activates Nucleotide-binding protein, Leucine-rich repeat, Pyrin domain containing 3 (NLRP3) inflammasome, which in turn increases IL-1beta in the adult rat hippocampus. We also reported that administration of purinergic type 2X7 receptor (P2X7R), a receptor for ATP, antagonist (A-804598) ameliorates both the increase of NLRP3 inflammasome and IL-1beta in the hippocampus, as well as decreased neurogenesis and depressive-like

behaviors caused by stress. P2X7R, when combined with ATP, triggers K^+ efflux and leads to the formation of a large multi-protein complex termed the NLRP3 inflammasome responsible for the processing of pro-IL-1beta to mature IL-1beta that is then released. These findings indicate that the NLRP3 inflammasome plays a critical role in the stress-induced inflammatory response and depressive-like behaviors. Recently, it was reported that beta-hydroxybutyrate (BHB), which is a ketone body that supports mammalian survival during states of energy deficiency in peripheral tissues and the brain, suppresses activation of the NLRP3 inflammasome in response to ATP by preventing K^+ efflux and reducing NLRP3 oligomerization. Thus, we hypothesized that BHB could produce antidepressant effects via inhibition of NLRP3 inflammasome activation and neuro-inflammation in the hippocampus, particularly in response to chronic psychological stress. In the present study, we found that peripheral BHB administration prevents depressive- and anxious-like behaviors induced by a rodent model of chronic unpredictable stress. We also confirmed that peripheral administration results in elevated BHB levels in the hippocampus, supporting the possibility that the antidepressant effects of BHB are mediated by direct actions in brain. Furthermore, we confirmed that peripheral administration of BHB inhibits the induction of mature IL-1beta elevation in the adult rat hippocampus in response to immobilization stress. These findings are consistent with the hypothesis that inhibition of the NLRP3 inflammasome represents a relevant and efficacious approach for the development of novel antidepressant treatments.

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Poster

073. New Molecular Targets in Animal Models of Depression

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 73.02/WW7

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Ryanodine receptor type 2, a potential therapeutic target for bipolar disorder & major depressive disorder

Authors: *J. YAO, F. HIESS, R. WANG, S. W. CHEN;
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Abstract: Bipolar disorder (BD) and major depressive disorder (MDD) are mood disorders that impact approximately 10% of adults in the United States, which represent an enormous burden on our society. Despite years of intensive studies, the molecular basis of these disorders remains undefined, and there are no long-term effective treatments for BD and MDD. One emerging area

of investigation involves endoplasmic reticulum (ER) calcium homeostasis. Increased evidence indicates that the inositol 1,4,5-trisphosphate receptor type 1 (IP3R1), one of the Ca^{2+} releasing channels in the ER membrane, plays an important role in the pathogenesis of BD and MDD. However, the role of ryanodine receptor type 2 (RyR2), another member of the Ca^{2+} release channel family, in these mood disorders are unclear.By using GFP-tagged RyR2 mice, we found that RyR2 is highly expressed in brain regions that are involved in emotion control, including the prefrontal cortex (PFC), amygdala (AMYG), and hippocampus (HIPP). In open field (OF) test and elevated plus maze (EPM) test, we found that mice with RyR2 suppression-of-function (SOF) mutation show depression-like behavior, while mice with gain-of-function (GOF) RyR2 mutation exhibit mania-like activity. In order to see whether manipulating RyR2 function will affect neuron activity, we performed patch-clamp recording to measure the frequency of action potential (AP) firing, the threshold and latency of current injection-induced AP, and the frequency and amplitude of miniature excitatory postsynaptic current (mEPSC). Interestingly, we found that pyramidal neurons in layer II/III of PFC and CA1 region of HIPP brain slices from SOF-RyR2 mice are less active compared to WT, in contrast, GOF-RyR2 mutation renders the neurons hyperactive. Furthermore, recordings of long term potentiation (LTP) from the same regions showed that SOF-RyR2 mutant requires stronger stimulation than WT for triggering LTP. Interestingly, we were unable to induce LTP in the same region in the GOF-RyR2 mice. High frequency stimulation (HFS) may no longer potentiate the synaptic activity in already hyperactive neurons. Further studies are needed to determine whether SOF- and GOF-RyR2 mutations also alter intracellular Ca^{2+} homeostasis in an opposite manner. Together, these data suggest that RyR2 may play an important role in pathogenesis of BD and MDD. Thus, targeting RyR2 may represent a potential therapeutic approach for treating BD and MDD.

Disclosures: **J. Yao:** A. Employment/Salary (full or part-time): Eyes High Postdoc Fellowship of University of Calgary. **F. Hiess:** None. **R. Wang:** None. **S.W. Chen:** None.

Poster

073. New Molecular Targets in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIMH R01MH106500

Title: Reduced Slc6a15 in nucleus accumbens D2 expressing neurons mediates depression susceptibility

Authors: *L. M. RIGGS[1], R. CHANDRA[1], T. C. FRANCIS[1], H. NAM[1], H. SUN[2], C. A. TAMMINGA[3], G. TURECKI[4], M. LOBO[1];
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Abstract: Depression is a devastating illness whose complex etiology makes it difficult to treat. Transporters that are encoded by the solute carrier 6 (Slc6) gene family are ideal therapeutic targets for depression, given their regulation of neurotransmitter homeostasis. However, the development of novel therapeutics will depend on improving our understanding of how genetic factors promote depression susceptibility through cell-type specific molecular adaptions. Although previous research has shown that decreased expression of Slc6a15 (a neutral amino acid transporter) is associated with depression susceptibility, no study has examined this relationship within nucleus accumbens (NAc) medium spiny neuron (MSN) subtypes (D1 vs. D2). Given our previous characterization of Slc6a15 as being a D2-MSN enriched gene, we examined the role of D2-MSN Slc6a15 in mediating negative behavioral states (i.e., social avoidance). First, we show that mRNA expression of Slc6a15 is significantly reduced in the NAc of medicated and unmedicated depressed suicide victims. These results are consistent with our preclinical data showing significant reductions of Slc6a15 mRNA in the NAc of susceptible mice following chronic social defeat stress (CSDS) - a paradigm that produces behavioral and molecular adaptations that resemble clinical depression. In order to examine whether these observations are driven by reductions of Slc6a15 selectively within D2-MSNs, we isolated ribosome-associated mRNA Slc6a15 transcripts from D2-MSNs following the CSDS procedure. We show significant reductions in Slc6a15 mRNA in D2-MSNs of mice susceptible to CSDS, whereas no mRNA changes are detected in NAc D2-MSNs of resilient mice, relative to non-stressed controls. In order to determine the role of Slc6a15 in stress susceptibility, we selectively manipulated the expression of Slc6a15 in D2-MSNs by bilaterally injecting Cre-inducible double-floxed inverted open reading frame (DIO) adenoassociated viruses (AAV) into the NAc of D2-Cre mice. Virus-mediated Slc6a15 miRNA knockdown in D2-MSNs led to enhanced susceptibility to subchronic social defeat stress (SSDS), which supports the hypothesis that Slc6a15 cell-type specifically confers vulnerability to depression. Interestingly, we show enhanced resilience to CSDS when Slc6a15 is overexpressed in D2-MSNs, which resembles an antidepressant restoration of normal behavior. When taken together, our results show that reduced Slc6a15 expression within the NAc mediates susceptibility to depression, and that this effect occurs selectively within the D2-MSN population.

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Poster

073. New Molecular Targets in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

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Title: Haploinsufficiency of the affective disorders associated Cacna1c gene results in altered emotional behavior in rats: Developmental trajectories and inflammatory markers

Authors: *M. D. BRAUN[1], T. M. KISKO[1], R. KAYUMOVA[1], C. RAITHEL[1], C. HOHMEYER[3], M. RIETSCHEL[3], S. H. WITT[3], R. K. W. SCHWARTING[1], H. GARN[2], M. WÖHR[1];
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Abstract: The neurobiological mechanisms underlying affective disorders, i.e. major depressive disorder and bipolar disorder, are not fully elucidated yet. Genetic and environmental risk factors contribute critically to their etiology, but the exact pathophysiological pathways how these risk factors interact and influence brain structure and function remain to be uncovered. Important genetic factors include the gene *CACNA1C*. Here, we used the newly generated *Cacna1c* rat model to study its role in regulating phenotypes relevant to affective disorders. Firstly, behavioral phenotypes displayed by *Cacna1c* heterozygous (+/-) rats and wildtype littermate controls were compared in a sex-dependent manner. Secondly, to study environmental effects on inflammatory markers, *Cacna1c* +/- rats and wildtype littermate controls were exposed to post-weaning social isolation as a model for maltreatment or social plus physical enrichment modeling beneficial environments. Our results show that *Cacna1c* +/- rats are viable, yet the average number of rat pups born per litter is smaller for *Cacna1c* +/- than for wildtype females, with genotypes being evenly distributed. Interestingly, *Cacna1c* +/- females displayed less maternal licking/grooming, while nursing behavior did not differ. Consistent with the idea that reduced maternal licking/grooming results in an anxious phenotype, offspring of *Cacna1c* +/- females emitted more isolation-induced ultrasonic vocalizations (USV) in the first week of life, as compared to offspring of wildtype females. In addition, *Cacna1c* +/- pups emitted fewer isolation-induced USV than wildtype controls. No genotype differences were seen in body weight gain, temperature regulation and somatosensory reflexes, with both genotypes following normal early developmental patterns. In adulthood, exploratory behavior in the open field was reduced and anxiety-related behavior in the elevated plus maze was enhanced in both male and

female *Cacan1c* +/- rats. Finally, cytokine measurements revealed that post-weaning social isolation mostly increased proinflammatory markers, while social plus physical enrichment mainly led to opposite effects. Such changes were most prominently seen in wildtype controls, with relatively minor environmental effects being evident in *Cacna1c* +/- rats. Together, our findings indicate that *Cacna1c* is involved in the regulation of behavioral phenotypes relevant to neuropsychiatric disorders and that the responsivity to environmental changes at the level of inflammatory markers is reduced in *Cacna1c* +/- rats. Funding by the Deutsche Forschungsgesellschaft (DFG), FOR 2107: GA 545/5-1, SCHW 559/14-1, WO 1732/4-1.

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Poster

073. New Molecular Targets in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

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Title: Effects of kappa-opioid receptor antagonist on depression-like behavior

Authors: *A. WILLIAMS, B. C. TRAINOR;
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Abstract: Stressful experiences are a risk factor for the development of mood disorders such as depression and anxiety. Kappa opioid receptors (KOR) play an important role in mediating behavioral responses to stress and may represent a novel target for antidepressants. Social defeat activates KOR through the action of dynorphin, which in turn promotes aversion and depression-like behaviors. Previous research targeting KOR as a therapeutic option have focused on using long-acting antagonists such as norBNI. Although the use of these antagonists show promising antidepressant properties, the extremely long action of these compounds make them impractical for clinical use. Thus short-acting KOR antagonists may be a more viable option for the treatment of mood disorders. We examined the effect of the short-acting KOR antagonist AZ-MTAB on the development of depression-like phenotypes following social defeat stress in the monogamous California mouse (*Peromyscus californicus*). Mice underwent three episodes of

defeat or control conditions and were treated with either 10mg/kg dose of AZ-MTAB or vehicle prior to each episode. Autogrooming behavior was recorded immediately before the first and last episode of social defeat. Preliminary data indicate that AZ-MTAB immediately prior to defeat had short and long term effects. Mice exposed to defeat and vehicle showed significant increases in autogrooming behavior immediately prior to a third episode of defeat (indicating increased anxiety), and decreased sucrose preference two weeks after the last episode of defeat. Mice exposed to defeat and AZ-MTAB did not show these behavioral changes. These results show that temporary inhibition of KOR during stress blocks long lasting behavioral changes. Previous research shows that physiological effects of KOR differ between males and females, yet most of the research being done currently use only male rodents. We are currently repeating this experiment in female California mice to test for sex differences in behavior that may be mediated by KOR function.

Disclosures: **A. Williams:** None. **B.C. Trainor:** None.

Poster

073. New Molecular Targets in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

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Title: Reduced enkephalin signaling in the nucleus accumbens D2 - MSN circuit regulates depression-like phenotype in social defeat stress

Authors: *H. NAM, R. CHANDRA, T. C. FRANCIS, S. DAS, M. K. LOBO;
Dept. of Anat. and Neurobio., Univ. of Maryland, Baltimore, Baltimore, MD

Abstract: Enkephalins are primary endogenous ligands for delta opioid receptors (DORs) and are highly enriched in D2-medium spiny neurons (MSNs) in the nucleus accumbens (NAc) and dorsal striatum. Enkephalins are highly implicated in depression, as preproenkephalin knockout and DOR knockout mice display anxiety- and depression-like phenotypes. Further, enkephalinase inhibitors can act as antidepressants. However, the specific the role of enkephalins, selectively in the D2-MSN microcircuit in NAc - ventral pallidum (VP) pathway, in depression is not fully investigated. To provide insight into enkephalin function in this circuit we use an animal model of depression, the 10-day chronic social defeat stress paradigm. Following the defeat sessions, animals were either categorized as susceptible (displaying depression-like behavior) or resilient to social defeat stress according to their performance in a social interaction

test. Compared to the control and the resilient animals, the susceptible animals showed reduction in enkephalin levels in the VP. To determine if the reduced enkephalin levels cause depression-like behavior through disrupted Enk-DOR signaling, we treated the animals that experienced chronic social defeat stress with a DOR agonist SNC80. Preliminary studies demonstrate this treatment was able to reverse the depression-like conditions in susceptible animals. In order to investigate further into the mechanisms that reduce levels of enkephalins in the depressed conditions, we analyzed levels of different peptidases that can act as enkephalinases. Our qRT-PCR results demonstrate an increase in mRNA levels of peptidases angiotensin-converting enzyme (ACE) and analyl aminopeptidase (ANPEP) in susceptible animals compared to the controls, which may account for the decrease in enkephalin levels. Overall, our data implicate that depression-like behavior induced by social defeat stress is caused by reduced DOR signaling resulting from lowered level of enkephalins, which may be mediated through elevated expression of enkephalinases.

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Poster

073. New Molecular Targets in Animal Models of Depression

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Title: Hippocampal α7 nAChR is a critical component for cholinergic regulation of anxiety and depression-like behaviors in C57BL/6J male and female mice.

Authors: ***T. MOSE**[1], Y. S. MINEUR[2], S. BLAKEMAN[2], S. A. NEWBOLD[2], M. R. PICCIOTTO[2], M. R. PICCIOTTO[2];
[1]Yale Univ., New Haven, CT; [2]Psychiatry, Yale Univ. Sch. of Med., New Haven, CT

Abstract: Evidence from pre-clinical and clinical studies has highlighted the link that exists between cholinergic signaling, anxiety and depression. We previously showed that blockade of

acetylcholinesterase (AChE; the enzyme responsible for hydrolyzing ACh) with physostigmine, can induce behaviors related to anxiety and depression in mice. This effect can be recapitulated by knocking down AChE only in the hippocampus and can be reversed by systemic injection of nicotinic antagonist and several partial agonists. Furthermore, knockdown of either the β2 or α7 subunit of the nicotinic acetylcholine receptor (nAChR) in the amygdala is sufficient to induce an antidepressant-like effect in mice. These results demonstrate that signaling at multiple nAChRs can mediate the effects of ACh on behaviors related to anxiety and depression. Previous studies have mainly focused on β2- and β4-containing nAChRs, and nicotinic compounds can also target α7 nAChRs. We therefore used more specific pharmacological agents to determine the contribution of α7 nAChRs to behaviors related to anxiety and depression. Administration of the α7 nAChR agonist GTS-21 increased anxiety-like behavior in male and female C57BL/6J mice in the light dark box, and increased immobility in the tail suspension and forced swim tests in male, but not female, mice. Conversely, the α7 nAChR antagonist methyllycaconitine (MLA) induced anxiolytic and antidepressant-like effects in male mice in the tail suspension and forced swim tests, but this effect was limited in female mice. Local knockdown of the α7 nAChR subunit in the hippocampus was confirmed by equilibrium binding using α-bungarotoxin, and was sufficient to prevent behavioral phenotypes induced by physostigmine in male and female mice. Interestingly, α7 nAChR knockdown in the hippocampus did not result in significant behavioral effects in the absence of pharmacological challenge. These results suggest that ACh can signal through α7 nAChRs in the hippocampus to regulate behaviors related to anxiety and depression. Further, these studies provide evidence for sex differences in signaling through these receptors that may be relevant for development of novel treatments for mood disorders.

Disclosures: **T. Mose:** None. **Y.S. Mineur:** None. **S. Blakeman:** None. **S.A. Newbold:** None. **M.R. Picciotto:** None. **M.R. Picciotto:** None.

Poster

073. New Molecular Targets in Animal Models of Depression

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 73.08/WW13

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: p11, a possible mood regulator, is essential for dopamine responses to reward in the nucleus accumbens.

Authors: *Y. HANADA[1], Y. KAWAHARA[1], Y. OHNISHI[1], T. SHUTO[1], M. KUROIWA[1], N. SOTOGAKU[1], Y. SAGI[2], P. GREENGARD[2], A. NISHI[1];

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Abstract: p11, a member of the S100 protein family, regulates depression-like behaviors and responses to antidepressants. A recent study showed that the nucleus accumbens (NAc) is a key brain region of p11 action. Dopamine (DA) releasing neurons projecting to the NAc and prefrontal cortex (PFC) are responsible for aspects of reward- and cognition-related behaviors, respectively, and their functions are assumed to be impaired in depression. The present study investigated the role of p11 in the DA responses to rewarding stimuli. For this aim, the effects of rewarding stimuli (cocaine, palatable food and sexual interaction) on the extracellular DA levels in the NAc and PFC were evaluated in p11 knockout mice using in vivo microdialysis. All the rewarding stimuli induced an increase in DA levels in the NAc and PFC in wild-type mice. DA responses to rewarding stimuli in the NAc were attenuated (cocaine) or abolished (food and sexual interaction) in p11 knockout mice. DA responses in the PFC in p11 knockout mice were similar to those in wild-type mice. These results demonstrate that p11 is essential for the DA response in the NAc, but not in the PFC, to rewarding stimuli. The dysregulation of mesolimbic DA neurons might be involved in depression-like behaviors in p11 knockout mice.

Disclosures: Y. Hanada: None. **Y. Kawahara:** None. **Y. Ohnishi:** None. **T. Shuto:** None. **M. Kuroiwa:** None. **N. Sotogaku:** None. **Y. Sagi:** None. **P. Greengard:** None. **A. Nishi:** None.

Poster

073. New Molecular Targets in Animal Models of Depression

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 73.09/WW14

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: JSPS Grant 24590183

Title: Depressive phenotype in N-acetylaspartate synthetase Shati/Nat8l-overexpressed mice

Authors: *Y. MIYAMOTO[1], K. FU[1], N. IEGAKI[1], K. SUMI[1], Y. FURUKAWA-HIBI[2], S.-I. MURAMATSU[3], T. NABESHIMA[4], K. UNO[1], A. NITTA[1];
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Abstract: Both *N*-acetylaspartate (NAA) and *N*-acetylaspartylglutamate (NAAG) are present at high concentrations in mammalian brain, and are considered to serve for various neuronal actions. Recently, several reports demonstrated that the contents of those endogenous substances

alter in the postmortem brain of patients with mental disorders, such as depression and schizophrenia. We previously identified a molecule Shati/Nat8l, which is containing a well-conserved *N*-acetyltransferase sequence, from the brain of psychosis animal model. Shati/Nat8l synthesizes NAA from L-aspartate and acetyl-CoA, and NAA is subsequently condensed with glutamate to produce NAAG. In the present study, we investigated the roles of NAA and/or NAAG in depression-like behaviors using Shati/Nat8l-overexpressed mice. After exposing the forced swimming stress, the expression levels of Shati/Nat8l mRNA elevated in the dorsal striatum of wild-type mice. We therefore generated striatal Shati/Nat8l-overexpressed mice by microinjecting Shati/Nat8l gene-contained adeno-associated virus vectors into the dorsal striatum. The contents of NAA, but not NAAG, were elevated in the dorsal striatum of the Shati/Nat8l-overexpressed mice. On the other hand, the content ratio of NAAG/NAA was low, and the expression levels of NAAG synthetase were enhanced in the dorsal striatum of these mice compared with control mice. The Shati/Nat8l-overexpressed mice revealed prolonged immobility time in both the forced swimming and tail suspension tests. The Shati/Nat8l-overexpressed mice also exhibited decreased sociability in the three chambers social interaction test. These three emotional impairments in the Shati/Nat8l-overexpressed mice were ameliorated by treatment with antidepressant drug fluvoxamine, an inhibitor of selective serotonin reuptake. In addition, those emotional impairments were restored by treatment with LY341495, a potent antagonist of the group II metabotropic glutamate receptors including mGluR3 as the target of NAAG. These observations defined depressive phenotype in the Shati/Nat8l-overexpressed mice, suggesting that the NAA-NAAG-mGluR3 pathway regulated by Shati/Nat8l in the dorsal striatum serves for motivation and sociability via regulation of the serotonergic neuronal system.

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Poster

073. New Molecular Targets in Animal Models of Depression

Location: Halls B-H

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Program#/Poster#: 73.10/WW15

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH Grant R01MH106500

Title: Transcriptional regulation of dendritic complexity mediates susceptibility to social stress

Authors: ***T. C. FRANCIS**[1], R. CHANDRA[1], P. KONKALMATT[2], L. M. RIGGS[1], A. SERAFINI[3], M. LOBO[1];

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Abstract: The nucleus accumbens (NAc) integrates emotionally salient stimuli and is highly involved in behavioral deficits produced by chronic social defeat stress (CSDS). Current evidence suggests dopamine 1 (D1) receptor expressing medium spiny neurons (MSNs) of the NAc are central to these alterations. Previously, we established that repeated stimulation of D1-MSNs restores normal social interaction and sucrose preference in mice susceptible to CSDS. Using quantitative RT-PCR, we found this stimulation significantly reduces NAc expression of the transcription factor and neuronal plasticity regulator early growth response 3 (Egr3). Further, using transgenic D1-Cre mice crossed with RiboTag mice (D1-RiboTag) to isolate ribosome-associated mRNA specifically in D1-MSNs, we found increased Egr3 expression in mice susceptible to, but not resilient to CSDS. Egr3 expression remained unchanged in D2 receptor expressing MSNs. Cre-dependent adeno-associated virus (AAV) overexpression of Egr3 in the NAc of D1-Cre mice promoted susceptibility to subthreshold social defeat stress. In contrast, knock-down of Egr3 in D1-Cre mice utilizing a Cre-dependent AAV micro RNA (miR) construct promoted resilience to CSDS and prevented anhedonia outcomes. D1-MSNS of susceptible mice displayed reduction in frequency of mEPSCs, enhancement in intrinsic excitability and changes in dendritic arborization, an effect blocked by Egr3-miR mediated knockdown. These results suggest Egr3 regulates stress-induced dendritic morphology through structural molecules that regulate dendritic atrophy, such as RhoA. RhoA expression was significantly up-regulated in D1-MSNs of susceptible, but not resilient D1-RiboTag mice. Chromatin immunoprecipitation demonstrated enhanced Egr3 binding to the promoter of RhoA, revealing RhoA up-regulation is mediated by Egr3 in CSDS mice. RhoA inhibition by the specific inhibitor Rhosin, via systemic administration or specific infusion in to the NAc, blunted the behavioral and electrophysiological effects of CSDS. These results establish a novel role for social stress-induced transcriptional control of dendritic structure through Egr3 regulation of RhoA in NAc D1-MSNs.

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Poster

073. New Molecular Targets in Animal Models of Depression

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Program#/Poster#: 73.11/WW16

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: P50 MH096890 (Epigenetic Mechanisms in Depression)

Title: Anxiety and depression in Setdb1 histone H3K9 methyltransferase mutant mice

Authors: *Y. JIANG[1], E. LOH[2], B. KASSIM[1], I. MAGRO[1], B. JAVIDFAR[1], I. PURUSHOTHAMAN[2], L. SHEN[2], S. AKBARIAN[1];
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Abstract: Histone methyltransferases specific for the histone H3-lysine 9 residue, including Setdb1 (Set domain, bifurcated 1)/Eset/Kmt1e are associated with repressive chromatin remodeling. Setdb1 is highly expressed in brain during early development and plays an important role in maintaining stem cell status, but potential effects on neuronal function and behavior remain largely unexplored. We previously reported that transgenic mice (*CK-Setdb1*) with increased Setdb1 expression in adult forebrain neurons show antidepressant-like phenotypes. Here, we explore Setdb1 in a loss-of-function model with conditional knockout mice. *Setdb1-CK-cKO* mice underwent ablation of Setdb1 specifically in CaMK II-alpha active postmitotic neurons. *Setdb1-CK-cKO* deficient mice, opposite to the behavioral phenotype previously reported for *CK-Setdb1* overexpressors, showed elevated level of anxiety and depression-like phenotype in multiple behavioral paradigms, including open filed, light dark and forced swim. Meanwhile, *Setdb1-CK-cKO* mice show decrease in brain weight, and cognitive deficits in radial-arm, object recognition and passive avoidance tests. To test whether such behaviors could be rescued by re-introducing Setdb1 to knockout neurons, we generated Setdb1-resuce mice by crossing *CK-setdb1* transgenic mice with *Setdb1-CK-cKO* mice. Strikingly, brain weight, anxiety, and depression-like phenotypes were fully reversed in Setdb1-rescue mice, indistinguishable from wildtype. Interestingly, *Setdb1-CK-cKO* brain showed loss of perforant path (PP) projection to hippocampus and additional subtle changes in connectivity. RNAseq from prefrontal cortex revealed differential transcriptome in *Setdb1-CK-cKO* as compare to littermate wildtype controls, showing 215 significantly unregulated and 106 downregulated genes (adjP<0.05). Experiments are in process to map epigenomic profiling in *Setdb1-CK-cKO* neurons using ChIPseq on FACS sorted neuronal nuclei from both native and fixed mouse cortical tissues. In conjunction with data from RNAseq, our goal is to identify a select set of target genes or functional genomic elements that contribute to the regulation affective and motivational behaviors through Setdb1-mediated repressive chromatin remodeling in mature brain.

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Poster

073. New Molecular Targets in Animal Models of Depression

Location: Halls B-H

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Program#/Poster#: 73.12/WW17

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NSERC

Title: TNF-alpha antagonism restores deficits in neurogenesis and hippocampal and prefrontal cortex-dependent memory in an animal model of depression

Authors: *K. BRYMER[1], E. FENTON[1], H. J. CARUNCHO[2], L. E. KALYNCHUK[3];
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Abstract: Chronic stress decreases neurogenesis within the hippocampus and impairs both hippocampal and prefrontal-cortex-dependent memory. Furthermore, chronic exposure to stress promotes the release of cytokines, which in turn exacerbate the stress response and disrupt cognition. Prominent among these is the cytokine TNF-alpha, which is preferentially expressed in the hippocampus and pre-frontal cortex. Importantly, the TNF-alpha antagonist etanercept is known to increase neurogenesis within the hippocampus. We hypothesized that treating chronically-stressed rats with etanercept could have antidepressant effects, and could reverse stress-induced deficits in cognition and neurogenesis. To test this hypothesis, we examined the effect of repeated corticosterone (CORT) treatment and concurrent TNF-alpha antagonism (i.e., with etanercept) on cross-modal memory and forced-swim test (FST) behavior. Additionally, we examined object-location and object-in-place memory--tasks that are dependent on the hippocampus and pre-frontal cortex, respectively. Finally, we examined hippocampal neurogenesis through doublecortin (DCX) immunohistochemistry. Rats received either 21 days of daily CORT injections (40 mg/kg) or vehicle injections. Rats also received semi-weekly injections of etanercept (0.8 mg/kg). Behavioral testing began on day 22. CORT increased depression-like behavior and impaired both object-location and object-in-place memory. Importantly, CORT rats treated with etanercept behaved like vehicle rats. CORT also decreased the number of DCX+ cells in the granule cell layer and dendritic complexity within surviving DCX+ cells, but etanercept restored both measures to control levels. These novel results clearly demonstrate that etanercept has antidepressant effects, which are accompanied by the restoration of normal levels of neurogenesis and both hippocampal-dependent and prefrontal-cortex-dependent memory. These results highlight an important role for the immune system in the pathogenesis of depression.

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Poster

073. New Molecular Targets in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIMH

NARSAD Young Investigator Award

Title: Tet1 in nucleus accumbens regulates stress responses

Authors: *J. FENG[1,2], C. PENA[3], I. PURUSHOTHAMAN[3], O. ENGMANN[3], D. WALKER[3], O. ISSLER[3], A. BROWN[1], M. DOYLE[3], E. HARRIGAN[3], E. MOUZON[3], V. VIALOU[3], L. SHEN[3], M. M. DAWLATY[4], R. JAENISCH[5], E. J. NESTLER[3];
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Abstract: Depression is a leading cause of disease burden, yet current therapies fully treat less than 50% of affected individuals. Increasing evidence implicates epigenetic mechanisms in depression and antidepressant action. Here, we examined a possible role for the DNA dioxygenase, ten eleven translocation protein 1 (TET1), in depression-related behavioral abnormalities. We show that chronic social defeat stress, an ethologically validated mouse model of depression, decreased *Tet1* expression in nucleus accumbens (NAc), a key brain reward region, in stress susceptible mice. Surprisingly, selective knockout of *Tet1* in NAc neurons of adult mice produced antidepressant-like effects in several behavioral assays. To identify *Tet1* targets that mediate these actions, we performed mRNAseq on NAc after conditional deletion of *Tet1* and found that immune-related genes are the most highly dysregulated. Interestingly, many of these genes are also upregulated in NAc of resilient mice after chronic social defeat stress. Together, these findings link *Tet1* to stress responses and identify novel targets for future antidepressant drug discovery efforts.

Disclosures: J. Feng: None. **C. Pena:** None. **I. Purushothaman:** None. **O. Engmann:** None. **D. Walker:** None. **O. Issler:** None. **A. Brown:** None. **M. Doyle:** None. **E. Harrigan:** None. **E. Mouzon:** None. **V. Vialou:** None. **L. Shen:** None. **M.M. Dawlaty:** None. **R. Jaenisch:** None. **E.J. Nestler:** None.

Poster

073. New Molecular Targets in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: MOST 104-2320-B-004-001

Title: Ablation of interferon inducible IFITM genes leads to anxiety- and depression- like behaviors

Authors: *S.-K. CHEN[1], W.-K. LIN[1], H.-C. CHANG[1], Y. WEE[3], P.-W. CHU[2];
[1]Inst. of Neurosci., [2]Dept. of Psychology, Natl. Chengchi Univ., Taipei, Taiwan; [3]Dept. of Pathology, Univ. of Utah, Salt Lake City, UT

Abstract: *Ifitm* genes are a group of interferon inducible genes. These genes are transcriptional activated upon interferon stimulation and play important roles in interferon meditated antiviral functions, including limiting viral entry and replication. However, the biochemical properties of these proteins are not fully understood. In addition to immune cell lineages, *Ifitm* genes are widely expressed in various tissues and cell type, including in central nervous system (CNS) in both embryonic stages and adult brains. Although the biological functions of these proteins besides immune system are still elusive, elevated IFITM proteins in schizophrenia patient brains, especially cerebral cortex, suggests a role of these proteins in modulating neural physiology. We recently revealed the behavioral abnormalities in *IfitmDel* mutants, in which 5 of the 6 *Ifitm* genes are deleted. These mutants exhibit anxiety- and depression- like phenotype in elevated plus maze and tail suspension test. The results of histological analyses, such as Nissl staining and immunohistochemical analysis of various neuron types, show no anatomical defects, detectable neuron count differences and morphological alterations of neurons in *IfitmDel* mutant brains, implying that these proteins are not crucial for the development of CNS. The expression levels of neuropeptides related to stress response and anxiety or depression status, such as corticotrophin releasing factor (CRF) and neuropeptide Y (NPY), are altered in mutant hypothalamus, suggesting hypothalamic influences on the behavioral phenotypes. Although no obvious differences in different types of neurons can be found, morphological changes of microglia and the trends of increased proinflammatory cytokines are detected in the hypothalamic regions in *IfitmDel* mutant brains, implying that hypothalamic inflammation and microglial activations might be involved in the abnormal behaviors. The cellular mechanisms underlying the behavioral phenotype induced by loss of IFITM proteins will be further determined in the future studies.

Disclosures: **S. Chen:** None. **W. Lin:** None. **H. Chang:** None. **Y. Wee:** None. **P. Chu:** None.

Poster

073. New Molecular Targets in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Program Grant NI Centre for Stratified Medicine

PhD Studentship, Dept of Education and Learning

Title: Expression of inflammatory cytokine genes in the Disc1 mouse model of major mental illness

Authors: C. R. LAPSLEY[1], B. LANG[3], D. ST. CLAIR[3], C. MCCAIG[3], A. J. BJOURSON[1], *E. K. MURRAY[2];
[1]Northern Ireland Ctr. for Stratified Med., [2]Univ. of Ulster, L'Derry, United Kingdom; [3]Univ. of Aberdeen, Aberdeen, United Kingdom

Abstract: Disc1 is a balanced chromosomal translocation and a significant risk gene for major mental illness, including schizophrenia and depression. There is considerable overlap between the Disc1 interactome and the downstream signal transduction pathways involved in response to immune challenge, and a role for Disc1 as a mediator of neuro-immune interplay has been suggested. To date, the effect of mutations in the Disc1 gene on immune status has not been investigated. In the current study, we investigated the inflammatory profile in the brains and blood of adult wild type and Disc1tr mice to determine if baseline levels of immune markers altered in the Disc1tr mouse brain. Cortex and hippocampus samples were dissected from adult Disc1 mice (n=10 5M, 5F) and WT littermates (n=10 5M, 5F). Gene expression levels were then measured for four inflammatory genes (IL-6, TNF-a, INF-g and IL-1b) by qPCR using commercially available assays. Each cDNA sample was run in triplicate for each assay and relative gene expression was calculated using the ΔΔCT method. Cytokine levels will be examined using multiplex ELISA in hippocampus, cortex and blood. No significant effects of sex were detected in expression levels of any of the four inflammatory genes; data from males and females were therefore combined. No significant differences were detected in expression levels of any of the four genes in the hippocampus between wild type and Disc1tr mice. In the cortex, IL-1B, IL-6 and TNF-a were significantly decreased in Disc1tr compared to wild type mice ($p<0.05$). IFN-y expression was also lower in the cortex but this did not reach statistical significance. These findings suggest that baseline immune status in the brain is altered by the Disc1tr mutation. Future studies will examine the effect of immune challenge on cytokine expression levels in Disc1 and wild-type mice.

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Poster

073. New Molecular Targets in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Role of Heat shock protein 105 on depression-like behavior in mice

Authors: *Y. UTAKA, R. TANOUE, Y. MORITA, S. YAMAMOTO, N. HASHIKAWA-HOBARA, N. HASHIKAWA;
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Abstract: Aim Changes in the Heat shock proteins (HSPs) are involved in neurological disease. However, the relationship between HSPs and depression-like behavior is still unclear. In the present study, we examined whether oral geranylgeranyl acetone (GGA), known as HSPs inducer, administration induced an anti-depressant effect in a social defeat stress model in mice. We also investigated the possible molecular mechanisms involved, particularly focusing on hippocampal neurogenesis and neurotrophic factor expression.
Methods C57B6/J male mice were submitted to social defeat stress for 15 consecutive days. Every day, each experimental mouse was introduced into the home cage of an aggressor male ICR mouse for 10 min, during which time the experimental animal was physically defeated. Following the 10 min defeat, each experimental mouse was separated from aggressor and placed across a plastic separator with holes, where they remained in sensory contact with the ICR aggressor for the remainder of the 24 hours. After the stress exposure, depression-like behaviors were assessed by the forced swim test, tail suspension test, social interaction test and sucrose preference test. *HSPs* (*Hsp60, 72, 90, 105*) and neurotrophic factor (*Bdnf*, *Cntf*, *Nt-3*, *Ngf*) mRNA levels in the hippocampus were analyzed by qRT-PCR. Furthermore, hippocampal neurogenesis was assessed by immunofluorescence staining using doublecortin antibody. Moreover, to clarify whether HSPs are involved in behavioral development, GGA (0.34%) was orally administered during stress exposure. In addition, to elucidate the effects of HSP105 knockdown on depression-like behavior, we injected *Hsp105*-siRNA into the mouse brain on day 1, 7 and 14 during 15-day stress exposure.
Results and Discussion After stress exposure, mice were exhibited depression-like behavior and decrease of *Hsp105* and *Bdnf* mRNA levels in the hippocampus when compared with control mice. GGA administration recovered the behavioral dysfunctions and hippocampal neurogenesis

and *Bdnf* mRNA level. Moreover, HSP105 knockdown inhibited the GGA-mediated anti-depressant effect. These results suggest that HSP105 in the mouse hippocampus is associated with depression-like behavior through hippocampal neurogenesis.

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Poster

073. New Molecular Targets in Animal Models of Depression

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Fondecyt Regular 1130904 (PR)

Fondecyt Regular 1120240 (DV)

Title: Role of $Ca_v1.2$ calcium channel in hippocampal neurons of animals with depression-like behaviors

Authors: *C. MORENO NARANJO[1,2], P. HARDY[1], D. PINO[1], M. BASUÑAN[1], T. HERMOSILLA[2], D. VARELA[2], P. ROJAS[1];
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Abstract: Major depression is one of the most common mood disorder worldwide, being a serious public health problem in most countries. The biological study of depression has elucidated partly the development of this condition. Previous studies has been demonstrated that hippocampal neurons from depressive patients show a reduction in dendritic arborization and number of dendritic spines, nevertheless, the mechanisms responsible at the molecular level for these changes are still to be defined. $Ca_V1.2$ calcium channels are the principal pathway for calcium influx in neuronal soma, and have been associated to several cellular processes such as

changes in neuronal morphology, and excitation-transcriptional coupling. These processes involve changes in gene expression for at least three pathways: activation of Calmodulin, nuclear translocation of Nuclear Factor Activated T- cells (NFAT) and nuclear translocation of C-terminus region of the $Ca_V1.2$. Our working hypothesis is that changes in morphology and function in hippocampal neurons from animal models of depression are due to regulation in gene expression dependent on signaling through $Ca_V1.2$. In order to test this hypothesis we use chronic restraint stress (CRS) to generate a model of chronic stress-induced depression. Behavioral parameters such as anhedonia, change in social interaction and behavioral despair, demonstrate that these animals share characteristic of mayor depression, as has been reported for this model previously. By using electrophysiological and molecular biology techniques, we are currently studying changes in $Ca_V1.2$ calcium channel expression and subcellular localization that could be related to the described pathways that modulate gene expression. When comparing the expression level in control and CRS animals we have obtained a significant increase of $Ca_V1.2$ calcium channel in whole hippocampal samples and increase in expression of C-terminal region associated to the transcriptional factor. Our results support the idea that $Ca_V1.2$ is a key player in the development of depression in an animal.

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Poster

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Hope for Depression Research Foundation

Pritzker Neuropsychiatric Disorders Research Consortium

Title: Neural cell adhesion molecule peptide mimetics modulate affective behavior

Authors: *C. A. TURNER[1], S. J. WATSON, Jr.[2], H. AKIL[2];
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Abstract: Recent studies have highlighted the importance of the fibroblast growth factor (FGF) family in modulating emotionality. Moreover, ligands that activate FGF receptors, such as FGF2, have antidepressant and anxiolytic effects in animal models. Neural cell adhesion molecule (NCAM) is also known to bind to and activate FGF receptors. Therefore, this study assessed the ability of NCAM peptide mimetics to modulate anxiety-like and depression-like behavior in rodents. Anxiety-like behavior was measured on the elevated plus-maze and depression-like behavior was measured on the forced swim test. Specifically, we assessed the acute and chronic effects of three different NCAM peptide mimetics that have previously been shown to have effects on learning and memory in rodents. One peptide increased anxiety-like behavior in the EPM acutely. Chronically, it decreased depression-like behavior in the FST. The second peptide decreased depression-like behavior both acutely and chronically. The third peptide increased anxiety-like behavior acutely. Chronically, it decreased anxiety-like behavior. These findings lend support for the idea that NCAM peptides can modulate emotionality in animal models. Moreover, some peptides may be more beneficial in treating major depressive disorder, whereas others may be more beneficial in treating anxiety disorders.

Disclosures: **C.A. Turner:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The authors are members of the Pritzker Neuropsychiatric Disorders Research Consortium, which is supported by the Pritzker Neuropsychiatric Disorders Research Fund L.L.C. **S.J. Watson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The authors are members of the Pritzker Neuropsychiatric Disorders Research Consortium, which is supported by the Pritzker Neuropsychiatric Disorders Research Fund L.L.C. **H. Akil:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The authors are members of the Pritzker Neuropsychiatric Disorders Research Consortium, which is supported by the Pritzker Neuropsychiatric Disorders Research Fund L.L.C..

Poster

073. New Molecular Targets in Animal Models of Depression

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 73.19/XX2

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Spanish Ministry of Economy and Competitiveness, SAF2012-35183 and SAF-68346 (FA)

Instituto de Salud Carlos III PI13/01390 (AB) co-financed by the European Regional Development Fund “A way to build Europe”.

Title: siRNA-mediated suppression of astroglial glutamate transporters GLT-1 and GLAST in infralimbic, but not prelimbic cortex, induces depressive-like behaviors in mice

Authors: N. FULLANA[1,2,3], A. FERRÉS-COY[1,2,3], E. RUIZ-BRONCHAL[3,1,2], A. BORTOLOZZI[3,1,2], *F. ARTIGAS[1,2,3];
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Abstract: Major depression has been associated with alterations of the monoaminergic systems. However, recent evidence suggests that dysregulations of glutamatergic neurotransmission in the prefrontal cortex (PFC) are involved in the pathophysiology of depression. Previous studies showed that astrocytic glutamate transporter, GLT-1, blockade induces anhedonia, impaired memory and *c-fos* expression in rat PFC. Given the role of ventral cingulate areas in mood regulation, we hypothesized that reduced expression of GLAST and GLT-1 in the infralimbic cortex (IL) would induce depressive-like behaviors in mice due to dysfunctional glutamatergic neurotransmission. We knocked-down the expression of GLAST and GLT-1 to evoke an increase of excitatory neurotransmission in IL cortex. We microinjected small interfering RNA (siRNA) targeting GLAST or GLT-1 unilaterally into prelimbic (PrL) or IL cortices of mice and examined cellular and behavioral effects. Moreover, the extracellular levels of 5-HT in dorsal raphe nucleus (DRN) were determined by *in vivo* microdialysis. Local unilateral GLAST-siRNA microinfusion in the mouse IL (4.2 nmol) reduced selectively GLAST mRNA and protein levels to ~80% of control mice. GLAST knockdown mice exhibited depressive-like behaviors in the tail suspension test (TST, 125% of controls) and in the forced swim test (FST, 128% of controls) and anhedonia in the sucrose of preference test (SPT, 50% of controls). Likewise, intra-IL infusion of GLT-1-siRNA (4.2 nmol) reduced GLT-1 expression (~70% of controls), increased immobility time also in the TST (130% of controls) and in FST (118% of controls) and reduced preference for sucrose in SPT (56% of controls). In contrast, reduced GLAST or GLT-1 expression in the PrL cortex did not affect behavioral responses to stress. Moreover, GLAST- and GLT-1-siRNA administered animals showed a reduction in baseline 5-HT extracellular levels in the DRN (49% of controls), even if the responsiveness of the neurons to veratridine was not altered. The present results suggest that astroglial regulation of excitatory neurotransmission in the IL cortex plays a major role in mood control. GLAST and GLT-1 down-regulation impairs the normal clearance of synaptically released glutamate. Given the reciprocal connectivity between the ventral PFC and many cortical and subcortical areas, an increased excitatory function in IL likely translates into downstream alterations of other brain areas involved in major depression, such as the monoamine nuclei. Overall, these findings improve our understanding of the pathophysiology of major depression, and help to identify novel targets in antidepressant drug development.

Disclosures: **N. Fullana:** None. **A. Ferrés-Coy:** None. **E. Ruiz-Bronchal:** None. **A. Bortolozzi:** None. **F. Artigas:** None.

Poster

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH F30MH100835

NIH R01MH090264

Title: A cell-type specific role for nucleus accumbens neuroligin-2 in depression and stress susceptibility

Authors: *M. HESHMATI[1], H. ALEYASIN[1], C. MENARD[1], M. E. FLANIGAN[1], M. L. PFAU[1], P. H. GOFF[1], G. E. HODES[1], A. TAKAHASHI[1], A. LEPACK[1], L. BICKS[1], D. J. CHRISTOFFEL[2], R. CHANDRA[3], A. K. FRIEDMAN[1], G. TURECKI[4], M.-H. HAN[1], M. LOBO[3], I. MAZE[1], S. A. GOLDEN[5], S. J. RUSSO[1];
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Abstract: Neuroligin-2 (NLGN-2) is a postsynaptic cell adhesion protein that plays an integral role in the inhibitory synapse. Mutations in the neuroligin gene family have been associated with neuropsychiatric disorders like autism and schizophrenia; however, relatively little is known about NLGN-2. Transcriptional profiling of the neuroligin gene family in postmortem nucleus accumbens (NAc) of patients with major depressive disorder revealed a decrease in NLGN-2. Reverse translation of these findings in the chronic social defeat stress model in mice supported an overall decrease in NAc NLGN-2 in mice that are susceptible to stress. A cell-type specific analysis of NLGN-2 transcription in D1 receptor-positive (D1-MSNs) compared to D2 receptor-positive cells (D2-MSNs) revealed that NLGN-2 mRNA is reduced selectively in D1-MSNs of stress susceptible mice.
To further explore the cell-type specific contribution of NAc NLGN-2 to stress resiliency and coping behavior, we used a Cre-conditional RNA interference approach to knockdown NLGN-2 in either D1-MSNs or D2-MSNs of transgenic mice. Cell-type specific knockdown of NLGN-2 in NAc D1-MSNs results in decreased dominance behavior and promotes susceptibility to social defeat stress. NLGN-2 knockdown in NAc D2-MSNs causes anxiolysis, increased dominance behavior, and the engagement of coping strategies to mediate increased stress resiliency. Overall, we demonstrate a unique role for NAc NLGN-2 in major depressive disorder and the engagement of behavioral strategies to promote dominance and active stress resiliency. These findings establish a novel role for NAc NLGN-2 in stress susceptibility, providing a molecular basis for the development of cell-type specific depression therapies.

Disclosures: **M. Heshmati:** None. **H. Aleyasin:** None. **C. Menard:** None. **M.E. Flanigan:** None. **M.L. Pfau:** None. **P.H. Goff:** None. **G.E. Hodes:** None. **A. Takahashi:** None. **A. Lepack:** None. **L. Bicks:** None. **D.J. Christoffel:** None. **R. Chandra:** None. **A.K. Friedman:** None. **G. Turecki:** None. **M. Han:** None. **M. Lobo:** None. **I. Maze:** None. **S.A. Golden:** None. **S.J. Russo:** None.

Poster

073. New Molecular Targets in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Science Foundation Ireland Grant

Title: Effects of norbinaltorphimine (nor-BNI) on depressive-like and habit formation behavior in the IFN-α-induced depression model

Authors: ***J. ROUINE**, C. K. CALLAGHAN, S. M. O'MARA;
Psychology, Trinity Col. Inst. of Neurosci., Dublin 2, Ireland

Abstract: Depression is a heterogeneous condition that affects approximately 350 million people worldwide. Current research is focussing on the development of novel antidepressant compounds with differing mechanisms of action to standard tricyclic or selective reuptake inhibitors. Kappa opioid receptor (KOR) antagonism has been reported to have antidepressant-like properties. The current study examined the effects of norbinaltorphimine (nor-BNI), a potent KOR antagonist, in a progressive ratio schedule and forced swim test (FST) in an IFN-α-induced depression model. Male Wistar rats (3mth, 350-400g, n=8 per group) were injected with recombinant human IFN-α (170,000 IU s.c., 3 times per week for 4 weeks). nor-BNI (10 mg/kg, s.c.) was tested in the progressive ratio schedule of reinforcement (operant responses required to dispense a sucrose pellet reward) at 30 min and 24 h. nor-BNI was tested in the FST, a measure of behavioral despair, at 24 h. The effects of acute administration of nor-BNI were measured on brain-derived neurotrophic factor (BDNF) levels in prefrontal cortex, hippocampus and nucleus accumbens. IFN-α treatment increased breaking point (maximum responses per reinforcement), while reducing nose-pokes in the progressive ratio schedule compared to saline treated controls, indicating habitual lever pressing behavior, and not goal-directed behavior, in this cohort. Immobility score in the FST was increased following IFN-α treatment in comparison to saline treated controls. nor-BNI administration reduced breaking point in IFN-α treated animals when tested at 24 h compared to saline treated controls. nor-BNI administration significantly reduced immobility score in IFN-α treated animals compared to saline treated controls, without affecting

locomotor activity. nor-BNI prevented IFN-α-induced reductions in BDNF levels in prefrontal cortex, hippocampus and nucleus accumbens. These data indicate kappa opioid receptors mediate, at least in-part, depressive-like behavior in the IFN-α-induced depression model and provide more evidence that KOR antagonists could potentially be used as novel antidepressants.

Disclosures: J. Rouine: None. **C.K. Callaghan:** None. **S.M. O'Mara:** None.

Poster

073. New Molecular Targets in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: CIHR MOP142308

Title: Dissecting the contribution of intracellular estrogen receptor subtypes to chronic stress resilience in female mice

Authors: *R. MAHMOUD, J. A. CHAITON, C. CHOW, S. E. LIEBLICH, L. A. M. GALEA; Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Chronic stress has profound effects on the brain, is intimately linked to the development of cognitive deficits, and is associated with an increased vulnerability for neuropsychiatric disorders such as depression and anxiety. Prior research indicates that females may be more resilient to some of the deleterious effects of chronic stress, and that estradiol may at least partially afford this resilience. However, the molecular mechanisms through which estradiol may impart resilience against chronic stress are poorly understood, in part due to the complexity of estradiol signaling. The aim of this study was to investigate the contribution of estrogen receptor (ER) subtypes to estradiol-mediated resiliency, using the Chronic Unpredictable Stress (CUS) rodent model. Adult female mice (C57BL/6) were ovariectomized or sham operated, and chronically treated with the ERα-selective agonist propylpyrazole-triol (PPT), ERβ-selective agonist diarylpropionitrile (DPN), estradiol (E2), or vehicle (Oil). Mice from each treatment group were assigned to CUS or non-CUS conditions. All mice were assessed for depressive- and anxiety-like behaviour (forced swim test, tail suspension test, sucrose preference test, and novelty suppressed feeding), hippocampal neurogenesis, neuroinflammatory markers (cytokine levels and microglial activation), and neuroendocrine function (dexamethasone suppression test). Preliminary results suggest that the ERβ-selective agonist DPN may alleviate depressive-like behaviour in the tail suspension test. Thus, estrogen

receptor subtypes may differentially modulate the effects of estradiol in the context of chronic stress, shedding light on the potential cellular mechanisms of estradiol-mediated stress resilience.

Disclosures: R. Mahmoud: None. **J.A. Chaiton:** None. **C. Chow:** None. **S.E. Lieblich:** None. **L.A.M. Galea:** None.

Poster

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Hope for Depression Research Foundation

Title: Esr1 is an upstream regulator of pro-resilient transcriptional changes in mouse models of depression

Authors: *Z. S. LORSCH, R. BAGOT, I. PURUSHOTHAMAN, D. WALKER, O. ISSLER, B. LABONTÉ, H. KRONMAN, P. J. HAMILTON, C. J. PEÑA, M. E. CAHILL, L. SHEN, E. J. NESTLER;
Neurosci. and Friedman Brain Inst., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Numerous studies have identified wide-ranging transcriptional changes associated with Major Depressive Disorder in humans and depression-like behavior in animal models. However, analysis of the mechanisms by which these multiple transcriptional changes occur has been limited. In this study, we performed upstream regulator analysis on RNA sequencing data from male mice following ten days of Chronic Social Defeat Stress (CSDS). We identified Esr1 as one of the strongest predicted upstream regulators of pro-resilient transcriptional changes in the nucleus accumbens (NAc). Accordingly, we found that Esr1 mRNA levels were decreased in the NAc of susceptible, but not resilient, mice. Esr1, which encodes the nuclear estrogen receptor-alpha, has been shown to play a role in development of the dopaminergic reward system but its specific involvement in depression is unknown. To test the putative role of Esr1 in depression, we overexpressed Esr1 in the NAc and exposed mice to established stress paradigms. Overexpression of Esr1 reduced depression-like behavior in both male and female mice. Further,

overexpression of Esr1 was associated with unique transcriptional changes in both stressed mice and unstressed controls. As a whole, these findings suggest that Esr1 is an upstream regulator of pro-resilient transcriptional changes in the NAc with significant implications for depression-like behavior.

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Poster

073. New Molecular Targets in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH Grant MH100583

NIH Grant MH076929

Title: Loss of SIRT1 in hippocampal and cortical glutamatergic neurons leads to depressive-like behaviors and hypoexcitability of pyramidal neurons

Authors: *Y. LEI, J. J. YOU, J. G. WANG, X. Y. LU;
Dept. of Pharmacol., UT Hlth. Sci. Ctr. At San Antonio, San Antonio, TX

Abstract: SIRT1 is a key regulator of cellular metabolism. It has been recently identified as a genetic marker linked to major depressive disorder. However, the underlying neural mechanisms are largely unknown. In this study, we investigated the effects of SIRT1 in forebrain glutamatergic neurons on depression-related behaviors. Both male and female mice lacking SIRT1 in hippocampal and cortical glutamatergic neurons showed normal growth and locomotor activity. Male SIRT1 conditional knockout mice displayed behavioral despair in the forced swim test and anhedonia in the saccharin preference test without affecting anxiety behavior. In contrast, female SIRT1 conditional knockout mice didn't exhibit significant behavioral phenotype in the same behavioral tests. Whole-cell patch-clamp recordings were made from layer V pyramidal neurons located in the prelimbic and infralimbic areas of the medial prefrontal cortex (mPFC) and CA1 pyramidal neurons in the ventral hippocampus. We found that intrinsic excitability of pyramidal neurons were decreased in a region-specific manner in SIRT1 conditional knockout mice. These results suggest that SIRT1 in mPFC and hippocampal

glutamatergic neurons is involved in the modulation of depressive-like behaviors and is essential for normal neuronal excitability.

Disclosures: **Y. Lei:** None. **J.J. You:** None. **J.G. Wang:** None. **X.Y. Lu:** None.

Poster

073. New Molecular Targets in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH Grant R001MH094405-01

Title: Cell-type specific actions of SIRT1 in the nucleus accumbens mediate depression-like behaviors

Authors: ***H.-D. KIM**, J. HESTERMAN, T. CALL, S. CAROTENUTO, K. ARMENTA, D. FERGUSON;
Basic Med. Sci., The Univ. of Arizona Col. of Med., Phoenix, AZ

Abstract: Major depressive disorder (MDD) is a leading cause of disability and 20% of individuals will suffer from a major onset of clinical depression during their lifetime. The nucleus accumbens (NAc), a key region in the reward circuitry, integrates rewarding and aversive information from various brain regions, thus the NAc is thought to contribute to the pathophysiology and symptomatology of depression. A majority of the neurons in the NAc are GABAergic medium spiny neurons (MSNs) which are primarily composed of two subtypes enriched with dopaminergic D1- or D2-type receptors, but the distinct roles of these two populations in depression are still elusive. Recently, it has been demonstrated that *Sirt1*, a well-characterized deacetylase, is involved in addiction and depression-like symptoms and that *Sirt1* expression is increased in the NAc of depressed (susceptible) mice. To dissect cell-type specific functions of SIRT1 in the NAc, we used chronic social defeat stress to model depression, combined with a Cre-inducible viral vector system and SIRT1 conditional knock-out mice. We found that selective modulation of *Sirt1* expression in a specific subtype of MSNs differentially regulates anxiety- and depression-like behaviors. Indeed, overexpression of *Sirt1* in D1-MSNs, but not in D2, significantly promotes depression-like behaviors. Electrophysiological recordings also demonstrate that SIRT1 differentially modulates neurophysiological response of the MSNs subpopulations in the NAc. Based on our morphological analysis and cell-type specific transcriptomics using RiboTag mice, we believe that changes in neuronal structure and *Sirt1* downstream gene signaling network might be involved in this modulation mechanisms. Taken

together, we demonstrate that *Sirt1* signaling differentially regulates depression-like behaviors in a cell-type specific manner, implicating a novel candidate therapeutic target for antidepressants.

Disclosures: H. Kim: None. **J. Hesterman:** None. **T. Call:** None. **S. Carotenuto:** None. **K. Armenta:** None. **D. Ferguson:** None.

Poster

073. New Molecular Targets in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: TonEBP regulates psychotomimetic behaviors of mice through modulating brain dopaminergic neurotransmission

Authors: *S. KIM[1], H. PARK[2], S. PARK[1], Y. KIM[1], H. KWON[3];
[1]Dongguk Univ. Intl. Hosp., Ilsandong-Gu, Goyang-Si, Gyeonggi-Do, Korea, Republic of; [2]Seoul Natl. Univesity, Seoul, Korea, Republic of; [3]Ulsan Natl. Inst. of Sci. and Technol., Ulsan, Korea, Republic of

Abstract: Tonicity enhancer element binding protein (TonEBP), also known as nuclear factor of activated T-cells 5 (NFAT5), is the osmosensitive transcription factor, which plays a crucial role in protection of renal medullary cells against hyperosmotic stress, urinary concentration, the adaptive immune response. TonEBP is expressed abundantly in the brain, while the functions in nervous systems have not been understood yet. We examined the role of TonEBP on the behaviors in terms of mood and psychotomimetic domains and the behavios-related regulatory mechanisms. TonEBP heterozygote (+/-) mice did not show significant differences compared to wild type (WT) mice in forced swim test, sucrose preference test, and open field activity. When chronic unpredictable stress (CUS) has been applied, WT mice demonstrated depression-like behavioral phenotypes, while TonEBP (+/-) mice showed attenuated response to CUS compared to WT. The reponse to cocaine injection was also in TonEBP (+/-) mice compared to WT. While overexpression of TonEBP gene in mice striatum using adenovirus iuduced the increased behavioral response to cocaine. The dopamine level was reduced in the striatum of TonEBP (+/-) mice compare to WT. TonEBP gene knockdown using siRNA showed reduced expression of dopamine decarboxylase and tyrosine hydroxylase genes. The findings suggest the important role of TonEBP gene in dopamine neurotransmission and related psychotomimetic behaviors in the brain.

Disclosures: S. Kim: None. **H. Park:** None. **S. Park:** None. **Y. Kim:** None. **H. Kwon:** None.

Poster

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH Grant R001MH094405-01

Title: Sirt1 mediates depression-like behaviors in the nucleus accumbens

Authors: *T. CALL[1], H.-D. KIM[1], J. HESTERMAN[1], S. MAGAZU[1], E. KEELEY[2], K. ARMENTA[1], R. NEVE[3], E. NESTLER[2], D. FERGUSON[1];
[1]Basic Med. Sci., Univ. of Arizona Col. of Med., Phoenix, AZ; [2]Icahn Sch. of Med. at Mount Sinai, New York, NY; [3]MIT, Cambridge, MA

Abstract: Depression is a recurring and life threatening illness that affects up to 120 million people worldwide. In the present study we show that chronic social defeat stress, an ethologically validated model of depression in mice, increases SIRT1 levels in the nucleus accumbens (NAc), a key brain reward region. Increases in SIRT,1 a well-characterized class III histone deacetylase, after chronic social defeat suggest a role for this enzyme in mediating depression-like behaviors. When resveratrol, a pharmacological activator of SIRT1, was directly infused bilaterally into the NAc, we observed an increase in depression- and anxiety-like behaviors. Conversely, intra-NAc infusions of EX-527, a SIRT1 antagonist, reduced these behaviors; EX-527 also reduced despair-like behaviors in stress-naïve mice. Next, we directly increased SIRT1 levels in the NAc by use of viral-mediated gene transfer and observed an increase in depressive- and anxiety-like behaviors when mice were assessed in the open field, elevated plus maze, and forced swim tests. Using a Cre-inducible viral vector system to selectively overexpress SIRT1 in dopamine D1 or D2 subpopulations of medium spiny neurons (MSNs) in the NAc, we found that SIRT1 promotes depressive-like behaviors only when overexpressed in D1 MSNs, with no effect seen in D2 MSNs. Conversely, selective ablation of SIRT1 in the NAc, using viral-Cre in floxed *Sirt1* mice, resulted in decreased depression- and anxiety-like behaviors. Together, these results demonstrate that SIRT1 plays an essential role in the NAc in regulating mood-related behavioral abnormalities and identifies a novel-signaling pathway for the development of innovative antidepressants to treat major depressive disorders.

Disclosures: **T. Call:** None. **H. Kim:** None. **J. Hesterman:** None. **S. Magazu:** None. **E. Keeley:** None. **K. Armenta:** None. **R. Neve:** None. **E. Nestler:** None. **D. Ferguson:** None.

Poster

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIH Grant T32 ES07051

Title: Role of pde1b in depressive-like behavior: a mouse model

Authors: *J. R. HUFGARD[1], M. T. WILLIAMS[2], C. V. VORHEES[2];
[1]Univ. of Cincinnati Med. Ctr., Cincinnati, OH; [2]Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstract: Depression is one of the most prevalent mental disorders, yet the underlying mechanism remains unknown and current treatments are frequently ineffective. Secondary messengers, such as cAMP and cGMP, are implicated in depression and schizophrenia and are regulated by phosphodiesterases (PDE). There are 11 PDE families each with multiple isoforms. PDE4 inhibitors were efficacious for depression but had excessive side effects. Many of the other isoforms have never been explored for their role in neuropsychiatric disorders. PDE1b is highly expressed in the striatum and hippocampus, regions linked to depression, therefore, we created a Pde1b floxed mouse and bred it to a cytomegalovirus cre line mouse to make a global knockout (KO) mouse. This mouse shows a phenotype that is resistant to the induction of depressive-like behaviors in both the tail suspension test (TST) and the forced swim tests (FST). Immunohistochemistry for RNA and protein has shown that Pde1b is localized to post-synaptic serotonin and dopamine positive cells in the striatum and hippocampus. The cell specificity for the PDE1b phenotype was further explored using pre- and postsynaptic serotonin and dopamine Cre drivers to create serotonin transporter (SERT), dopamine transporter (DAT), and dopamine receptor 1a (Drd1a) specific Pde1b KO animals. The resistance to depressive phenotype was not observed in either the SERT or DAT KO animals; the Drd1a KO mice are being tested. To further explore the mechanism of Pde1b we are using D1 agonists and antagonists directly targeted to the striatum in conjunction to determine the regional specificity of the phenotype. FST and TST are the behavioral readouts along with and changes in cAMP, cGMP, and PKA. (Supported by NIH T32 ES07051)

Disclosures: J.R. Hufgard: None. **M.T. Williams:** None. **C.V. Vorhees:** None.

Poster

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Program#/Poster#: 73.29/XX12

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Morc1, a gene associated with early-life stress and depression, in the rodent brain.

Authors: *N. B. FREUND, A. MUNDORF, D. BEYER;
Universitaetsklinikum Tuebingen, Tuebingen, Germany

Abstract: Recent studies point to an important role of the MORC family CW-type zinc finger 1 (MORC1)-gene in early life stress and depression. A multidimensional, cross-species, epigenetic study revealed MORC1 DNA hypomethylation following early life stress[1]. MORC1 was also significantly associated with major depressive disorder in a gene-based association analysis[1]. This relation of MORC1 and depression was furthermore confirmed in an animal model[2]. Mice with a functional knock-out of MORC1 demonstrated a depressive-like phenotype in several behavioral tests[2]. Despite this clear evidence for a neurobiological function of MORC1, little is known about its function. MORC1 is involved in spermatogenesis and was found in cancer tissue, but its role in the brain is still unknown. In a first step to reveal the neurobiological influence of MORC1 on stress and depression, we started characterizing MORC1 in the rodent brain. Immunohistochemical staining revealed the distribution of MORC1 protein in motoric and somatosentoric areas but also regions involved in mood regulation like nucleus accumbens, hippocampus and amygdala. Quantitative PCR furthermore detected MORC1 mRNA in the medial prefrontal cortex, dorsal raphe nucleus and hippocampus. The distribution within the brain and its structure[3] indicate that MORC1 plays an important role in mood regulation by influencing transcription rates within mood pathways. This ability puts this gene in a position to predispose the brain for the development of major depressive disorder as a result of early life stress. A better understanding of this relationship between stress and MORC1 will improve our general understanding of the connection between stress and the development of major depressive disorder.

1. Nieratschker, V. *et al.* MORC1 exhibits cross-species differential methylation in association with early life stress as well as genome-wide association with MDD. *Transl. Psychiatry* **4,** e429 (2014).
2. Schmidt, M. *et al.* Morc1 knockout evokes a depression-like phenotype in mice. *Behav. Brain Res.* **296,** 7-14 (2016).
3. Li, D.-Q., Nair, S. S. & Kumar, R. The MORC family: new epigenetic regulators of transcription and DNA damage response. *Epigenetics* **8,** 685-693 (2013).

Disclosures: N.B. Freund: None. **A. Mundorf:** None. **D. Beyer:** None.

Poster

073. New Molecular Targets in Animal Models of Depression

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: INCT-CNPq

Title: Susceptibility and resilience to the effects of prolonged social defeat in adolescent male mice: a study of neuronal nitric oxide synthase

Authors: *S. CHIAVEGATTO, L. S. RESENDE, M. N. MUSCARA, S. A. TEIXEIRA, M. R. ARAUJO, P. E. N. S. VASCONCELOS, J. F. S. CARRILLO;
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Abstract: The brain does not reach full maturity until adulthood making adolescents especially vulnerable to the effects of stressors. Exposure to bullying is a risk factor for major depression (MD), however, it is unclear why some individuals are more susceptible than others. Nitric oxide (NO), an important neurotransmitter in the SNC, is mainly synthetized by the neuronal NO synthase (nNOS). Recent studies have shown that paroxetine, a selective serotonin reuptake inhibitor, also inhibits nNOS implicating the NO in the pathophysiology of MD.Thus, our objective was to investigate the effects of chronic social defeat stress (SD) on emotional behaviors, on nNOS protein and gene expression in distinct brain areas, and on systemic metabolism of NO in adolescent male mice. C57BL/6 (30 day-old) were subjected to daily bouts of SD with an aggressive adult male CD-1 mouse for 10 days. Control mice were exposed to the same situation without the aggressive encounter. Twenty-four hours after the last defeat episode, mice were evaluated in the social interaction and sucrose preference (SP) tests. Approximately 60% of adolescents displayed social avoidance ($p<0.0001$) and reduced SP (anhedonia) ($p<0.001$; “susceptible”). The remaining 40% were similar to control group ($p>0.05$; “resilient”). Brain tissues [hippocampus (HC), prefrontal cortex (PFC), dorsal striatum (DS)] and trunk blood were collected for molecular and biochemical analyses. Western blot and RNA-seq tests showed an increase of nNOS protein levels ($p<0.05$) and mRNA ($p<0.01$, FDR=0.06), respectively, in the HC of susceptible mice. In the PFC and DS, no alterations were found ($p>0.05$). Additionally, resilient mice showed increased serum levels of total nitrite and nitrate (NOx) ($p<0.05$). Our data suggest that prolonged psychosocial stress induces depressive and anxiety like-behaviors in adolescent male mice. Moreover, susceptibility to depression, but not resilience, is associated with increase of nNOS gene and protein expression selectively in the HC. Our data indicate an interesting association between hippocampal nNOS and NO systemic metabolism with vulnerability and resilience to depression post-bullying in male adolescents.

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Poster

074. Treatment of Depression: Exercise and Physical Therapies

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Support: NRF Grant 2014R1A2A2A01003079

MHW HI12C0021

Title: Social interaction rescued abnormal mood and attention behaviors caused by acute stress in adolescent mice through ERK1/2 modulation

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Abstract: Multiple stressors are intertwined with work, school and living conditions. Finding reasonable strategy to cope up with stresses has become an important issue to the current society. In the present study, we evaluated the stress-buffering effect of social interactions on a molecular signal related to abnormal behaviors induced by an hour of acute restraint stress in adolescent ICR mice. Interestingly, one hour of restraint stress induced the activation of ERK1/2, which was reduced in the stress group subjected to social interaction with conspecific mice. We also examined the effects of social interaction on behavioral changes induced by restraint stress, assessed through forced swimming test, and Y-maze test. The abnormal behaviors in the stress group were normalized by the addition of social interaction with conspecific mice. To specify the roles of ERK1/2 in these stress-induced abnormal behaviors, we investigated stress-induced behaviors and ERK1/2 level in prefrontal cortex using brain-penetrant ERK1/2 inhibitor, SL327. Fascinatingly, this ERK1/2 inhibitor rescued abnormal attention behavior and mood behavior. These results suggest that social interactions could alleviate the acute restraint stress-induced behavioral abnormalities in mice, as well as the overt ERK1/2 activation in the medial prefrontal cortex. Moreover, ERK1/2 might be an important target in social buffering effects against stress.

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Poster

074. Treatment of Depression: Exercise and Physical Therapies

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Philanthropic support

Title: Repetitive transcranial magnetic stimulation (rTMS) side effect characterization for treatment resistant depression. Non-inferiority rTMS trial

Authors: *C. DOBEK[1], J. THAM[1], C. NORTHCOTT[1], J. DOWNAR[2], Z. J. DASKALAKIS[3], A. DIPINTO[1], R. W. LAM[1], D. M. BLUMBERGER[3], F. VILA-RODRIGUEZ[1];
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Abstract: Introduction rTMS is a non-invasive neurostimulation treatment used for Treatment Resistant Depression (TRD). Conventional 10 Hz treatment (HFL) takes 37 minutes to administer, however a newer 50 Hz intermittent theta burst stimulation (iTBS) takes 3 minutes. This pilot study compares the two excitatory protocols targeting the Left DLPFC, to determine if iTBS is just as effective, and has no difference in the tolerability and safety profile compared to HFL. This study provides an in depth characterization and accurate timing depiction of the side effect profiles for both treatments on a daily basis.**Method** 20 TRD patients were medically screened for history of seizures, neurological insult, among other exclusion criteria prior to entry. Participants received 20-30 treatment sessions and were randomly selected to receive either treatment course. Before each treatment, technicians inquired about hours of sleep, alcohol/substance use, and medications as additional screening for seizure risk factors. Technicians also systematically recorded side effects and adverse events (AE) reported.A daily questionnaire of the following 10-items (pain, tingling, burning, fatigue, nervousness, headache, disturbed visual perception/ sleep/ concentration, visual flash) was completed 1) immediately post-treatment and 2) emailed home to be completed before the next treatment. **Results** Out of 541 treatments, common side effects include headache (5.2%) and fatigue (0.74%) with majority reporting mild severity. Of 20 participants, 12 reported at least 1 incidence of headache, fatigue (3), or sleep issues (3). Only 2.4% of treatments required an analgesic for headache. Side effects were reported more frequently within the first 10 treatments with a steady decrease over time, and were comparable between treatment conditions. Most side effects reported were self-limited and lasted < 24hrs.Both treatment conditions have similar side effect and AE reporting trajectories with HFL treatment reporting slightly higher rates of disturbed concentration, fatigue, and nervousness (1-2.5 points out of 9 point likert scale). No treatment-related serious

AE or seizure was reported during either treatment. **Conclusion** If iTBS treatment is as effective and safe as the HFL, it will drastically reduce treatment time, increase rTMS clinic capacity, and significantly reduce associated cost. A more cost-effective treatment option is a compelling argument to include rTMS in health insurance coverage. A thorough medical screening process in combination with a standardized pre-treatment risk assessment for rTMS-induced seizures may decrease the risk of seizures and other side effects.

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Poster

074. Treatment of Depression: Exercise and Physical Therapies

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIMH K-23

Title: Magnetic seizure therapy in geriatric depression: neurophysiological characteristics

Authors: *M. LY[1], S. B. ROWNY[2], L. CASAL-ROSCUM[2], J. GOWATSKY[2], P. SEHATPOUR[2], J. PRUDIC[3], D. C. JAVITT[2], C.-M. A. CHEN[1];
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Abstract: Magnetic seizure therapy (MST) is under development as an alternative to conventional electroconvulsive therapy (ECT) for the treatment of severe depression. ECT has long been established as an efficacious treatment for severe depression, but is known for its adverse cognitive effects. Both its efficacy and side effects are influenced by the site of seizure induction and the extent of stimulation, which are difficult to control. MST uses high frequency repetitive transcranial magnetic stimulation (rTMS) to induce seizures, offering better control over the spatial distribution of stimulation and reduced side effects. The neurophysiological characteristics of MST have been studied with two-channel electroencephalogram (EEG) in non-human primates, where the ictal period showed significantly greater power than the two subsequent post-ictal periods for four frequency bands (delta, theta, alpha, beta). Our ongoing trial of MST uses 64-channel EEG recordings of geriatric patients with severe depression who received treatment three times per week for six to 18 sessions. EEG recordings were collected during and immediately after the ictal period for the second and penultimate treatment sessions. Power within delta, theta, alpha, beta, and gamma frequency bands was calculated for ten frontal channels. Preliminary results are consistent with past research in non-human primates. Global power was higher during the ictal period compared to both of the post-ictal periods. This pattern was significant within the theta, alpha, beta, and gamma frequency bands but not for delta, as delta power was highly variable across sessions. Preliminary results also suggest a difference in response over the course of MST treatment, where all frequency bands showed increased power during the ictal period of the penultimate session compared to the second treatment session. To date, this is the first simultaneous high-density EEG-MST study in humans. Our findings of the neurophysiological characteristics of the seizures induced by MST provide insight into mechanisms by which MST may be a safer and more favorable treatment for severe depression in geriatric patients.

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Poster

074. Treatment of Depression: Exercise and Physical Therapies

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Improved outcomes in treatment-resistant depression with concurrent rTMS and CBT

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Abstract: The STAR*D trial demonstrated that only about half of patients suffering from depression are expected to remit entirely with adherence to medication. While perhaps more effective, electroconvulsive therapy (ECT) is not as well-tolerated as repetitive transcranial magnetic stimulation (rTMS). Though cognitive behavioral therapy (CBT) has been demonstrated an effective adjunct therapy with medication, relatively few data have been published on the effectiveness of concurrent rTMS and CBT. The present study investigates the viability this combination.Only patients with treatment-resistant unipolar depression who had failed 4 or more different antidepressant medications were recruited for this open label adjunct TMS study. At this time, 49 patients (35 female, 14 male; 44 Caucasian, 4 African American, 1 Hispanic) were recruited for the study; 34 patients participated in the full 9-week course of 36 rTMS sessions. CBT was offered to all and administered for the vast majority of patients. MADRS, HAM-D, and PHQ-9 scores were recorded during each week as well as during a final follow-up visit 2-4 weeks later. Mental status examination was conducted during each visit. CBT and rTMS treatment modalities were administered by the same mental health nurse practitioner. A Neuronetics NeuroStar TMS machine was utilized to administer left prefrontal rTMS. CBT methods included introduction of the cognitive model, psychoeducation, identification of cognitive distortions and core beliefs, and assistance with dysfunctional thought record worksheets. Preliminary analyses were conducted with Microsoft Excel. Depression scale scores with improvement of < 50% were recorded as “no response”; 50-60% as “response”; and > 60% as “remission.” When results were normalized to the number of participants who successfully followed-up, 26 patients (~75%) were responders and 8 (~25%) were non-responders. Of the responders, 14 patients (42%) demonstrated remission, 12 (34%) met criteria for response only. Since the non-CBT group is small, formal statistics have not been conducted at this time. Limitations of this study include that it is a retrospective analysis of an open-label clinical

treatment sample and there was variability in the length of time that CBT was administered during each treatment session. However, when compared with other results in the literature, which shows average remission/response rates in the 20-30/40-50% range, rTMS + CBT appears to demonstrate greater efficacy than rTMS or CBT alone. Also, this combination therapy may demonstrate efficacy similar to ECT with higher tolerability. Ongoing side-by-side comparisons with other modalities seems merited.

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Poster

074. Treatment of Depression: Exercise and Physical Therapies

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: Temerty Family Grant through the CAMH Foundation

Title: Using seizure adequacy measures to inform clinical decisions in magnetic seizure therapy

Authors: *F. A. BACKHOUSE[1,2], Y. NODA[3], J. DOWNAR[4], Z. J. DASKALAKIS[3], D. M. BLUMBERGER[3];
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Abstract: Major Depression is a debilitating disorder and resistance to pharmacotherapy is common and leads to treatment resistant depression (TRD). The most effective treatment for TRD, 'Electroconvulsive Therapy' (ECT), involves applying a direct electrical current to the brain in order to elicit a therapeutic seizure. The underlying mechanism of how this seizure leads to clinical improvement is not fully understood, but prevailing theories involve mechanisms of neural plasticity. Because of the adverse cognitive side effects of ECT, an alternative convulsive therapy, Magnetic Seizure Therapy (MST), is currently under investigation. Electrophysiological (EEG) recordings of the treatment seizures in ECT have been previously used to predict response to treatment. However, these characteristics have yet to be examined in MST to determine whether they can predict who will respond to treatment. Moreover, the effects of MST frequency on the seizure characteristics have yet to be investigated. We examined EEG seizure characteristics in a sample of depressed subject receiving MST at three separate frequencies: 25

(n=10), 50 (n=10) and 100 (n=10) Hz. The polyspike phase amplitude and duration, slow wave phase amplitude and duration, post-ictal suppression, global seizure strength and symmetry were evaluated across a course of treatment in all subjects. Using a binary logistic regression to predict response, none of the EEG seizure variables were significant $p>.05$. Overall, the average EEG seizure duration significantly decreased from baseline (M=71.54 seconds) to the last treatment (M=36.20 seconds) $t(22)=4.068$, $p<.001$. Slow wave duration also significantly decrease over time from the baseline (M=57.32 seconds) to the final treatment (M=19.23 seconds) $t(18)=2.741$,$p=.013$, likely driving the total decreased duration effect. When analyzing the data by frequency, a non-significant trend in the average polyspike amplitude increased with treatment frequency for the 25 Hz (M=57.69 μV, SD=26.82), 50 (M=65.56 μV, SD=25.72) and 100 Hz (M=64.17 μV, SD=35.02) groups $F(1)=7.332$, $p=.073$. Moreover, suppression ratings showed trend increases with increased frequency $F(1)= 6.795$, $p=.080$. Although none of the variables were significant predictors of response we observed changes in the quality and duration of seizures during a course and across frequencies. Polyspike amplitude and suppression ratings may be significant predictors of response. Future research with a larger sample size is needed to elucidate the role of seizure adequacy in informing treatment decisions with MST.

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Poster

074. Treatment of Depression: Exercise and Physical Therapies

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NARSAD Young Investigator P&S Fund Award 2014 (ID#:22516)

Title: Next-generation sequencing of the rat hippocampal miRNome following chronic electroconvulsive stimulation

Authors: *K. M. RYAN[1,2], P. SMYTH[3], G. BLACKSHIELDS[3], O. SHEILS[3], D. M. MCLOUGHLIN[1,2];
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Abstract: Background: MicroRNAs (miRNAs), a class of endogenous small RNA species, may contribute to the development of depression and its treatment. MiRNAs control about half of all

protein-coding genes in mammals and are found in abundance in the brain where they play a role in a variety of functions such as neurogenesis, cell specification, apoptosis and synaptic plasticity. We previously reported that chronic treatment with electroconvulsive stimulation (ECS), the animal model equivalent of electroconvulsive therapy (ECT), alters expression of miRNAs associated with the neurotrophin BDNF in rat brain and blood. To gain further understanding of the effects of chronic ECS on hippocampal miRNAs, we used the hypothesis-neutral approach of Next-Generation Sequencing here. **Methods:** Male Sprague-Dawley rats (*n*=8 per group) received either "sham" or "real" ECS (100pulses/s; pulse-width=0.5ms; duration=0.7s; 75mA) daily for 10 days. Hippocampal RNA was run on a SOLiD^{TM} sequencing platform. Differential expression analysis was performed using DESeq2. Confirmation and specificity studies were carried out using individual stem-loop primers and quantitative real-time polymerase chain reaction (qRT-PCR). Drosha and Dicer1 mRNA levels were assessed using qRT-PCR. mRNA target and pathway analyses were performed using miRWalk and DAVID. **Results:** DESeq2 analysis identified significant differential expression ($p<0.001$) in six miRNAs in the hippocampus following ECS after correcting for multiple comparisons. qRT-PCR confirmed significant changes in four of the six miRNAs, including miR-212 which we previously reported to be altered by ECS. These changes were confirmed to be hippocampal specific. Hippocampal levels of Drosha and Dicer1, which regulate production of mature miRNAs, were unaltered. Preliminary miRWalk and DAVID analyses predict that these miRNAs target mRNA species involved in inflammatory, glutamatergic, neurotrophic and intracellular signalling pathways, cytoskeletal regulation, axon guidance and long-term potentiation. **Conclusions:** Alterations in miRNA expression may be informative about the mechanism of action of ECS/ECT and in turn may give insight into the neurobiology of depression. MiRNAs have already shown tremendous promise in treating other diseases such as Hepatitis C. Ultimately, a better understanding of the role of miRNAs in depression and its treatments may also lead to novel therapeutic targets.

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Poster

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MEXT/JSPS KAKENHI 16H02892

Title: An open pilot study of non-pharmacological augmentation therapy in major depressive patients using inaudible high-frequency sounds

Authors: ***M. HONDA**[1], R. YAGI[2], N. KAWAI[3], O. UENO[1], Y. YAMASHITA[1], T. OOHASHI[3];
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Abstract: Due to the fact that major depressive disorder (MDD) patients often fail to achieve remission with the initial treatment of antidepressants, in order to improve outcome, a number of augmentation options including non-pharmacological treatments have been used. The aim of the current study is to explore a potential non-pharmacological augmentation option using high-frequency sounds above the human audible range (max. 20 kHz). In the previous studies, we have found that sounds containing inaudible high-frequency components activate the midbrain and diencephalon and evoke various physiological, psychological and behavioral responses, which referred to as hypersonic effect. To test the safety and efficacy of inaudible high-frequency sound on MDD, an open pilot trial was conducted.Ten MDD outpatients diagnosed with the Diagnostic and Statistical Manual for Mental Disorders, fourth edition (DSM-IV) criteria entered an open trial receiving the inaudible high-frequency sound stimulation therapy. Participants received 3-28 sessions of sound stimulation therapy. In each session, during participants sat on a comfortable seat and watched a movie showing natural environment, the tropical rain forest sounds including inaudible high-frequency components were presented for 20-minute. At the same time, spontaneous fluctuations of alpha power in electro encephalogram (EEG) was measured from the occipital regions, which is recognized as an index of the emergence of the hypersonic effect. Efficacy and tolerability were assessed using the State-Trait Anxiety Inventory (STAI), EEG, patients' subjective reports of side effects.Throughout the trial, there was no reports of side effects and a rejection of attribution for any reason, indicating the safety of the inaudible high-frequency sound stimulation. Remarkably, the anxiety scores of STAI were significantly decreased after the sessions. In addition, spontaneous fluctuations of alpha power in EEG was significantly increased, indicating the emergence of the hypersonic effect in MDD patients. Moreover, there was a significant negative correlation between the alpha power and the scores of STAI.These results suggest that the fluctuations of alpha power may be a useful biomarker reflecting anxiety states of MDD patients. Moreover, the current results suggest that the inaudible high-frequency sound stimulation may be a potential non-pharmacological augmentation option for some depressed patients with anxiety. In order to show clinical effect, further controlled trials will be necessary.

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Poster

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Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: DFG Grant KFO 247

BMBF Grant GCBS

Title: Anti-depressant deep brain stimulation - How controlled animal experiments help to improve stimulation protocols in the clinic

Authors: *M. VOGET[1,2], J. RUMMEL[1,2], F. WIESKE[1], R. HADAR[1], S. EWING[1], A. SARTORIUS[3], A. A. MATHÉ[4], B. VOLLMAYR[3], C. WINTER[1];
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Abstract: Deep brain stimulation (DBS) of limbic targets has been advanced as a treatment for otherwise therapy-resistant major depression with initial reports showing promising results. Recently however, major randomized controlled trials fail to replicate these findings, demonstrating a need for refined DBS protocols. While the challenges involved in this line of research must eventually be met at a clinical level, model rodents meeting criteria for construct, face and predictive validity may aid in redefining issues at the proof-of-concept level.
Using the Flinders sensitive line (FSL) and congenital learned helpless (cLH) rats and their respective controls, we investigated the anti-depressant-like effects of DBS to the ventromedial prefrontal cortex (vmPFC, rodent equivalent to the Cg25 region) and the nucleus accumbens (Nacc) applying chronic-intermittent or chronic-continuous stimulation protocols. We found vmPFC-DBS to outperform Nacc-DBS in anti-depressant efficacy. Chronic continuous stimulation did not add further benefit compared to chronic-intermittent DBS, suggesting that continuous stimulation overrides the involved neural network. Further, the otherwise therapy-resistant cLH rats did not benefit from DBS at all.
These data reflect the current clinical literature and are still unsatisfactory. A way to advance from this point could be to focus on individual patient's symptom profiles. This would give guidance as to where, when and how to stimulate most efficiently. Further, in Parkinson research, it has already been shown that adaptive DBS is more effective than conventional continuous DBS. In order to apply adaptive stimulation protocols also in depression, we now need to systematically investigate into neurophysiological and neurochemical biomarkers of the

disorder.
For that purpose, we study electrophysiological and biochemical characteristics of several brain areas in FSL rats using in-vivo local field potential recordings and microdialysis. We are comparing data for baseline pathological states versus conditions under therapeutic doses of fluoxetine. That way identifying specific biomarkers that go along with depressive symptomatology, we will eventually be able to redefine DBS protocols.

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Poster

074. Treatment of Depression: Exercise and Physical Therapies

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Support: AIHS CRIO

Title: Cellular mechanisms of simulated deep brain stimulation (sDBS) in rat infralimbic cortex

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Abstract: Chronic, high frequency (>100 Hz) electrical stimulation, known as deep brain stimulation (DBS), of the subcallosal cingulate gyrus (SCG) including Brodmann area 25 (BA25) may be a promising therapy for treatment-resistant depression. However the neural mechanisms underlying the clinical benefit are unknown. The ventral division of the medial prefrontal cortex (vmPFC), particularly infralimbic cortex (IL), is the homologous region in rat based on anatomical connections and cytoarchitectural features. vmPFC DBS in rats shows antidepressant-like effects in the forced swim test and is used as a model to study mechanisms involved in SCG DBS. Therefore we investigated the cellular mechanisms of simulated DBS (sDBS) in layer 5 IL pyramidal neurons, using *in vitro* whole-cell patch clamp recordings. Single pulse stimulation was used to ensure the synaptic connection between the two sites of electrical stimulation and recording. sDBS applied inside the IL layer 5 induced a prolonged afterdepolarization that was dependent on stimulation frequency and current amplitude (n=14). The high frequencies (>100 Hz) used clinically for SCG DBS and a minimum current intensity of 222.7 ± 13.8 μA could reliably induce these afterdepolarizations. In contrast, sDBS applied in the subcortical white matter, although delivered at a higher intensity of 500.0 ± 42.7 μA, failed to induce any persisting membrane potential changes in layer 5 IL pyramidal neurons (n=5)

using similar parameters. Based on our previous work indicating that sDBS-induced afterdepolarization in basal ganglia involved cholinergic activation of muscarinic receptors, we next tested cholinergic involvement in IL neurons by application of atropine (2.4 μM, non-selective muscarinic receptor antagonist, n=6). Despite significant cholinergic projections from basal forebrain as well as the presence of cholinergic interneurons in IL, cholinergic blockade only abolished the afterdepolarization in 1 neuron, partially blocked it in another neuron, and did not affect afterdepolarization in the 4 remaining neurons. Therefore these preliminary data suggest that (i) sDBS in grey and white matter produce different cellular effects, and (ii) sDBS-induced afterdepolarizations must be mediated by different neuromodulators, in addition to acetylcholine. Key words: DBS, infralimbic cortex, patch clamp, rat. Funding: AIHS CRIO project

Disclosures: F. Luo: None. **Z.H.T. Kiss:** None.

Poster

074. Treatment of Depression: Exercise and Physical Therapies

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 74.10/YY1

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: Mechanical stimulation at PC6 acupoint suppresses stress-induced depressive behavior in rats

Authors: *J.-Y. MOON, S.-Y. KANG, O. KWON, Y.-H. RYU;
Korea Inst. of Oriental Med., Daejeon, Korea, Republic of

Abstract: Acupunture is widely used for treating many psychological disorders such as depression. Previous studies reported that repeated restraint stress induces depressive behavior in rats. Here we examine the effect of mechanical stimulation of PC6 or GV20 acupoint using mechanical acupuncture instrument (MAI) in stress induced depression model. Our results showed that repeated restriantstress (2hr/day, for 14 days) caused significant depressive-like behaviors, such as reduced body weight gain, prolonged duration of immobility in forced swimming test (FST) and decrease in the number of entries into open arms during the elevated pluse maze (EPM) compared to normal rats. In addition, corticosterone levels in plasma were increased and expression of BDNF and phosphorylated CREB (pCREB) were downregulated in hippocampus. After confirming the depressive behavior on 14 days after restraint, we treated machanical stimulation at PC6, GV20 or non-acupoint with MAI 30min prior to repeated restraint stress for 7 days. Daily mechanical stimulation at PC6 acupoint significantly reduced the duration of immobility in the FST and attenuated a decrese in the number of entries into open

arms during the EPM compared to GV20 or non-acupoint stimulation on day21. In addition, acupoint stimulation at PC6 reduced increased corticosterone level in plasma. However, the treatment did not affect decreased BDNF expression or pCREB in hippocampus. These results indicate that repeated stress induces depressive behavior and the acupuncture at PC6 is more effective than GV20 or non-acupont. However, the antidepressive effects are not related to BDNF or pCREB downregulation in hippocampus. Our study supports clinical use of acupuncture depending on the acupoint in depression.

Disclosures: **J. Moon:** None. **S. Kang:** None. **O. Kwon:** None. **Y. Ryu:** None.

Poster

074. Treatment of Depression: Exercise and Physical Therapies

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 74.11/YY2

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Title: The potential protective role of communal nesting on the behavioral consequences of prenatal infection in adult mouse offspring

Authors: ***M. CHAVEZ**[1], H. NORRIS[1], K. L. D'ANNA-HERNANADEZ[2];
[2]Psychology, [1]California State Univ. San Marcos, San Marcos, CA

Abstract: Proper formation of the fetal brain during the prenatal period is important for mental health outcomes in adulthood. In rodent models, prenatal insults such as infection during the second trimester have been linked to the disruption of neural development and alterations in depression and anxiety-like behaviors as well as impaired cognitive function in offspring in a sex-dependent manner. While the prenatal environment can alter fetal development, the postnatal environment has also been observed to affect offspring development and behavior. Communal nesting (CN) is an enriched perinatal environment that increases neurogenesis and promotes sociability in both male and female offspring, but this rearing style differentially affects anxiety- and depression-like behaviors between males and females. Currently, the effect that an enriched perinatal environment has on offspring prenatally exposed to LPS is unknown. The current study was designed assess the potential protective role of CN on male and female offspring prenatally exposed to LPS when tested on measures of anxiety, depression, and memory performance. Prenatal LPS administration was found to alter the number of self-grooming and on nest bouts with LPS exposed pregnant dams self-grooming more and spending more time on nest than saline treated dams. However, total time retrieving pups and the latency to first approach pups was not altered suggesting that prenatal LPS administration on PND 9 does not negatively affect these maternal behaviors in the postnatal period. Offspring behavioral data is forthcoming.

Disclosures: **M. Chavez:** None. **H. Norris:** None. **K.L. D'Anna-Hernanadez:** None.

Poster

074. Treatment of Depression: Exercise and Physical Therapies

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 74.12/YY3

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: OTRES RISE Grant GM-64783

Title: The effects of mild chronic variable stress and hypocretin in lactating dams during the postpartum period

Authors: ***A. J. CASTANEDA**, E. LANE, M. CHAVEZ, H. NORRIS, M. HAMLIN, K. L. D'ANNA-HERNANDEZ;
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Abstract: Postpartum depression is disruptive to maternal care and has negative health consequences for both mothers and infants, but the underlying neuromechanisms of the disorder are not well understood. Hypocretin (HCRT), a hypothalamic excitatory neuropeptide, has been shown to have direct influence separately on stress regulation, maternal care, and depressive-like symptoms in rodents. However, the potential role of hypocretin in postpartum depression and regulation of maternal behavior is not clear. The current study addressed the role of HCRT in the postpartum period in three ways: 1) creation of a chronic variable stress model (CVS) of postpartum depression, 2) antagonism of the HCRT system in the postpartum period and 3) labeling of HCRT-active cells in brain regions related to maternal behavior in response to pup-related stimuli. Results show that dams exposed to CVS postnatal days 1-3 reduced latencies to retrieve pups in a T-maze ($p = 0.041$), and show reduced immobility on the forced swim test ($p = 0.002$) compared to relative controls. However, intraperitoneal injections of a HCRT receptor 1 antagonsist (15 and 30 mg/kg) do not alter depressive-like behaviors on a forced swim test. Lastly, a separate cohort of dams was exposed to pups, pup bedding, no pups or a pup-like item. Brains were then processed for cells double-labeled for cFos and prepro-HCRT and analysis is ongoing. This study will provide information on the potential role of HCRT associated with depressive-like symptoms and maternal care during lactation.

Disclosures: **A.J. Castaneda:** None. **E. Lane:** None. **M. Chavez:** None. **H. Norris:** None. **M. Hamlin:** None. **K.L. D'Anna-Hernandez:** None.

Poster

074. Treatment of Depression: Exercise and Physical Therapies

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 74.13/YY4

Topic: G.04. Mood Disorders: Depression and Bipolar Disorders

Support: NIMH Grant R15 MH101698-01A1

Title: Contingency training influences neurobiological responses to environmental threats and cognitive uncertainty in male and female rats: a potential rat model of behavioral therapy

Authors: *M. H. KENT[1], S. SCOTT[3], S. LAMBERT[4], E. KIRK[2], B. TERHUNE-COTTER[2], B. THOMPSON[2], S. NEAL[2], B. DOZIER[2], M. BARDI[2], K. LAMBERT[2];
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[3]Psychology, Arizona State Univ., Tempe, AZ; [4]Biol., Furman Univ., Greenville, SC

Abstract: Despite the availability of multiple modes of treatment strategies, depression remains one of the most prevalent disorders in the world (Ledford, 2014). The use of cognitive-behavioral therapies produce outcome effectiveness scores ranging from 42-66%, a higher rate than pharmacological approaches (i.e, 22-40% Anthes, 2014). Research with a rat model of behavioral therapy [i.e, effort-based reward (EBR) contingency training] suggests that training focused on building associations between physical effort and desired outcomes enhances neurobiological indices of resilience in male rats (Lambert, 2006; Lambert et al., 2014). In the current study, both male and female Long-Evans rats were exposed to either six weeks of EBR training or non-contingent training prior to 10 days of exposure to chronic unpredictable stress (CUS; n=8 or 9 for each of the four groups). Subsequently, all animals were exposed to a problem-solving task and then trained in a spatial learning/foraging task, the dry land maze (DLM). Following habituation training and test trials, rats experienced a probe trial in which the expected reward was removed to create a prediction error (cognitive uncertainty). Results indicated that, during CUS exposure, contingency-trained males exhibited higher Dehydroepiandrosterone/Corticosterone ratios (indicative of healthier stress responses); an effect not observed in females. Additionally, contingency training increased exploratory and information seeking in the probe trial whereas a *sex x contingency* interaction indicated that contingency training differentially influenced on-task problem-solving performance in males and females. Following the probe trial, brains were exposed to immunocytochemistry to determine the effects of sex and contingency training on fos-, BDNF-, glucocorticoid receptor- and NPY-immunoreactivity in limbic and cortical areas involved in emotional and cognitive responses. Contingency training decreased BDNF-ir in the hippocampus CA1 and lateral habenula, implicating differential neuroplasticity responses in the training groups. Further, coordinated fos-ir between the retrosplenial cortex and several limbic areas (e.g, amygdala) corroborates past

research implicating this area as a modulating area in spatial tasks—emphasizing the value of the DLM probe task (Alexander & Nitz, 2015). In sum, the current findings confirm that behavioral training is associated with sex-specific neurobiological effects; however, further assessments are necessary to more accurately determine the therapeutic potential for the EBR behavioral training/therapy model. Supported by NIMH award 1 R15 MH101698-01A1 to KGL

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Poster

075. Anxiety Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 75.01/YY5

Topic: G.05. Anxiety Disorders

Support: DFG PE2077/3-1

Title: Under watchful eyes: social observation modulates feedback processing in social anxiety disorder

Authors: ***J. PETERBURS**[1], R. VOEGLER[1], C. BELLEBAUM[2], T. STRAUBE[1];
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Abstract: Intense fear of (social) performance situations and negative evaluation is a core symptom of social anxiety disorder (SAD). Evidence for altered feedback processing in SAD has recently been accumulating, yet little is known about the impact of contextual factors. The present study investigated processing of feedback during probabilistic learning in SAD patients (N = 22) and healthy controls (N = 20) using electroencephalography (EEG) and event-related potentials (ERPs). Crucially, subjects performed the task in two contextual conditions, A) under social observation (with an observer present and a camera running), and B) in a control condition with no observation (and the camera switched off).
Results showed no group differences in overall learning rates. Social observation differentially affected patients and controls: social observation increased avoidance learning in controls but not in patients, while patients showed increased avoidance learning in the control condition.
The feedback-related negativity (FRN), an ERP components linked to fast processing of performance feedback, was increased to negative feedback under social observation in patients, and to negative feedback in the control condition for controls. This finding is in line with hypersensitivity to social negative evaluative information in SAD. Interestingly, behavioral

results appear to suggest that social observation may suppress manifestation of this hypersensitivity in overt behavior.
Amplitudes of the P300, an ERP component associated with cognitive appraisal, attention and evaluation, were generally decreased under social observation and increased in response to negative feedback, the latter result likely reflecting more frequent occurrence of positive feedback due to successful learning. Interestingly, patients as compared to controls showed increased P300 amplitudes under social observation only, possibly indicating increased attentional orientation towards and salience of feedback in the presence of an observer in SAD, a notion that is in accordance with SAD psychopathology.
Overall, the present study adds to a growing body of evidence for altered performance monitoring in SAD.

Disclosures: **J. Peterburs:** None. **R. Voegler:** None. **C. Bellebaum:** None. **T. Straube:** None.

Poster

075. Anxiety Disorders

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Program#/Poster#: 75.02/YY6

Topic: G.05. Anxiety Disorders

Support: NIH Grant ZIAMH002798

Title: Impact of anxiety on neural and behavioral responses to incentives

Authors: *B. FUCHS, C. GRILLON, M. ERNST, A. GORKA;
Natl. Inst. of Mental Hlth., NIH, Bethesda, MD

Abstract: Background: Potential rewards and losses motivate behavior and activate reward processing brain regions, including the striatum. Exposure to threat has been shown to reduce neural responses to gain in the striatum (Choi et al., 2014). However, it is unclear how anxiety impacts neural processing of loss. Therefore, this study aims to examine the impact of state anxiety, induced by threat of shock, on neural and behavioral responses to anticipation of gain and loss.
Methods: In an MRI scanner, healthy adults (N=34) performed a timed task. Participants were instructed to respond to a target, presented after either an incentive cue, to gain or avoid losing money, or a neutral cue devoid of monetary incentive. Correct responses depended on the response speed. The task was completed under alternating blocks of threat and safe conditions, where threat was instantiated by administration of an unpredictable mild electric shock to the wrist. Effects of threat and incentive type were assessed using 2x3 repeated measures ANOVAs.

Results: Behaviorally, responses to targets were significantly faster on incentive than neutral trials. Across incentive and neutral trials, reaction times were longer under threat of shock than safety. Neurally, whole brain analyses revealed that, compared to neutral trials, incentive trials were associated with greater BOLD responses in bilateral ventral striatum (VS), ventral tegmental area, and bilateral insula during target anticipation. Threat of shock increased BOLD responses during target anticipation in left caudate and right dorsolateral prefrontal cortex. A priori ROI analyses revealed enhanced left VS activation under threat during gain trials, and to a lesser extent, loss trials.
Conclusions: Threat increased VS responses during all types of trials. This finding is in contrast to prior work (Choi et al., 2014) reporting that threat decreased VS activation to gain. This discrepancy may reflect methodological differences between the two paradigms, such as (1) the inclusion of loss trials in our paradigm; or (2) that the threat in our paradigm was unpredictable rather than predictable. Notably, a priori ROI analyses suggest that unpredictable threat impacts neural responses to the anticipation of gain and loss. Increased activity during the threat condition may reflect the conflict between anxious anticipation and the processing of gains and losses. These interpretations will need to be explored in future work.

Disclosures: **B. Fuchs:** None. **C. Grillon:** None. **M. Ernst:** None. **A. Gorka:** None.

Poster

075. Anxiety Disorders

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Program#/Poster#: 75.03/YY7

Topic: G.05. Anxiety Disorders

Support: NIH Grant ZIAMH002798

Title: Susceptibility to 7.5% CO_2-evoked panic is associated with hypersensitivity to unpredictable threat

Authors: ***J. Y.-T. LIU**, N. L. BALDERSTON, M. ERNST, C. GRILLON;
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Abstract: A core feature of panic disorder (PD) is a heightened anticipatory anxiety, as indexed by anxiety-potentiated startle. However, it is unknown whether this abnormality is specific to the diagnosis of PD or would manifest along a continuum of panic sensitivity (PS). This study examines this question using exposure to carbon dioxide (CO_2) shown in preclinical studies to induce panic symptoms.
The present study investigated associations among PS, as assessed with behavioral and

autonomic responses to CO_2, psychophysiological (startle potentiation) and brain measures of anticipatory anxiety in healthy individuals. We hypothesize that PS is associated with startle potentiation to threat of shock and brain measures of anticipatory anxiety in healthy individuals. Healthy individuals completed a CO_2 challenge consisting of 5 minutes baseline (room air), 8 minutes challenge (7.5% CO_2), and 5 minutes recovery (room air). PS was quantified using physiological measures (N=49) and item-optimized scores of subjective panic symptoms (N=60) during the challenge period. Participants also completed the "NPU threat" task consisting of alternating blocks of safe conditions (no (N) shock) and two threat conditions -predictable (P) shock signaled by a cue and unpredictable (U) shock. Anticipatory anxiety during the threat of predictable (P) and unpredictable (U) shock was compared to safe periods when no (N) shock could be administered in two separate sessions, one to assess startle potentiation (N=60) and the other to investigate brain activity (fMRI: N=46).
Behaviorally, participants displayed increased physiological and subjective symptoms of panic under CO_2 inhalation, as well as increased startle and subjective distress during both threat conditions. Subjects also showed a pattern of brain activity commonly observed in studies of fear and anxiety, including significant threat-evoked activation in the dorsal anterior cingulate cortex, dorsolateral prefrontal cortex, and anterior insula. Importantly, subjective awareness of panic symptoms under CO_2 significantly correlated with subjective anxiety and fear in threat conditions. In addition, fear-potentiated startle significantly correlated with subjective reports of palpitations under CO_2.
Although preliminary, these results suggest a potential link between panic sensitivity and anxious arousal. These results highlight utility of CO_2 inhalation in inducing panic symptoms, and future analyses will explore the link between panic sensitivity and the network underlying fear and anxiety.

Disclosures: **J.Y. Liu:** None. **N.L. Balderston:** None. **M. Ernst:** None. **C. Grillon:** None.

Poster

075. Anxiety Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 75.04/YY8

Topic: G.05. Anxiety Disorders

Support: NIH Grant ZIAMH002798

Title: Threat of shock engages the dlPFC during a wide variety of cognitive paradigms.

Authors: ***N. L. BALDERSTON**, A. HSIUNG, J. LIU, M. ERNST, C. GRILLON;
Natl. Inst. of Mental Hlth., NIH, Bethesda, MD

Abstract: Threat of shock (TOS) is a well validated, translational procedure that reliably increases anxious arousal, causes worry, and affects cognition. In a typical TOS experiment, subjects are exposed to alternating blocks where they are safe or at risk for receiving an unpleasant electrical stimulation. Because the shock can be delivered infrequently, it is possible to conduct a wide variety of tasks during TOS, making it an ideal paradigm to experimentally test the effects of elevated state anxiety on cognitive processes and their underlying neural mechanisms. Resting state studies have shown that TOS changes functional connectivity, in particular by increasing coupling between the amygdala and dmPFC. However, it is unclear how subjects recruit the resources necessary to overcome these network changes when faced with task demands. One possibility is that they recruit prefrontal cognitive control resources when they encounter task-based stimuli under TOS.

We tested this hypothesis across several TOS studies with independent cohorts from ongoing studies. In Study 1 (n = 37), subjects were exposed to blocks of no threat (N), predictable threat (P), and unpredictable threat (U) in the standard NPU task. Simple geometric shapes served as cues, and predicted the shock only in the predictable condition. In Study 2 (n = 36), subjects viewed pictures of common household items during blocks of safety and (unpredictable) threat, and made a simple perceptual judgement. In Study 3 (n = 21), subjects completed a Sternberg working memory (WM) paradigm. On each trial, subjects were presented with a series of letters, and were required to maintain these letters for a brief interval.

During all tasks, we collected multi-echo fMRI data, and processed the data using standard practices. At the single subject level, we conducted the general linear model using the onsets and durations for trials in the individual conditions. We conducted group level ANOVAs to examine the effect of threat on stimulus evoked BOLD activity.

In all studies we found a significant cluster of activity over the right dlPFC as a function of threat. Across studies this region showed increased cue evoked activity during threat blocks relative to safe blocks. Importantly, this region has been repeatedly shown to be involved in both cognitive control, and WM. Taken together, these results suggest that cognitive control processes are engaged by even low load tasks during periods of elevated state anxiety. Additionally, in a previous study we found that anxiety patients show reduced WM-related activity in this region, suggesting that these individuals may have a reduced capacity for engaging these mechanisms.

Disclosures: **N.L. Balderston:** None. **A. Hsiung:** None. **J. Liu:** None. **M. Ernst:** None. **C. Grillon:** None.

Poster

075. Anxiety Disorders

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Program#/Poster#: 75.05/YY9

Topic: G.05. Anxiety Disorders

Support: NIH Grant ZIAMH002798

Title: Anxiety enhances right dorsolateral prefrontal cortex activity during retrieval in behavioral pattern separation paradigm

Authors: *A. HSIUNG, N. BALDERSTON, M. ERNST, C. GRILLON;
Natl. Inst. of Mental Hlth., NIH, Bethesda, MD

Abstract: Forming distinct representations for items, places, and events is crucial for everyday life. This function is commonly impaired in individuals with anxiety disorders, who often overgeneralize details of a fearful situation to a non-fearful situation. Behavioral pattern separation (BPS), the ability to discriminate among items or experiences in episodic memory based on subtle differences, could elucidate possible mechanisms underlying this fear overgeneralization. Previously, we showed that BPS was impaired when items were retrieved under threat of shock, suggesting that fear overgeneralization in anxiety disorders may be linked to impaired BPS; however the neural underpinnings mediating this effect remain unclear. The current study explored changes in neural activity as participants performed BPS under periods of threat and safety. We hypothesized that differences in activation of the hippocampus, particularly in the dentate gyrus, could help explain the effect of anxiety on BPS.

Subjects (n = 36) viewed images of common household items at encoding and retrieval in blocks of safety and threat in a 2 x 2 factorial design during fMRI. In retrieval, participants judged whether the items they saw were new, old, or altered. New items were images not presented during encoding, old items were identical in encoding and retrieval, and altered items were images that had been rotated slightly from encoding to retrieval. To assess BPS and brain activity, we analyzed responses in retrieval as a function of encoding block (threat or safe), retrieval block (threat or safe), and image type (old or altered).

Participants reported greater feelings of anxiety in threat blocks compared to safe for both blocks. Additionally, threat during retrieval increased activity in the left anterior insula and the dorsal anterior cingulate cortex (dACC), across all stimuli. Threat also increased activation in the right dorsolateral prefrontal cortex (dlPFC), in response to altered (but not old) images. No behavioral differences were found across conditions.

Activation of the left anterior insula and dACC as a function of threat replicate previous work suggesting these regions play a vital role in emotional expression. Although we did not see our hypothesized differences in the hippocampus, we saw robust activation in the dlPFC, an area important for cognitive control that is impaired in anxiety patients. This elevated dlPFC activity to altered images may reflect cognitive control processes needed to perform a difficult task during periods of elevated state anxiety. Accordingly, impairment in this skill may explain the fear overgeneralization in anxiety patients.

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Poster

075. Anxiety Disorders

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Program#/Poster#: 75.06/YY10

Topic: G.05. Anxiety Disorders

Support: DFG STR 987/3-2

DFG SFB/ TRR 58: C06, C07

Title: Investigation of grey matter volume in Social Anxiety Disorder: an independent replication

Authors: *M. R. STEFANESCU[1], S. BOEHME[1], T. STRAUBE[2], U. LUEKEN[1];
[1]Dept. of Psychiatry, Psychosomatics and Psychotherapy, Univ. Hosp. Wuerzburg, Wuerzburg, Germany; [2]Inst. of Med. Psychology and Systems Neurosci., Univ. Hosp. Muenster, Muenster, Germany

Abstract: Due to its prevalence, early onset and in its precursor function for subsequent comorbid disorders, social anxiety disorder (SAD) is associated with a high individual burden and socioeconomic costs. Evidence is accumulating on altered frontolimbic circuity function. Compared to functional neuroanatomy, however, brain morphometric alterations are relatively understudied. Moreover, independent replications are missing. The purpose of this study was to investigate gray matter volume changes in two independent German samples. Matched SAD patients and healthy comparison (HC) subjects (Jena group: n = 33 SAD and n = 33 HC; Muenster group: n = 33 SAD and n = 33 HC; 19 women and 15 men in each group) were compared regarding gray matter volumes (GMV) using Voxel-Based Morphometry (VBM12). In the Jena group, SAD patients compared to controls showed a reduction in GMV in the left middle parts of frontal gyrus, precuneus, middle occipital gyrus, parahippocampal and lingual gyrus, inferior parietal lobule and in the right middle temporal gyrus. In the Muenster group, SAD patients compared to controls showed a reduction in GMV in the left superior frontal gyrus, supramarginal gyrus, left middle and superior temporal gyrus, bilateral parts of insula, the right cuneus and medial part of the superior frontal gyrus. Present results point towards reduced gray matter volumes in SAD patients as replicated by an independent site encompassing the left middle and superior frontal gyrus, regions of insula and middle temporal gyrus. In conclusion, findings illustrate gray matter volume reductions in fear circuitry and object processing brain regions as a correlate of SAD that was partly replicated in a second, independent sample.

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Poster

075. Anxiety Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 75.07/YY11

Topic: G.05. Anxiety Disorders

Title: Not everything that looks like change is change: anxious individuals show over-reactivity to uncertainty in a change point detection task

Authors: *H. HUANG, M. PAULUS;
Laureate Inst. for Brain Res., Tulsa, OK

Abstract: Differentiating between fluctuations that occur by chance or by an underlying statistical regularity that signals environment change is an important task that profoundly affects adaptive behavior. It has been shown that anxious individuals may not appropriately differentiate between those situations (Browning et al. 2015). This study examined whether anxiety leads to a non-adaptively increased reactivity by manipulating environmental volatility in a visual search task. 181 subjects (0<= Overall Anxiety Severity and Impairment Scale <=20) participated this study and were divided into Non-anxious group (N = 90, OASIS<9) and Anxious group (N = 91, OASIS>=9). Anxious subjects showed significantly higher lose-shift rate regardless of the underlying probability, i.e. higher lose-shift rate even at the most likely location, while no significant difference in their first choices was found, i.e., percentage of trials that started the searching at the most likely location. Using a dynamic R-W model with a base learning rate and a value-based sensitivity rate, we found that anxious individuals had significantly higher base learning rate and lower sensitivity rate. In addition, base-learning rate positively correlates with lose-shift rate, but not the optimality of the first choices (inverted U shape). Taken together, this indicates anxious subjects are not optimal in two aspects: 1) higher base learning rate caused by over-reactivity to uncertainty, 2) lower sensitivity to true changes in the environment. Thus, clinically anxious individuals non-adaptively respond to random fluctuations and are less able to detect changes in the environmental condition when they - in fact - have occurred.

Disclosures: H. Huang: None. **M. Paulus:** None.

Poster

075. Anxiety Disorders

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Program#/Poster#: 75.08/YY12

Topic: G.07. Other Psychiatric Disorders

Support: CONACYT-Fronteras de la Ciencia 2015-02-1078

Title: Relationship between cholesterol & number of suicide attempts

Authors: *O. H. HERNANDEZ[1,2], B. CARMONA-RUIZ[3], F. A. LEON-CRUZ[3];
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Abstract: Background: Suicidal behavior remains a major challenge for psychiatrists. The WHO estimates that approximately 900,000 people die each year by suicide worldwide. The role of lipids in mental health has aroused the interest of the scientific community, due to its association with the oligomerisation kinetics of synaptic membrane proteins (G protein-coupled receptors) in some psychiatric disorders (depression, schizophrenia). Together with sphingolipids and gangliosides, cholesterol is concentrated in detergent-resistant microdomains of the membrane called lipid rafts, which have been implicated in signal transduction. It has been reported that glutamatergic, gabaergic, dopaminergic, serotoninergic, cholinergic and purinergic neurotransmissions are affected by cholesterol depletion in lipids rafts. Many studies support the association between low serum cholesterol and higher risk of suicidal behavior; however, other studies have found no relationship. In this controversy, the number of suicide attempts has never been considered.
Objective: The aims of this study were to determine whether serum cholesterol and triglycerides are related to the number of suicide attempts; to explore whether the type of attempt (violent, non-violent) affects such association and analyze lipid levels as risk factors for multiple attempts.
Method: This is an observational, transversal, prospective, and analytical study. 81 patients with one or more suicide attempts were included. Socio-demographic, clinical and biochemical variables (cholesterol, triglycerides) were measured. Data were analyzed using descriptive, X^2, odds ratio and Spearman correlations tests. Results: The number of suicide attempts was negatively correlated with levels of cholesterol and triglycerides, but not with BMI, alcoholism, depression, type of attempt or gender. The use of poisoning substances was prevalent in women, but hanging was more common in men. There is a high risk of having multiple attempts in patients with low lipid levels.
Discussion and conclusion: The results support the use of cholesterol and triglycerides as

biological markers of suicidal behavior. It is important to categorize the number of suicide attempts. We are running experiments to test if electrophysiological parameters of brain waves (P100, P200) are related to lipid levels and number of suicide attempts.

Disclosures: O.H. Hernandez: None. **B. Carmona-ruiz:** None. **F.A. Leon-cruz:** None.

Poster

075. Anxiety Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 75.09/YY13

Topic: G.05. Anxiety Disorders

Support: CUNY Collaborative Incentive Research Grant (CIRG)

PSC-CUNY Enhanced Research Award

NIH MBRS-Rise Program at Hunter College Grant# GM060665

Doctoral Student Research Grant, City University of New York: Graduate Center

Title: Increased prefrontal activation to negative stimuli associated with clinical improvement following emotion regulation therapy for generalized anxiety disorder

Authors: *H. A. RAAB[1], C. F. SANDMAN[2], S. H. SEELEY[3], E. GARCÍA-LESY[2,4], D. M. FRESCO[5], D. S. MENNIN[2,4], C. LISTON[6,7,8];
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Abstract: Generalized anxiety disorder (GAD) is characterized by excessive worry, which may result from deficits in adaptively regulating emotions. Emotion regulation therapy (ERT) may be an effective treatment for GAD as it trains the use of cognitive control strategies to modulate emotions. The prefrontal cortex (PFC) plays a critical role in mediating these cognitive processes to down-regulate negative emotion. We aim to elucidate behavioral and neural changes associated with ERT in patients with GAD. Eighteen young adults with GAD (mean age=21.96 years, 15 females) were recruited from the NYC area and participated in 16 weekly sessions of ERT. We assessed neural activity (fMRI), clinician severity, and self-report measures pre- and post-ERT. Functional neuroimaging data was acquired on a Siemens 3T MRI scanner while patients performed an emotion regulation task. During the task they viewed aversive or neutral

images. For each trial, patients were instructed to either “LOOK” at the image as they normally would or “DECREASE” their negative emotion (only for aversive images). Following each image, patients rated the strength of their negative emotion. There was no difference in the emotional intensity ratings for aversive images on “DECREASE” versus “LOOK” trials. This was true both pre- and post-ERT. There was a main effect of treatment for aversive but not neutral images. Following ERT, patients reported significantly lower ratings of negative emotion to aversive images compared to pre-treatment. Accordingly, our fMRI analyses focused on the contrast of aversive versus neutral images. Prior to ERT patients had increased activation for aversive as compared to neutral images in posterior visual processing regions of the brain but no difference in PFC. Following ERT, PFC regions, implicated in emotion regulation, including the inferior frontal gyrus (BA 45 and 46) and middle frontal gyrus (BA 6, 8, 9), as well as fusiform gyrus (all $p<.05$, corrected) had increased activation for aversive as compared to neutral images. Increases in neural activity in BA 9 and 45 from pre- to post-treatment correlated with lower ratings of self-reported anxious arousal and improved attentional control ($p<.05$). These results suggest increased activity in PFC regions following ERT is associated with improvements in clinical severity and emotion regulation abilities in GAD patients. Future research will investigate how changes in brain activity during emotion regulation relate to improvements in clinical severity during a 3-month follow-up period.

Disclosures: H.A. Raab: None. **C.F. Sandman:** None. **S.H. Seeley:** None. **E. García-Lesy:** None. **D.M. Fresco:** None. **D.S. Mennin:** None. **C. Liston:** None.

Poster

075. Anxiety Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 75.10/YY14

Topic: G.07. Other Psychiatric Disorders

Title: Effects of benzodiazepine receptor agonists on pentobarbital-induced sleep latency in lipopolysaccharide-treated mice

Authors: *Y. KITAMURA[1], S. HONGO[1], K. OTSUKI[1], Y. YAMASHITA[1], A. MACHIDA[1], H. KANZAKI[1], I. MIYAZAKI[2], M. ASANUMA[2], T. SENDO[1];
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Abstract: Benzodiazepine receptor agonists are the most frequently administered type of drug for insomnia. The prevalence of insomnia has recently increased. Accordingly, the prescription rate of benzodiazepine receptor agonists has risen. However, benzodiazepine receptor agonists

are known to induce postoperative delirium in surgical patients. Delirium involves an acute change in cognition and psychiatric symptoms. Namely, it can change benzodiazepine receptor function under inflammatory conditions. In this study, we sought to investigate whether benzodiazepine receptor agonists influence pentobarbital-induced sleep latency using a mouse model of lipopolysaccharide (LPS)-induced inflammation. [METHODS] Male ICR mice were administered LPS (300 μg/kg, i.p.). We assessed pentobarbital-induced sleep latency at 24 hours after the LPS treatment. [RESULTS] LPS administration resulted in increased serum levels of tumor necrosis factor-α and interleukin 6 at 1 and 2 hours, but not 24 hours. Benzodiazepine receptor agonists (diazepam and brotizolam) and a gamma-aminobutyric acid $(GABA)_A$ receptor agonist (muscimol) produced significantly greater increases in pentobarbital-induced sleep latency in LPS-treated mice than in saline-treated mice. These effects were blocked by bicuculline, a $GABA_A$ receptor antagonist. Hippocampal $GABA_A$ receptor α3 mRNA expression was significantly increased at 24 hours after the administration of LPS. [DISCUSSION and CONCLUSION] The findings of the current study suggest that benzodiazepine receptor agonists enhance pentobarbital-induced sleep latency to a greater extent in inflammatory conditions than in normal conditions, and these effects might be related to $GABA_A$ receptor expression.

Disclosures: **Y. Kitamura:** None. **S. Hongo:** None. **K. Otsuki:** None. **Y. Yamashita:** None. **A. Machida:** None. **H. Kanzaki:** None. **I. MIyazaki:** None. **M. Asanuma:** None. **T. Sendo:** None.

Poster

075. Anxiety Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 75.11/ZZ1

Topic: G.07. Other Psychiatric Disorders

Title: Are gulf war illness related symptoms unique to gulf war i veterans?

Authors: ***K. LEI**[1], V. METZGER-SMITH[2], J. JAVORS[3], S. GOLSHAN[4], A. LEUNG[5]; [1]Univ. of California San Diego, La Jolla, CA; [2]Veterans Med. Res. Fndn., San Diego, CA; [3]Occup. Med., Veterans Admin. San Diego Healthcare, San Diego, CA; [4]Biostatistics Core, Veteran Admin. San Diego Healthcare Syst., La Jolla, CA; [5]Dept. of Anesthesiol., Univ. of California, Sch. of Medicine, San Diego, San Diego, CA

Abstract: Background: Public interest in Gulf War Veterans (GWV) has continued to rise due to their specific range of neurological and other health related complaints following their return. Now termed Gulf War Illness (GWI), many of these veterans experience pain, fatigue, neurological symptoms, gastrointestinal and skin problems, and respiratory issues. Past studies have suggested that this chronic multi-symptom illness is linked to certain chemical and

environmental exposures (White et al., 2016). Unfortunately, these studies have mainly focused on Gulf War I (GW-I) Veterans even though deployed military personnel from other GW periods exhibit very similar symptoms.
Objective: This study aims to assess the prevalence of GWI symptoms based on different periods of the war and durations of the deployment.
Methods: Information was extracted from physicians' intake note of who applied for the GW Registry at the Veteran Administration San Diego Healthcare System over a period of 24 months. This information was systematically extracted from the computerized patient record system and inputted into a categorized Excel spreadsheet for analysis. Chi-square was used for categorical data and Analysis of Variance was conducted for the continuous outcomes. All analyses were two-tailed, where applicable, with $\alpha = .05$ and Bonferroni for pairwise group comparisons using the SPSS v23.
Results: There were three groups created based on periods of war: Group One (n=164) served from 1990-1991 (GW-I), Group Two (n=156) served after 1991 (GW-II) and Group Three (n=47) served in both GW-I and GW-II. Group One was uniquely (8.5%) exposed to pyridostigmine ($P<0.001$), yet exhibited significantly less pain ($P=0.005$, 70.1% vs. 83.3%) and neurological symptoms ($P=0.013$, 64.0% vs.76.8%) than their counterparts from Group Two.
As the average tour length for GWV is 7 months, they were then categorized based on duration into Group A (≤7 months, n=138), Group B (8-11 months, n=55) and Group C (≥12 months, n=171) of deployment. Significantly ($P<0.001$) more pain symptoms were presented in Group C (86.5%) compared to Group A (68.8%). Group C (36.3%) was also more likely ($P=0.006$) to take nonsteroidal anti-inflammatory drugs (NSAIDs) to manage their pain than Group A (21.7%), as well as take more non-pain or mood related medications overall.
In addition, GWV who had been exposed to toxic exposures (n=40) had more significant ($P=0.042$) memory and attention problems (20.0%) than those who were not exposed (n=327, 9.5%).
Conclusion: The results of this study suggest that the range of symptoms constituting GWI may not be due to the chemical or environmental exposures, but rather the length of their duty.

Disclosures: K. Lei: None. **V. Metzger-Smith:** None. **J. Javors:** None. **S. Golshan:** None. **A. Leung:** None.

Poster

075. Anxiety Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 75.12/ZZ2

Topic: G.05. Anxiety Disorders

Title: Investigation of brain oscillation under academic stress in the classroom setting

Authors: ***S. LIU**[1], L.-W. KO[2], O. KOMAROV[3], C.-T. LIN[4], T.-P. JUNG[5];
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Abstract: Academic stress is considered as a source of negative emotions and commonly affects both students' behavior and cognitive abilities. Several studies have shown that different academic examinations, for example, unexpected or scheduled quizzes, midterms and final exams, can cause psychological consequences, such as an increase in the level of stress-related anxiety. Behavioral studies revealed that anxiety is a common consequence of stress that can lead to the loss of the attention, inefficient time management and lack of productivity. However, there are very few studies that use electroencephalography (EEG) to investigate physiological activities related to the effect of academic stress. This study implements the method of EEG to explore how students perceive and experience academic stress. The aim of this study is to investigate the phenomenon of brain oscillatory correlates of students' stress under naturalistic classroom settings. We recruited 26 college students and collected multiple physiological signals including synchronized recording of EEG, video monitoring, presentation of visual stimuli and collection of the participants' responses. Also the participants were asked to fill in the Depression Anxiety Stress Scales (DASS21) before each experimental session to evaluate their levels of long-term stress and stress-related anxiety. Based on the collected information, we observed correlations between the student's different stress levels, and changes in their EEG recordings. The experiments were performed under two conditions. All 26 students participated in experimental sessions under "non-stress" condition, and then 8 of them volunteered to participate in the "stress" part. The opened-eye resting state EEG data of the "stress" group were recorded during real academic examinations. The "non-stress" condition means that the participants didn't have any examinations during EEG recording. Emphasis were on the changes in brain activity as reaction to specific, real-life stressors. In this study, we processed the opened-eye resting state EEG data and obtained channel-based EEG topography for both "stress" and "non-stress" groups. The results show significant differences between "stress" and "non-stress" conditions. These changes in frontal region are associated with an increase of the theta and beta powers. In conclusion, we finding that frontal area may be predictive of stress hormonal responses.

Disclosures: **S. Liu:** None. **L. Ko:** None. **O. Komarov:** None. **C. Lin:** None. **T. Jung:** None.

Poster

075. Anxiety Disorders

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Program#/Poster#: 75.13/ZZ3

Topic: G.05. Anxiety Disorders

Support: Pilot translational research proposal grant, University of Cincinnati, Department of Psychiatry

NIH grant MH093362

Title: Translational evidence for dysregulation of acid-sensing TDAG8 receptor evoked inflammation in panic disorder

Authors: *L. L. VOLLMER, R. AHLBRAND, J. R. STRAWN, R. SAH;
Psychiatry and Behavioral Neurosci., Univ. Of Cincinnati, Cincinnati, OH

Abstract: Panic Disorder (PD) is a prevalent anxiety disorder characterized by repeated, unexpected attacks of intense fear as well as overwhelming respiratory and cardiovascular symptoms. Despite having a high prevalence and debilitating effects, the neurobiology of PD is still largely unknown. Several lines of evidence suggest that "acidosis" may relate pathoetiologically to panic disorder. CO_2 inhalation, a well-studied panicogen, causes brain acidosis. Recent evidence also shows significant comorbidity of panic disorder with chronic inflammatory conditions, such as arthritis, suggesting a potential role of inflammation in the pathophysiology of PD. Thus, converging homeostatic mechanisms involving acidosis and inflammation may underlie panic pathophysiology. Recently, using a rodent model we reported a role of microglial acid sensing receptor TDAG8 and pro-inflammatory cytokine IL-1β in CO_2-evoked fear (Vollmer et al, *Biological Psychiatry* 2016). Our studies suggest a primary role of innate immune cells in transducing acidosis to panic-associated behavior and physiology. However, the translational relevance of these "bench" findings to humans is not known. Given the potential association of panic disorder with inflammatory conditions we hypothesized that elevated inflammatory tone may be present in panic disorder patients, represented as increased TDAG8 expression as well as sensitized macrophage inflammatory responses. This pilot study evaluated: a) variance in acid sensor TDAG8 expression and acidosis-evoked cytokine release in monocytes from healthy volunteers, b) TDAG8 expression in monocytes collected from PD patients and healthy volunteers, and c) acidosis-evoked release of pro-inflammatory cytokines from macrophages cultured from these individuals. Baseline variance in TDAG8 expression was observed in macrophages from human volunteers without PD. Furthermore, selective acidosis-evoked release of IL-1β in cultured human macrophages was observed in agreement with our rodent data. This underscores the relevance of IL-1β in acidosis-evoked responses in innate immune cells. Ongoing studies are quantifying TDAG8 expression and macrophage pro-

inflammatory responses in patients with PD. Preliminary data indicate a potential age and gender specific changes in TDAG8 expression that is currently being replicated in a larger sample. Collectively, our studies support convergent acidosis-inflammatory mechanisms in panic disorder. TDAG8 associated inflammation may represent a potential marker of prognosis or risk in patients with PD or those at risk for the condition, and may inform novel treatment interventions.

Disclosures: **L.L. Vollmer:** None. **R. Ahlbrand:** None. **J.R. Strawn:** None. **R. Sah:** None.

Poster

075. Anxiety Disorders

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Topic: G.05. Anxiety Disorders

Support: NSF Grant DGE1122492

Title: Neurological predictors of treatment outcome in social anxiety disorder: a Neurosynth-aided review

Authors: ***J. CURTISS**[1], S. M. NOBLE[2];
[1]Clin. Psychology, Boston Univ., Boston, MA; [2]Interdepartmental Neurosci. Program, Yale Univ., New Haven, CT

Abstract: Social anxiety disorder (SAD) is among the most prevalent and debilitating psychiatric disorders (Kessler et al., 2005). Several efficacious treatments exist for SAD, including cognitive behavioral therapy and pharmacological interventions; however, such interventions are only modestly effective (Davidson et al., 2004). As such, there have been recent initiatives to enhance treatment for SAD by leveraging neuroimaging techniques to determine whether neurological markers predict successful remission (Hofmann, 2013). It is particularly important to identify neural predictors of treatment outcome in SAD to improve upon behavioral measures. Extant literature reveals an increase in the use of fMRI to predict treatment outcome for SAD; however, no systematic review has yet been conducted on this bourgeoning body of literature. To address this, we perform the first review of the literature with the aim of pooling evidence of markers of SAD outcome after three commonly-employed interventions: cognitive behavioral therapy (CBT), pharmacotherapy, and mindfulness-based therapies. Using PubMed, a search from the database inception until May 1st 2016 yielded 49 potential studies. Overall, we identified 10 studies relevant to the purpose of the current review. Our results suggest that activity and connectivity in frontal regions and the amygdala are crucial neurological

mechanisms underlying responsivity to the treatment of SAD. Specifically, successful treatment response for SAD was most commonly predicted by decreased activity and connectivity in the salience network (i.e., ACC and insula) and amygdala, as well as increased connectivity between the default mode network (e.g., ventromedial prefrontal cortex) and the amygdala. Several studies also suggest that the occipitotemporal regions may predict treatment response for SAD. We also used Neurosynth, an online aggregator of fMRI studies and coordinate-based meta-analysis tool, to further investigate the role of these regions in SAD. Results of the Neurosynth meta-analysis on 33 studies confirm that functional abnormalities in the amygdala, insula, and ventromedial prefrontal cortex are neural signatures of SAD. These studies attest to the utility of incorporating neuroimaging in the treatment of SAD and afford good impetus to extend this research to other disorders. Furthermore, the incorporation of neuroimaging can refine and optimize decisions about which treatment is most appropriate for a specific individual, which would complement recent initiatives toward personalized medicine.

Disclosures: J. Curtiss: None. **S.M. Noble:** None.

Poster

075. Anxiety Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 75.15/ZZ5

Topic: G.07. Other Psychiatric Disorders

Title: Compulsive behavior of sapap3 mutants in the signal attenuation task

Authors: *I. EHMER[1,2], L. CROWN[1], W. VAN LEEUWEN[1,2], I. WILLUHN[1,2], M. FEENSTRA[1,2], D. DENYS[1,2];
[1]Neuromodulation and Behavior, Netherlands Inst. For Neurosci., Amsterdam, Netherlands;
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Abstract: The neurobiological mechanism of compulsivity in obsessive-compulsive disorder (OCD) is unknown. Various hypotheses have been put forward, one of which is based on the idea that compulsive behavior results from impaired feedback processing (Joel and Avisar, 2001). The signal attenuation (SA) task for rats models this processing deficit by attenuating a feedback stimulus associated with the performance of a normal goal-directed response. Compulsive-like responding following SA is well characterized in normal rats, but has not yet been tested in genetic models of OCD-like behavior, such as the Sapap3 mutant mouse that presents with excessive grooming behavior (Welch et al., 2007). Here, we first demonstrate that the SA task can be implemented in mice. Subsequently, we tested the hypothesis that Sapap3 mutants show enhanced compulsive-like behavior in the SA model. Mice (24 wild-type (WT)

mice; 25 Sapap3 mutants; males, 3-6 months of age) learned that nose pokes lead to the presentation of a food reward and an audiovisual signal providing feedback. To simulate feedback deficiency experimentally, the signal was attenuated by presenting it without the corresponding outcome in half of the animals. Finally, operant responding of mice with and without feedback deficiency was compared in an extinction test. WT mice that underwent SA displayed significantly more uncompleted trials (UCT), extra nose pokes (ENP), and extra nose pokes per uncompleted trials (ENP-UCT) than WT mice that had experienced extinction only, and thus, were considered more compulsive-like. Analysis of Sapap3 mutant responses revealed a significantly higher number of ENP for animals that underwent SA, however, UCT and ENP-UCT did not differ from the regular extinction group. Comparison of the ENP-UCT between WT mice and Sapap3 mutants did not yield significant differences. Instead, Sapap3 mutants displayed significantly fewer completed trials (CT) and more no-poke trials compared to normal WT mice, indicative of enhanced extinction of the operant response. We show that the SA task produce similar behavior in mice as in rats, thereby introducing the opportunity to assess compulsive-like behavior due to defective feedback processing in various genetic mouse models. Contrary to our hypothesis, we found no evidence for increased compulsive-like responding or impaired extinction learning in Sapap3 mutants. This suggests that feedback processing during operant conditioning is not impaired in Sapap3 mice.

Disclosures: I. Ehmer: None. **L. Crown:** None. **W. van Leeuwen:** None. **I. Willuhn:** None. **M. Feenstra:** None. **D. Denys:** None.

Poster

075. Anxiety Disorders

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Topic: G.07. Other Psychiatric Disorders

Support: NIH Grant 5R21MH104829-02

Brain Research Foundation

Title: Identifying mechanisms underlying behavioral effects of putative OCD risk gene BTBD3

Authors: *S. L. THOMPSON[1,2], E. V. HO[1], M. E. KLINGER[2], M. J. RAMAKER[1], A. HAJEISSA[3], W. R. KATZKA[1], J. A. KNOWLES[4], S. C. DULAWA[1];
[1]UC San Diego, San Diego, CA; [2]Univ. of Chicago, Chicago, IL; [3]Univ. of Michigan, Ann Arbor, MI; [4]USC, Los Angeles, CA

Abstract: The first genome-wide association study for Obsessive-Compulsive Disorder (OCD) identified one genome-wide significant single nucleotide polymorphism (SNP), rs6131295, in the trio subset of the sample. This SNP regulates expression of the BTBD3 gene, which encodes a transcription factor implicated in activity-dependent dendritic patterning during development. However, nothing is known about the role of BTBD3 in regulating behavior. We have identified several behavioral deficits in BTBD3 KO mice that may be relevant to OCD and work toward identifying the mechanisms underlying these behaviors. Male and female BTBD3 wild-type (WT), heterozygous (HT) and knockout (KO) mice were assessed in the open field, dig test, and splash test. In addition, mice were assessed weekly for barbering. Behavioral deficits were found in all of these paradigms. Together, these phenotypes indicate hyperactivity, reduced exploratory drive, and perseverative/impulsive behavior. BTBD3 mice were next assessed for effects of OCD-effective and OCD-ineffective treatment on these behavioral deficits. Mice were pretreated with 10 mg/kg/day fluoxetine (OCD-effective), 20 mg/kg/day desipramine (OCD-ineffective), or vehicle in the drinking water for fourteen weeks. After four weeks of drug treatment, mice were assessed in the same tests as above. Barbering was assessed weekly throughout treatment. Fluoxetine reduced barbering in WT and HT but not KO mice starting at four weeks of treatment, whereas desipramine had no effect. This data indicate an interaction between BTBD3 expression and serotonin signaling and provides predictive validity for barbering as an OCD-like behavior. BTBD3 mice were then assessed for basic sensory and motor function. BTBD3 KO mice had no impairments in olfactory dis/habituation, olfactory memory, grip strength, or gait, but did exhibit excessive wheel-running in the home cage and impaired nest building. These data indicate that BTBD3 KO mice do not exhibit gross sensory or motor impairments that may confer the other behavioral deficits identified. To identify brain regions requiring BTBD3 expression for normal behavior, BTBD3 expression was knocked down in juvenile mouse hippocampus using an adeno-associated virus expressing shRNA against BTBD3. Knockdown mice were assessed in the open field and nest building test during adulthood. Knockdown mice built worse nests and trended toward hyperactivity in the open field, recapitulating two phenotypes found in the global knockout. These data indicate that BTBD3 expression in hippocampus may play a role in the mechanism underlying effects of BTBD3 on behavior.

Disclosures: **S.L. Thompson:** None. **E.V. Ho:** None. **M.E. Klinger:** None. **M.J. Ramaker:** None. **A. Hajeissa:** None. **W.R. Katzka:** None. **J.A. Knowles:** None. **S.C. Dulawa:** None.

Poster

075. Anxiety Disorders

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Topic: G.07. Other Psychiatric Disorders

Support: TSA Grant

AFSGT Grant

Title: Juvenile onset of stereotypy with loss of BDNF signaling in D1R expressing striatal neurons

Authors: *M. ENGELN[1], R. CHANDRA[1], A. LA[2], S. HAIDER[3], T.-C. FRANCIS[1], M.-K. LOBO[1];
[1]Anat. and Neurobio., Univ. of Maryland Baltimore, Baltimore, MD; [2]Univ. of Maryland, Col. Park, College Park, MD; [3]Johns Hopkins Univ., Baltimore, MD

Abstract: Imbalance between D1- vs. D2-receptor containing medium spiny neuron (MSN) basal ganglia output-pathways is implicated in stereotyped disorders including Tourette Syndrome (TS). Surprisingly, there is little information on the molecular role of MSN subtypes in TS or other stereotypy disorders. We have a mouse model with a deletion of TrkB (the BDNF receptor) in D1-MSNs (D1-Cre-flTrkB mice), in which a subset of mice display involuntary stereotypic behaviors beginning around 3 weeks of age. Consistent with an impaired GABAergic system in TS, these mice display a decrease in striatal GABA-A subunits accompanied by reduced inhibition in striatal D1-MSNs. We first characterized repetitive behaviors in D1-Cre-flTrkB mice with stereotypy (S), or with no stereotypy (NS), and D1-Cre control mice. Complete turns, head tics, rearing, and grooming are assessed weekly from ages 3 to 8 weeks. We found that D1-Cre-flTrkB-S mice display more complete turns at all ages compared to D1-Cre-flTrkB-NS and control mice. D1-Cre-flTrkB-S mice exclusively display head tics, which decline from juvenile to adult ages. We then selectively inhibited dorsal striatum D1-MSNs using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs; AAV-DIO-HM4(Gi)-mCherry) to evaluate if abnormal D1-MSN activity is responsible for stereotypic behaviors. Our preliminary data suggests that selective inhibition of D1-MSNs with DREADDs reduces circling behavior. Finally, since the transcription factor, early growth response 3 (Egr3) is regulated by BDNF and Egr3 transcriptionally regulates a subset of GABA-A subunits, we are examining Egr3 mRNA in all groups. We find that both Egr3 mRNA and protein levels are decreased in the striatum of D1-Cre-flTrkB mice. To investigate if this change is specific to D1-Cre-flTrkB-S mice we are using D1-Cre-flTrkB-RiboTag mice to measure Egr3 levels selectively in D1-MSNs. We also performed chromatin immunoprecipitation to examine Egr3 transcriptional regulation of GABA-A subunits in these mice. Finally, to determine if restoring Egr3 levels to D1-MSNs can rescue the stereotypy behaviors we injected AAV-DIO-Egr3-eYFP into the dorsal striatum of D1-Cre-flTrkB mice. Our findings demonstrate that D1-MSNs, through dysfunctional BDNF signaling, play a role in juvenile onset of stereotypy behaviors. The enhanced stereotypy behaviors potentially occur through reduced inhibition in D1-MSNs via altered Egr3 regulation of GABA-A subunits. Our ongoing studies can provide novel insight into the cell subtypes and molecular mechanisms underlying stereotypy disorders.

Disclosures: **M. Engeln:** None. **R. Chandra:** None. **A. La:** None. **S. Haider:** None. **T. Francis:** None. **M. Lobo:** None.

Poster

075. Anxiety Disorders

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Program#/Poster#: 75.18/ZZ8

Topic: C.03. Parkinson's Disease

Support: NIH F31 NS092190

NIH R01 NS089470

Title: Striatal D1-medium spiny neurons in L-Dopa-induced dyskinesias

Authors: *S. L. ALBERICO[1], T. LENCE[1], Y. KIM[2], N. NARAYANAN[2];
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Abstract: A major limitation in treating Parkinson's disease (PD) is the development of L-Dopa-induced dyskinesias (LID), which are disruptive involuntary movements that lead to a decreased quality of life in PD patients. Prior research demonstrates that mice lacking the D1-dopamine receptor (D1DR) do not develop LID. Here, we injected the D1DR antagonist SCH23390 (1 mg/kg, ip) and investigated changes in activity of medium spiny neurons (MSN) in the striatum of a 6-OHDA PD mouse model following LID development. We found that administration of SCH23390 modulated striatal activity and decreased some subtypes of dyskinesias. We studied this issue using computer-assisted tracking of LID. We further studied the role of the striatal D1DRs in D1-Cre mice by optogenetically inhibiting or stimulating D1DR expressing MSN in the striatum while simultaneously recording striatal activity during LID. We also investigated how activity of MSN related to striatal field potentials. These results may elucidate how D1DR expressing neurons in the striatum dysfunction during LID and further the understanding of the mechanism underlying LID.

Disclosures: S.L. Alberico: None. **T. Lence:** None. **Y. Kim:** None. **N. Narayanan:** None.

Poster

075. Anxiety Disorders

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Program#/Poster#: 75.19/ZZ9

Topic: G.07. Other Psychiatric Disorders

Support: Wellcome trust

Title: Functional shift in noradrenergic control over behaviour between the development and the expression of compulsivity

Authors: *A. BELIN-RAUSCENT[1], S. A. TORRISI[1], B. J. EVERITT[2], D. BELIN[1];
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Abstract: The neural basis of individual vulnerability to compulsive disorders remains largely unknown. Whereas most research on compulsivity has hitherto focused on dopaminergic or serotoninergic mechanisms, there is increasing evidence that noradrenergic processes influence impulse control and contribute to the development of impulsive/compulsive behaviours. Thus, in rats, atomoxetine, a selective noradrenergic reuptake inhibitor, decreases trait high impulsivity and the associated vulnerability compulsively to relapse to cocaine seeking. Atomoxetine also prevents, in highly impulsive rats, the development of an excessive, maladaptive and compulsive adjunctive behaviour as measured under a schedule-induced polydipsia procedure (SIP).
While the effect of atomoxetine on high impulsivity has been shown to depend upon noradrenergic mechanisms in the shell of the nucleus accumbens (AcbS), the precise neural locus of this noradrenergic control over the development, and expression of compulsive behaviour remains unknown.
Having demonstrated that chronic atomoxetine treatment prevents the development of compulsivity in the SIP procedure in highly impulsive rats known to be more vulnerable to develop compulsivity than low impulsive rats, we investigated whether atomoxetine could also influence the well-established expression of compulsivity.
Sprague Dawley rats were characterized as high or low compulsive based upon their water intake after 21 days of training under SIP. Rats then received daily intraperitoneal injections of either vehicle or atomoxetine for 3 weeks. In marked contrast to its influence on the development of compulsivity, atomoxetine potentiated the well-established expression of SIP in high compulsive rats only.
These results show that atomoxetine exerts opposite influence on the development and the expression of compulsivity, preventing the former while enhancing the later. This suggests that a shift in the nature of the noradrenergic control over behaviour occurs over the course of the development of compulsivity. Noradrenaline may therefore be involved in the switch from impulsivity to compulsivity.

Disclosures: **A. Belin-Rauscent:** None. **S.A. Torrisi:** None. **B.J. Everitt:** None. **D. Belin:** None.

Poster

075. Anxiety Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 75.20/ZZ10

Topic: G.07. Other Psychiatric Disorders

Support: Pinsent Darwin PhD Studentship in Mental Pathology

Wellcome Trust Senior Investigator Award (104631/Z/14/Z) awarded to T.W. Robbins.

Title: Evidence of maladaptive goal-directed system in obsessive-compulsive disorder (OCD) as indexed by abnormal instrumental contingency

Authors: *M. M. VAGHI[1,2], A. M. APERGIS-SCHOUTE[3,2], A. SULE[4], N. A. FINEBERG[5], R. N. CARDINAL[3,2], T. W. ROBBINS[1,2];
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Abstract: Obsessive-compulsive disorder (OCD) is regarded as a prototypical disorder of compulsivity in which actions persist despite being inappropriate to the situation and without relationship to the overall goal. To test this hypothesis formally, we used contingency degradation, a procedure devised to test whether instrumental behavior is guided by knowledge of the relationship between an action and its consequences. In this classic free-operant behavioral assay, the relationship between the action and its associated outcome is weakened by the additional delivery of non-contingent outcomes. Across species, action response rate declines selectively under contingency degradation when the outcome is no longer associated with that action. In humans a similar effect has been shown not only for action response rate by also for causal judgements. Moreover, the influence of contingency degradation is mediated by a cortico-striatal brain system, including the caudate nucleus, that has been shown to be abnormal in patients with OCD. We tested a sample of healthy volunteers and patients with OCD using a contingency degradation task in which we manipulated P (outcome | action) and P (outcome | no action) to produce different levels of positive, degraded, and negative contingency (when action is disadvantageous as it prevents the outcome from occurring). Both response rates and explicit causal judgements showed a systematic decline as objective contingency decreased. There was a significant interaction between contingency and group in the extent to which explicit causal judgements predicted response rates, suggesting that OCD patients were less aware of whether an action was advantageous or disadvantageous. In addition, for positive contingencies, there

was a trend for response rate to be less sensitive to contingency degradation in OCD patients. These data lend further support to the hypothesis that abnormal goal-directed regulation and more specifically altered instrumental contingency learning play a role in sustaining maladaptive OCD psychopathology.

Disclosures: **M.M. Vaghi:** None. **A.M. Apergis-Schoute:** None. **A. Sule:** None. **N.A. Fineberg:** None. **R.N. Cardinal:** None. **T.W. Robbins:** None.

Poster

075. Anxiety Disorders

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Topic: G.07. Other Psychiatric Disorders

Support: NIH Grant RO1MH104255

Title: Disruption of cognitive flexibility in models with disturbances in cortico-striatal circuit function: relevance to OCD

Authors: *E. E. MANNING[1], J. SHEN[1,2], B. BIZUP[1], M. TORREGROSSA[1], S. E. AHMARI[1]; [1]Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA; [2]Tsinghua Univ., Beijing, China

Abstract: Background: Obsessive Compulsive Disorder (OCD) is a chronic, severe mental illness, but despite its severity and prevalence, the pathophysiology remains unclear. Functional imaging studies in patients demonstrate increased activity in cortico-striato-thalamo-cortical (CSTC) circuits after symptom provocation; and we recently demonstrated that brief but repeated orbitofrontal cortex (OFC)-ventromedial striatum (VMS) optogenetic hyperstimulation can induce OCD-like increased grooming in mice (Ahmari et al., *Science* 2013). Studies in transgenic models [such as the SAPAP3 knockout (KO) mouse] also support the role of CSTC disturbances in the emergence of compulsive grooming. Disruption of flexible cognition plays a critical role in the symptoms observed in OCD, and yet the effects of OCD-relevant changes in CSTC function have not been addressed in relevant animal models. The current study characterizes cognitive flexibility in an operant reversal learning task in two independent animal models of OCD-relevant CSTC dysfunction; OFC-VMS hyperstimulation and SAPAP3 KO mice.
Methods: To generate OFC-VMS hyperstimulation, mice were injected with AAV-channelrhodopsin-EYFP or AAV-EYFP in the OFC, and implanted with fiberoptics in the VMS. Following at least 7 days of daily stimulation [5 minutes, 473nm, 1-10mW, 10Hz, 10msec pulse width], food restricted mice were tested on discrimination and reversal learning. Testing

occurred in operant chambers with two levers, and mice were first trained to press one lever to receive a food reward; after several days of training the contingency was reversed. Separate cohorts of SAPAP3 KO and wild-type (WT) littermates were tested in operant chambers under similar conditions. Data were analyzed using repeated-measures ANOVAs and post-hoc tests ($\alpha = 0.05$).
Results: mOFC-VMS stimulated mice and SAPAP3 KO mice showed a similar preference for the active lever to control groups during the discrimination phase. In contrast, both models showed impairments in cognitive flexibility following contingency reversal.
Conclusions: CSTC disruption is associated with impairments in flexible behaviour in a reversal learning paradigm. Further comparison of the neural mechanisms underlying impaired reversal in these two models may shed light on neural mechanisms underlying perseverative behavior and disruption of flexible cognition in OCD. Improved understanding of the neural mechanisms underlying disruption of cognition in OCD, using circuit-specific tools, may provide insight regarding new treatment targets for the disorder.

Disclosures: E.E. Manning: None. **J. Shen:** None. **B. Bizup:** None. **M. Torregrossa:** None. **S.E. Ahmari:** None.

Poster

075. Anxiety Disorders

Location: Halls B-H

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Program#/Poster#: 75.22/ZZ12

Topic: G.07. Other Psychiatric Disorders

Title: Voxel-wise predictors of response to stereotactic capsulotomy for OCD

Authors: *P. NANDA[1], G. P. BANKS[2], Y. PATHAK[2], D. L. PAULO[2], G. HORGA[2], M. Q. HOEXTER[3], Z. XU[4], A. C. LOPES[3], N. C. MCLAUGHLIN[5], B. GREENBERG[5], J. P. SHEEHAN[4], E. C. MIGUEL[3], A. A. GORGULHO[3], A. A. DE SALLES[3], E. T. FONOFF[3], S. A. RASMUSSEN[5], S. A. SHETH[2];
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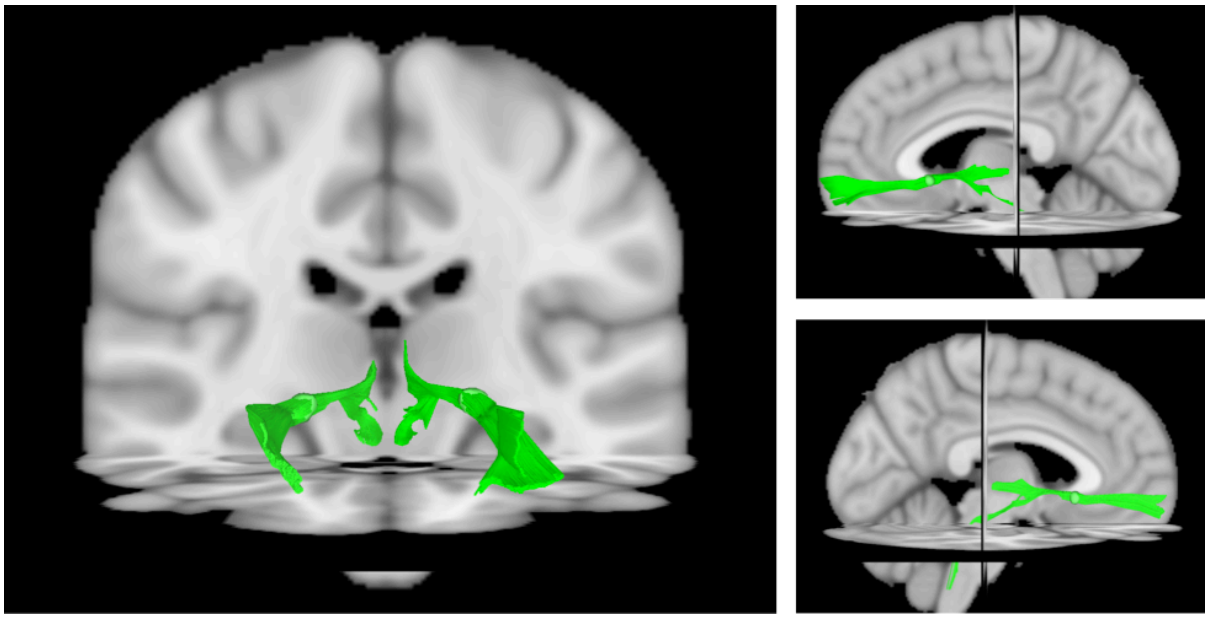
Abstract: Introduction: Whereas most patients with obsessive-compulsive disorder (OCD) are well controlled with pharmacological and behavioral therapy, 10-20% remain severe and refractory. Lesions in the anterior limb of the internal capsule (ALIC) have been used for decades to treat these patients. Recent stereotactic radiosurgical (SRS) studies have demonstrated significant symptom response in 30-70% of patients. One factor in response variability may be differences in brain networks affected by lesions. We used voxel-based statistics and diffusion

tensor imaging to identify successful targets and examine involved networks.
Methods: We analyzed postoperative volumetric T1 MRIs from 21 patients with refractory OCD who underwent SRS capsulotomy at 2 institutions. Data were analyzed using a voxel-based generalized linear model to identify an area predicting clinical response. Using an atlas from diffusion data of 842 Human Connectome Project controls, we identified fiber-tracts crossing this significantly predictive area ("predictive ROI").
Results: The statistical model identified right and left ALIC regions significantly differentiating responders from non-responders. The predictive ROI exhibited connections with orbitofrontal cortex (OFC) and thalamus.
Conclusions: SRS capsulotomy remains attractive for severe, refractory OCD. This analysis shows that lesions in right and left ALIC will more likely produce clinical response. Lesions here modulate a network connecting OFC and thalamus, supporting the hypothesis that aberrant frontal cortico-basal-thalamo-cortical positive feedback loops may underlie OCD. Prospective longitudinal imaging collection will help validate our understanding of affected networks, permit individualized analyses, and improve this approach's efficacy.
Figure: Tractography from predictive ROI



Disclosures: **P. Nanda:** None. **G.P. Banks:** None. **Y. Pathak:** None. **D.L. Paulo:** None. **G. Horga:** None. **M.Q. Hoexter:** None. **Z. Xu:** None. **A.C. Lopes:** None. **N.C. McLaughlin:** None. **B. Greenberg:** None. **J.P. Sheehan:** None. **E.C. Miguel:** None. **A.A. Gorgulho:** None. **A.A. De Salles:** None. **E.T. Fonoff:** None. **S.A. Rasmussen:** None. **S.A. Sheth:** None.

Poster

075. Anxiety Disorders

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Topic: G.07. Other Psychiatric Disorders

Support: Norman Prince Neuroscience Institute Postdoctoral Fellowship to TKJ

NSF IOB-1146334 to RDB

Title: Acquisition and extinction of avoidance behaviors: The role of context

Authors: *T. K. JACOBSON[1], J. R. PHILLIPS[1], R. D. BURWELL[1,2];
[1]CLPS, [2]Dept. of Neurosci., Brown Univ., Providence, RI

Abstract: Avoidance is a hallmark feature of many syndromes of pathological anxiety, including obsessive-compulsive disorder (OCD) and post-traumatic stress disorder (PTSD). Animal models of these syndromes typically use classical conditioning paradigms (i.e. fear conditioning in rats), but there are limitations to these approaches. Notably, most fear-conditioning studies focus on passive and reflexive defensive behaviors, such as freezing, and ignore active defensive behaviors, (i.e. active avoidance) evident in OCD and PTSD. Also, few studies attempt to model the role of context in the pathology and treatment of these disorders. The use of context to guide cognition and behavior is disrupted in PTSD and OCD, and the primary treatments for OCD and PTSD are forms of exposure therapy that are highly context dependent. Exposure to trigger stimuli outside the therapy context results in relapse and the return of pathological avoidance behaviors. Available evidence suggests that different neural circuits modulate active and passive defensive behaviors. Both circuits involve prefrontal regions and the periaqueductal gray. Passive behaviors (freezing) are supported by a prefrontal->amygdala->ventrolateral PAG pathway, whereas active behaviors (avoidance) are supported by a prefrontal->ventral striatum->dorsolateral PAG (dlPAG) pathway. Importantly, both pathways include connections with parahippocampal cortex (PHC), which is critical for representing spatial context. PHC and its rodent homolog, the postrhinal cortex (POR), have reciprocal connections with prefrontal regions and project to both striatum and the central nucleus of the amygdala. We have developed a novel paradigm, the Contextual Approach/Avoidance Task (CAAT), which is motivated by this circuitry and addresses limitations of existing models. CAAT models conditioning to anxiety-provoking stimuli, active avoidance, relapse (both renewal and reinstatement), and the context-dependency of extinction. Briefly, rats are implanted with stimulation electrodes in the dlPAG. Stimulation of the dlPAG causes unpleasant, anxiety-provoking sensations. After recovery rats are shaped on the CAAT. Unpleasant anxiety-like sensations are conditioned to the tone with several Tone->dlPAG stimulation pairings. Next rats are trained in avoidance learning. During a trial, the tone and a visual cue on the floor are presented simultaneously. Rats learn to avoid

dlPAG stimulation by approaching the cue. We will present data on acquisition, extinction, and renewal in the CAAT task. Strategies for minimizing the context-dependency of extinction in this model of anxiety disorders will be discussed.

Disclosures: **T.K. Jacobson:** None. **J.R. Phillips:** None. **R.D. Burwell:** None.

Poster

075. Anxiety Disorders

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Topic: G.07. Other Psychiatric Disorders

Support: Undergraduate Research Opportunities Program Wayne State University Research Funding

Title: Exploring activation-connectivity relationships in Obsessive-Compulsive Disorder: Conjoint assessment of task-based activation profiles and resting state functional connectivity

Authors: *H. PAREKH[1], K. RAMASESHAN[2], A. BURGESS[2], G. HANNA[3], P. ARNOLD[4], D. ROSENBERG[2], V. DIWADKAR[2];
[1]Wayne State Univ., Sterling Heights, MI; [2]Wayne State Univ., Detroit, MI; [3]Univ. of Michigan, Ann Arbor, MI; [4]Univ. of Calgary, Calgary, AB, Canada

Abstract: Background: OCD are characterized by dysfunctional activation profiles in frontal, striatal and thalamic regions when basic working memory-related processing is induced (Diwadkar et al., 2015). The dynamic properties of regional activations stand distinctly from independent assessment of resting state signals (e.g., Anticevic et al., 2014). These latter studies suggest that OCD patients are characterized by a mix of hypo- and hyper-connectivity in cortical, striatal and cerebellar networks. The relationship between task-based activation profiles and resting state functional connectivity (rsFC) within subjects is unclear. Exploring these relationships within subjects may illuminate understanding of the mechanisms of pathology in both the task-active and task-free states.
Methods: 17 healthy controls and 22 OCD subjects underwent fMRI (3T Siemens Verio) during which they performed a standard verbal working memory paradigm (0-back and 1-back conditions: 30s epochs, Rest: 20s epochs). In the same imaging session, resting state (rs) fMRI data were collected in the same subjects (eyes closed). Task based data were processed using typical methods (SPM8). First level activation maps (1-Back > 0-Back) were submitted to second level random effects analysis to identify group differences in activation (HC ≠ OCD). rsfMRI images were separately preprocessed (Whitfield-Gabrieli & Nieto-Castanon, 2012) using

typical methods.
Results: Activation analyses identified regions of hyper- and hypo-activity in OCD. Hyper-activity was observed in the parietal lobe and dorsal anterior cingulate cortex (dACC); hypo-activity in the parietal lobe, basal ganglia, middle frontal gyrus (middle FG), dorsal prefrontal cortex (dPFC), and thalamus. Significance peaks ($p < .05$) for each contrast were identified as separate networks of inquiry for rsfMRI connectivity. Within network pairs, we assessed significant differences between groups (HC ≠ OCD, $p<.05$, FDR). Notably, for each of the task-based networks assessed (hyper- and hypo-active), no differences in rsFC were observed.
Conclusion: Activity differences that exist between OCD and HC may index dynamic pathology in brain function. However, our results indicate that the relationship between task-induced activation and rsFC between the identified pathological nodes is at best uncertain. More assessment will be needed to elucidate relationships between task-active and resting FC (Deco et al., 2013), and their relevance for OCD associated brain network dysfunction.

Disclosures: H. Parekh: None. **K. Ramaseshan:** None. **A. Burgess:** None. **G. Hanna:** None. **P. Arnold:** None. **D. Rosenberg:** None. **V. Diwadkar:** None.

Poster

075. Anxiety Disorders

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Topic: G.07. Other Psychiatric Disorders

Support: NIH R00HD065832

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NIH U54EB020406

Title: Influence of Tourette Syndrome associated SNP on cortical thickness and behavior in children and young adults

Authors: *T. K. HSU, A. KRAFNICK, A. TOGA, K. CLARK;
USC Mark and Mary Stevens Neuroimaging and Informatics Inst., Keck USC Sch. of Med., Los Angeles, CA

Abstract: Background: Tourette syndrome (TS) is a neurologic developmental disorder with one of the highest familial recurrence rates among neuropsychiatric diseases with complex

inheritance pattern. A genome-wide association study (GWAS) study found multiple genetic markers with increased susceptibility to developing TS. The single nucleotide polymorphism (SNP) with the highest signal was rs7868992 which occurs in COL27A1, a fibrillary collagen gene located on chromosome 9q32-33. We undertook the current study to find correlations between the genetics, neuroanatomy and behavior. **Method**: Using neuroimaging and genetic data from the Pediatric Imaging, Neurocognition and Genetics Study (PING) from 409 subjects (209 M/ 200 F; ages 8-21), cortical thickness variations with genotype for SNP rs7868992 (COL27A1) was investigated using FreeSurfer with group analysis. Post-hoc correlation with cognitive abilities as measured by the NIH toolbox Flanker and Dimensional Change Card Sort (DCCS) test were used to examine relevant behavior within regions identified by the genotype analysis. **Results**: Significant variations with genotype was found for rs7868992 (COL27A1) within females in both the left and right hemispheres. In the left lingual and parsorbitalis regions significant differences were found between GA and AA genotypes ($p<0.01$, cluster extent corrected). In the right superiorparietal and lateraloccipital regions significant differences were found for GG and AA ($p<0.01$). In the right post central gyrus regions significant differences were noted for GG and GA as well ($p<0.01$). Significant correlation between cortical thickness and behavioral scores within the female population, specifically, an inverse relationship between lingual and pars orbitalis cortical thickness and behavioral scores. **Conclusion**: The data show that genetic variation in COL27A1 are related to significant changes in cortical thickness and behavioral scores in regions that may be relevant to the development of Tourette Syndrome. The effect was specific to females and further investigations are warranted to ascertain the cause.

Disclosures: T.K. Hsu: None. **A. Krafnick:** None. **A. Toga:** None. **K. Clark:** None.

Poster

075. Anxiety Disorders

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 75.26/AAA2

Topic: G.07. Other Psychiatric Disorders

Title: Attenuation of compulsivelike behavior through positive allosteric modulation of alpha4beta2 nicotinic acetylcholine receptors in noninduced compulsive-like mice.

Authors: *S. MITRA[1], M. MUCHA[2], S. N. KHATRI[4], R. GLENNON[6], M. K. SCHULTE[5], A. BULT-ITO[3];
[1]Dept. of Chem. Biochem., [2]Dept. of chemistry Biochem., [3]Biol. and Wild life, Univ. of Alaska Fairbanks, Fairbanks, AK; [4]Dept. of Pharmaceut. Sci., [5]Univ. of the Sci. Philadelphia, Philadelphia, PA; [6]Dept. of Medicinal Chem., Virginia Commonwealth Univ., Richmond, VA

Abstract: *Rationale:* Nicotinic *alpha4beta2* receptors are the most abundant subtypes of nicotinic acetylcholine receptors (nAChRs) expressed in brain regions implicated in OCD. These receptors are known to modify normal and addictive behaviors by modulating neuronal excitability. Desformylflustrabromine (dFBr) is a novel, positive allosteric modulator (PAM) of HS (high acetylcholine sensitivity) and LS (low acetylcholine sensitivity) alpha4beta2 nAChRs. The present study tested the hypothesis that positive allosteric modulation of alpha4beta2 receptors by dFBr will attenuate compulsive-like behavior in a non-induced compulsive-like mouse model. *Methods:* Male mice (*Mus musculus*) selected for compulsivelike nesting behavior (48 animals; 12 per group) received a subcutaneous injection of dFBr at 2, 4 and 6 mg/kg doses. Saline was used as a control (0 mg/kg). Compulsive-like nesting behavior was assessed after 1, 2, 3, 4, 5 and 24 hours, while marble burying was performed 2 hours after dFBr administration. Anxietylike behaviors were determined by the open field test 2 hours after dFBr administration.*Results:* dFBr dose dependently attenuated compulsive-like nesting and marble burying behavior. No effects of dFBr were observed for anxietylike open field behaviors. *Conclusions:* This research demonstrates a novel therapeutic target for OCD and OCD spectrum disorders through positive allosteric modulation of alpha4beta2 nicotinic receptors.

Disclosures: S. Mitra: None. **M. Mucha:** None. **S.N. Khatri:** None. **R. Glennon:** None. **M.K. Schulte:** None. **A. Bult-Ito:** None.

Poster

076. Addictive Drugs and Development

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 76.01/AAA3

Topic: G.08. Drugs of Abuse and Addiction

Title: Consideration of hemispheric asymmetry in addiction research

Authors: *H. W. GORDON;
Clin. Neurosci. Br., Natl. Inst. Drug Abuse, Bethesda, MD

Abstract: In a previously-published report (Gordon, 2016), it was demonstrated that two risk factors for addiction differentially activated the right and left hemispheres of the brain: impulsivity (assessed by response inhibition) activated areas in the right hemisphere; craving activated the left. This report extends these observations by demonstrating differential structural or activation effects on the right or left hemisphere by prenatal exposure to drugs of abuse or by ingestion by individuals themselves. Data were extracted from journal articles compiled from PubMed searches by pairing each drug of abuse with key words such as MRI or laterality. Then the brain structures and locations (i.e., right, left, bilateral) were tabulated. For prenatal exposure

across all substances (cocaine, nicotine, alcohol, cannabis, opiates), there were nearly 3 times as many unilateral (right or left) structures specifically mentioned than for (presumably equivalent) bilateral structures. There were nearly 4 times as many mentioned in individuals who were taking these drugs. However, contrary to the activation studies of impulsivity and craving, and in spite of the fact that unilateral structures were mentioned, the effects do not appear to favor one hemisphere more than the other. The most common anatomical effect for both prenatal exposure and/or adolescent or adult drug use was reduced gray matter volume or reduced cortical thickness, although for a few studies of prenatal alcohol exposure, thicker cortices were reported. Among individuals taking drugs, thinning or reduced volume seemed to affect the left cingulate and superior frontal gyrus more than the right, but affected the right orbital and medial frontal cortices more than the left. Functional imaging (BOLD signals) was also affected by prenatal exposure usually in either right or, more often, left sites. For the most part, there was increased activation, though reduced activation was also reported. The significance of these observations is that drugs of abuse affecting individuals exposed either prenatally or as adolescents or adults seem to target individual structures in either the right or left hemisphere. Why this is the case is unknown. Perhaps there is differential brain development or neurotransmitter activity. Up to now, not much attention has been paid to these asymmetries. Consideration of them may give clues as to the basis of drug abuse and its effect on the brain. [Gordon, HW, Laterality of brain activation for risk factors of addiction. Current Drug Abuse Reviews, 9:1-18; 2016]

Disclosures: H.W. Gordon: None.

Poster

076. Addictive Drugs and Development

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 76.02/AAA4

Topic: G.08. Drugs of Abuse and Addiction

Support: RISE GM-64783

MARC GM-08807

Title: Repeated administration of ketamine in adolescence produces affective changes that last into adulthood.

Authors: *T. ZAFAR, A. ROCHA, T. TOWNER, K. A. TRUJILLO;
Psychology, California State Univ. San Marcos, San Marcos, CA

Abstract: Ketamine (KET) was originally developed for clinical use as an anesthetic. Known as a 'dissociative drug,' it is popularly abused, especially by teens and young adults in club and rave settings. Ketamine produces out of body experience, cognitive disorganization, and hallucinations, as well as transient symptoms that mimic both positive and negative symptoms of schizophrenia. Moreover, because of its rewarding effects and broad availability, KET is often used by teens for recreational purposes. Behavioral sensitization is an increase in an effect of a drug after repeated adminstration. Also referred to as "reverse tolerance," sensitization is thought to be involved in the development of addiction. While there are reports of behavioral sensitization to ketamine in adult rats, little has been done to elucidate sensitization in adolescents, and potential differences in sensitization between adults and adolescents. The present study examined the locomotor effects of KET following repeated administration to adolescent and adult Sprague-Dawley rats. We hypothesized that ketamine would induce an increase in locomotor activity across days reflective of behavioral sensitization and that adolescent rats would differ in sensitization when compared to adults. Furthermore, we sought to determine if sensitization would persist into adulthood across two different behavioral paradigms. Animals received saline or KET (10 mg/kg s.c), once daily for seven days and locomotor activity was assessed each day to follow the development of locomotor sensitization. After a washout period in which the adolescent rats developed into adulthood, animals were given an injection of ketamine and ultrasonic vocalizations (USVs) were measured to assess the impact of early exposure on the rewarding effects of the drug. On day 1 of treatment, ketamine (10.0 mg/kg) induced a short-lived stimulant effect that was considerably greater in adolescents than adults. In addition, although both groups showed evidence of sensitization, the increase in response to ketamine across days was greater in adolescents than adults. After the washout period, the group that received repeated ketamine as adolescents showed the greatest number of USVs in response to ketamine, reflective of sensitization to the rewarding effect of the drug. Thus, while repeat administration of KET leads to behavioral sensitization, we found that this sensitization differs in adults and adolescents. Further research will help to determine the factors involved in KET sensitization in adults and adolescents, and may lead to better prevention and treatment for KET abuse and addiction in teenagers.

Disclosures: **T. Zafar:** None. **A. Rocha:** None. **T. Towner:** None. **K.A. Trujillo:** None.

Poster

076. Addictive Drugs and Development

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Program#/Poster#: 76.03/AAA5

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant T34GM008074

NIH Grant 8UL1GM118979-02

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NIH Grant 8RL5GM118978-02

Title: Oxycodone reward in male and female adolescent rats: effects of dose

Authors: *N. SOLLENBERGER[1], A. MANOOGIAN[2], H. K. PARK[2], A. R. ZAVALA[2]; [1]Psychology, California State University, Long Beach, Lakewood, CA; [2]Psychology, California State University, Long Beach, Long Beach, CA

Abstract: Oxycodone abuse among adolescents has increased in recent years. Surprisingly, little preclinical research has examined the rewarding effects of oxycodone in male and female adolescent rats. To this end, we examined the rewarding effects of oxycodone in adolescent rats using the established conditioned place preference (CPP) paradigm. Male and female rats were assessed for oxycodone-induced CPP using an 11-day CPP procedure beginning on postnatal day (PD) 40. During pre-conditioning and post-conditioning sessions, rats were tested for their baseline and final place preference, respectively, in 15-min sessions. During conditioning (PD 42-47), rats underwent daily 30-min sessions, during which they received alternating oxycodone (0, .033, 0.1, 0.3, 0.9 mg/kg) and saline injections in distinct compartments. Results indicated that regardless of sex, rats showed a significant shift towards the oxycodone-paired compartment at the higher doses, suggesting that the CPP model may be useful for understanding the neurobiology of oxycodone abuse in adolescence.

Disclosures: N. Sollenberger: None. **A. Manoogian:** None. **H.K. Park:** None. **A.R. Zavala:** None.

Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH R01 DA034185

Title: Early-life experience decreases opioid self-administration in adulthood through anti-inflammatory IL-10 signaling in nucleus accumbens

Authors: *M. J. LACAGNINA[1], A. M. KOPEC[1], S. S. COX[1], C. WELLS[2], S. SLADE[2], P. M. GRACE[3], L. R. WATKINS[3], E. D. LEVIN[2], S. D. BILBO[1];
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Abstract: Opioid drug abuse is a serious public health concern with few effective therapeutic strategies. There is evidence that opioids can activate glial cells within the nucleus accumbens (NAc), and the subsequent pro-inflammatory cytokine and chemokine response may contribute to drug reward and relapse. We have previously shown that a neonatal handling procedure in rats (which promotes enriched maternal care) reduces glial activation in response to an acute morphine challenge and attenuates morphine conditioned place preference. We hypothesize that these responses may be driven in part by increased expression of the anti-inflammatory cytokine interleukin-10 (IL-10). Here, we employed a model of opioid self-administration to interrogate the effects of early-life experience on later-life drug taking for male Sprague-Dawley rats. Neonatal handling attenuated intravenous self-administration of remifentanil (a short-acting synthetic opioid) in a dose-dependent manner, as handled rats self-administered less remifentanil by the final day of drug acquisition. Transcriptional profiling revealed a unique suppression of chemokine signaling molecules in the NAc of handled rats. To determine if anti-inflammatory IL-10 could explain this effect, we delivered bilateral intracranial injections of naked plasmid DNA encoding IL-10 (pDNA-IL-10) or a control plasmid along with the transgene adjuvant D-mannose into the NAc of naïve rats. Treatment with pDNA-IL-10 reduced the number of remifentanil infusions acquired in a dose-dependent fashion, an effect similar to handled rats. Importantly, neither handling nor pDNA-IL-10 treatment altered operant responding for food or sucrose pellets compared to controls. These collective observations suggest that neuroimmune signaling in the NAc can be influenced by early-life experience, and these signaling mechanisms may be important in the development of self-motivated opioid drug acquisition. In addition, these results demonstrate that *in vivo* manipulation of anti-inflammatory IL-10 represents a promising target for future investigations.

Disclosures: M.J. Lacagnina: None. **A.M. Kopec:** None. **S.S. Cox:** None. **C. Wells:** None. **S. Slade:** None. **P.M. Grace:** None. **L.R. Watkins:** None. **E.D. Levin:** None. **S.D. Bilbo:** None.

Poster

076. Addictive Drugs and Development

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 76.05/AAA7

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA (DA027525)

Title: The effects of prenatal stress on cocaine reward, cocaine locomotion and sensorimotor processing are heritable in the BXD recombinant inbred strain panel

Authors: *J. R. BAGLEY, R. BOZADJIAN, L. BUBALO, K. L. PLOENSE, P. A. VIEIRA, T. E. KIPPIN;
UC Santa Barbara, Santa Barbara, CA

Abstract: Early life stress has been implicated in a number of psychiatric conditions, including addiction and schizophrenia, and appears to be involved in complex gene X environment interaction leading to pathology. To dissect this interaction, we have been examining the impact of prenatal stress (PNS) on cocaine-seeking/locomotion and sensorimotor processing in mice with different genetic backgrounds. Previously, we found that PNS in C57BL/6 (B6) but not DBA/2 (D2) increased the magnitude of cocaine-induced conditioned place preference (CPP) and cocaine induced locomotion whereas PNS in D2, but not B6, impaired pre-pulse inhibition (PPI) of acoustic startle. Identification of the alleles mediating these interactions is an important step in understanding the underlying neurobiology. In pursuit of this goal, we have characterized the effects of PNS on BXD recombinant inbred strains. These strains possess unique combinations of B6 and D2 alleles and may allow for identification of quantitative trait loci (QTLs) that mediate the effects of PNS. **Methods.** BXD strains were subject to timed mating followed by assignment to PNS and control groups. PNS dams were placed in a restraint stress protocol (1 hour restraint, 3 times daily) starting between embryonic day (E) 11 and 14 and continued until parturition. PNS may affect developmental outcomes by altering maternal behavior in the post-natal period. Accordingly, the frequency of pup-dam contact was measured in the first 10 post-natal days. Adult control and PNS offspring (8 to 9 weeks) were tested in a PPI procedure (110 dB startle, 74 and 90 dB PPI) followed by cocaine (10 mg/kg, I.P.) CPP. **Results.** We have found PNS by strain interactions, indicating the effects of PNS are heritable in the BXD panel. Specifically, PNS interacts with strain to alter cocaine acute locomotion, locomotion sensitization and CPP, acoustic startle response and PPI. PNS interacts with strain to alter pup-dam contact, with the most frequent effect being a reduction in contact. Interestingly, the PNS strain effect on maternal behavior correlates with the PNS strain effects on cocaine reward/locomotion, but not with startle or PPI, indicating the effects of PNS on cocaine related behaviors may be mediated by changes in maternal behavior in the post-natal period. Overall, these results suggest that the effects of PNS on cocaine reward/locomotion, sensorimotor processing and maternal stress response are heritable in the BXD panel. Future work will seek to identify QTLs, and ultimately polymorphisms, that mediate the effects of PNS. This research may serve to elucidate gene X environment interactions that lead to the development of psychiatric disorders.

Disclosures: J.R. Bagley: None. **R. Bozadjian:** None. **L. Bubalo:** None. **K.L. Ploense:** None. **P.A. Vieira:** None. **T.E. Kippin:** None.

Poster

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NIH grant 8RL5GM118978-02

Title: One-trial methamphetamine-induced locomotor sensitization in male and female adolescent rats

Authors: *B. SALINAS, E. BATES, B. SCHUESSLER, A. SU, L. TRAN, A. R. ZAVALA; California State University, Long Beach, Long Beach, CA

Abstract: One-trial locomotor sensitization is the process by which a single exposure to a drug leads to a progressively higher locomotor behavioral response during a subsequent drug exposure. In adult rats, the context, or the environment where the initial drug administration took place, plays a key role in the development of this phenomenon. One-trial sensitization in young rats (postnatal days (PD) 21 or younger) is characteristically different. For instance, young rats typically demonstrate one-trial sensitization to cocaine and methamphetamine (METH), but the sensitization is context-independent (i.e., rats exhibit sensitization regardless of where the drug was given). Few studies have examined one-trial sensitization in adolescent rats. Thus the present study examined one-trial sensitization in adolescent rats to determine the ontogeny of context-dependent METH sensitization. Adolescent (PD 48) male and female rats were pretreated with saline or 3.0 mg/kg METH (IP) and immediately placed in a novel test chamber where locomotor activity was measured for 60 min. To assess for context-dependent sensitization, rats that were previously given saline in the novel chamber were injected with saline or 3.0 mg/kg METH (IP) in their home cage 45 min after being returned from the novel chamber. In contrast, rats given METH in the novel chamber were injected with saline in the home cage. The next day (i.e., PD 49), rats from each group were given a challenge injection of METH (0, 0.15, or 0.3 mg/kg, IP) in the novel test chamber and their locomotor activity was assessed for 90 min. Results show that adolescent female rats largely exhibited a weaker sensitized behavioral response after administration of METH that was primarily expressed during the first 30 min of testing. In contrast, adolescent male rats pretreated with 3.0 mg/kg METH showed drug-induced context-dependent locomotor activity relative to control conditions.

This indicates that in adolescent males, one-trial sensitization is similar to adult one-trial sensitization in that they are both context-dependent, unlike younger rats (PD 21) who exhibit context-independent one-trial sensitization.

Disclosures: **B. Salinas:** None. **E. Bates:** None. **B. Schuessler:** None. **A. Su:** None. **L. Tran:** None. **A.R. Zavala:** None.

Poster

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P20-RR016467

G12MD007601

Title: Developmental methamphetamine exposure causes lasting changes to mesolimbic dopamine signaling.

Authors: ***D. TORRES**[1], S. BARAYUGA[1], R. RUELI[1], J. YORGASON[2], M. ANDRES[3], S. STEFFENSEN[4], F. BELLINGER[1];
[1]Cell and Mol. Biol., Univ. of Hawai'i at Manoa Dept. of Cell and Mol. Biol., Honolulu, HI; [2]Vollum Inst., Oregon Hlth. and Sci. Univ., Portland, OR; [3]Pacific Biosci. Res. Ctr., Univ. of Hawaii at Manoa, Honolulu, HI; [4]Dept. of Psychology, Brigham Young Univ., Provo, UT

Abstract: Abuse of Methamphetamine (METH), a neurotoxic psychostimulant that affects the dopaminergic system, has become a serious public health issue in the United States and is of particular concern regarding pregnant women who are users. While the teratogenic effects of drugs such as alcohol and cocaine have been extensively investigated, much less is known about the effects of prenatal methamphetamine exposure (PME). Recent findings show that PME can produce lasting deficits in attention, arousal and memory. Exposure during the third trimester of pregnancy can have particularly harmful consequences as it is during this period that the brain growth spurt begins and neurons are especially vulnerable to insult. In this study, we investigated the long-term consequences of early methamphetamine exposure using a mouse model of human third-trimester exposure. METH increases dopamine (DA) signaling by inhibiting DA reuptake and triggering reverse transport of DA through its transporters. DA neurons can release DA through action-potential dependent and independent mechanisms. We used fast-scan cyclic

voltammetry (FSCV) to measure DA responses to METH in adult mouse nucleus accumbens (NAc) brain slices following developmental METH exposure. PME model mice exhibited impaired basal DA reuptake kinetics compared to control mice. The reduced rate of DA reuptake indicates reduced levels of the dopamine active transporter (DAT). Developmental METH exposure did not alter peak evoked DA release either prior to or following acute METH application. METH-induced impairments of DA reuptake were attenuated, however, in comparison to controls. In contrast to evoked DA release, non-evoked DA release in response to acute Meth application was increased by developmental Meth exposure. Behavioral tests revealed increased hyperactivity in adulthood of PME model mice. This data suggests that METH exposure during this period of development causes long-lasting changes within the mesolimbic system. These changes alter behavior and physiological responses to METH in adulthood.

Disclosures: D. Torres: None. **S. Barayuga:** None. **R. Rueli:** None. **J. Yorgason:** None. **M. Andres:** None. **S. Steffensen:** None. **F. Bellinger:** None.

Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: China Medical University Hospital (DMR-105-028)

National Health Research Institutes (NHRI-EX105-10224NC)

National Health Research Institutes (NHRI-105A1-PDCO-1315161)

Title: The influence of prenatal buprenorphine exposure on the nociceptin/orphan FQ system in the locus coeruleus of offspring rats

Authors: *Y.-C. CHIANG[1,2], J. WU[1], C.-W. LEE[3,4], I.-K. HO[1,2];
[1]Ctr. for Drug Abuse and Addiction, China Med. Univ. Hosp., Taichung, Taiwan; [2]China Med. Univ., Taichung, Taiwan; [3]Div. of Basic Med. Sci., [4]Dept. of Nursing, and Chronic Dis. and Hlth. Promotion Res. Ctr., Chang Gung Inst. of Technol., Chiayi, Taiwan

Abstract: Abuse of addictive substances is a serious problem, which has significant impact on health, economy, public safety and society. Buprenorphine is a new maintenance agent for treating heroin addicts, that has benefits on less addictive liability and respiratory depression when compared to methadone. Buprenorphine has also been used to treat opioid addictive

pregnant women, yet the intrauterine effects of buprenorphine on the offspring are still unclear. This indicates that investigating the prenatal effects of buprenorphine on the offspring is an important and urgent issue. Buprenorphine is an agonist-antagonist mixed agent acting on both μ-opioid receptor and nociceptin/orphan FQ receptor (NOPR) at high doses (detoxification doses). Our previous studies showed that prenatally higher dose of buprenorphine (3 mg/kg/day)-exposed rats exhibited a marked change in the postnatally morphine-induced tolerance, and dopaminergic-mediated cellular pathways in the nucleus accumbens. In the current study, we showed that when treated only with vehicle, higher frequencies of withdrawal signs precipitated by NOPR antagonist J113397 (4 mg/kg) were exhibited in the prenatally buprenorphine-exposed offspring as compared with the prenatal vehicle-exposed subjects. The precipitated withdrawal symptoms could also be observed after postnatal treatment of buprenorphine in a dose dependent manner. The levels of mRNA and protein of NOPR in the locus coeruleus were also changed after prenatal or postnatal exposure to buprenorphine, but the nociceptin mRNA (precursor) and protein expressions were only changed in postnatal buprenorphine exposure condition. Since the protein kinase C (PKC) and beta-arrestin systems have been reported to play an important regulatory role in opioid receptors internalization, the PKC and arrestin expressions were also investigated in this study. Different protein expression patterns of beta-arrestin 1 and 2, and PKC δ and γ were obtained after buprenorphine re-exposure on prenatally buprenorphine-exposed rats. The study reveals that prenatal exposure to buprenorphine caused long-term and complex effects on postnatal buprenorphine-induced withdrawal and dependence on the offspring.

Disclosures: **Y. Chiang:** None. **J. Wu:** None. **C. Lee:** None. **I. Ho:** None.

Poster

076. Addictive Drugs and Development

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Program#/Poster#: 76.09/AAA11

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant AA012446

Title: Effects of late gestational cannabinoid exposure on behavioral development in rats

Authors: *B. ZAMUDIO[1], K. R. BREIT[2], J. D. THOMAS[3];
[1]Psychology, San Diego State Univ., Chula Vista, CA; [2]Psychology, [3]San Diego State Univ., San Diego, CA

Abstract: Given the recent legalization of marijuana for both recreational and medical use in several states, cannabis use has increased, even among pregnant women. The most psychoactive

constituent in cannabis, delta-9- tetrahydrocannabinol (Δ9-THC), crosses the placenta during maternal consumption and can directly affect the fetal brain. Some prospective clinical studies indicate that developmental cannabinoid exposure may alter long-term behavior in emotional and cognitive functioning domains. However, given the increasing potency in cannabinoid products available today and the novel "synthetic marijuana" market, fetal consequences may be more severe. The goal of this study was to investigate the effects of cannabinoid exposure on behavioral development. Using an animal model, the present study examined the effects of cannabinoid exposure during the brain growth spurt, a period of brain development during which there is a rapid increase in the number of CB1 cannabinoid receptors in multiple brain areas. Sprague-Dawley rats were randomly assigned to one of 3 groups receiving varying doses of the cannabinoid receptor agonist CP 55,940 (CP; 0.10, 0.25, 0.40 mg/kg/day), an agonist that activates both CB1 and CB2 cannabinoid receptors. Subjects received i.p. injections of CP or vehicle from postnatal day (PD) 4 through 9, a period of brain development equivalent to the human third trimester; control subjects were injected with DSMO or saline vehicle. From PD 12-20, motor development was assessed with a hindlimb coordination task, on PD 25, anxiety was measured with an elevated plus- maze, and on PD 40-46, spatial learning was assessed with the Morris water maze. Cannabinoid exposure altered the trajectory of motor development. Subjects exposed to CP were more successful in hindlimb coordination at an earlier age compared to controls. In contrast, there were no significant effects of CP on open arm entries within the elevated plus maze, although CP increased grooming behavior, suggesting that perinatal CP exposure has little effect on later anxiety. Finally, the highest dose of CP significantly impaired spatial memory, but only among the female subjects. These data suggest that cannabis exposure late in gestation alters brain and behavioral development, but in a domain- and sex-dependent manner. These data have important implications for children of women who consume cannabis during pregnancy. Further elucidation of the effects of prenatal cannabinoid exposure has important implications for public health that may guide public policy on cannabis, particularly for use during pregnancy.

Disclosures: **B. Zamudio:** None. **K.R. Breit:** None. **J.D. Thomas:** None.

Poster

076. Addictive Drugs and Development

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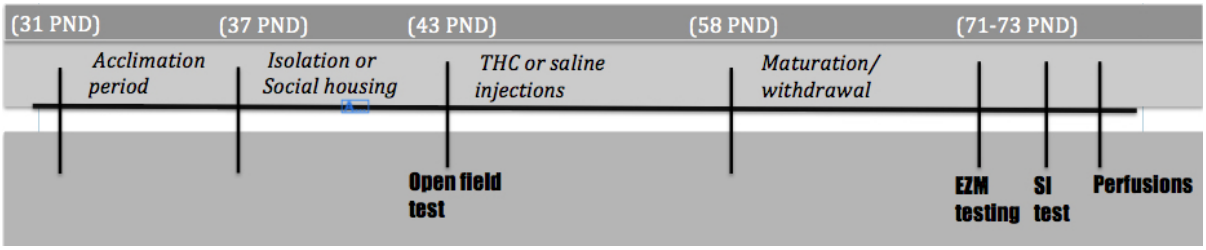
Program#/Poster#: 76.10/AAA12

Topic: G.08. Drugs of Abuse and Addiction

Title: Adolescent THC exposure following stress induces synaptic changes in adult rats

Authors: E. REPO, N. LANDRY, T. BENT, A. STILLAR, M. SAARI, *A. C. WEEKS; Nipissing Univ., North Bay, ON, ON, Canada

Abstract: The legalization and increased use of cannabis and its main constituent Δ9-tetrahydrocannabinol (THC), as a recreational and medicinal drug, has enhanced research interest related to the potential interactions of cannabis use and other psychological conditions. For example, adults and adolescents often report using cannabis as a way of coping with stress and anxiety. While the effects of marijuana use on the adult brain are fairly well understood, less is known about chronic use during adolescence. This study assessed anxiety levels and social interaction in adult rats after chronic THC was given following an isolation stressor event during the adolescent phase of development. Four experimental groups included: isolation/THC, no isolation/THC, isolation/vehicle, and no isolation/vehicle. Following one week in the housing condition (isolation or social housing), daily THC or vehicle injections were carried out for 15 days starting at post-natal day 43 (see Fig. 1). An open field test was completed after the isolation period, but before injections, to assess pre-THC anxiety levels. Following the injections, a withdrawal period, and a maturation delay, an elevated zero maze and a social interaction task were used to assess anxiety/fear related behavioural changes. The behavioural results from the zero maze indicated that chronic adolescent THC administration following isolation-induced stress caused adult rats to spend less time in the closed regions during the first minute in the maze. For the social interaction task, there was a main effect of the drug where animals treated with THC were found to groom other rats significantly less often. Following the behavioural tests, the rats were perfused for confocal microscopy. Pilot data from this analysis related to changes in synaptic protein levels in the nucleus accumbens suggests that THC leads to a decrease in synaptic compliment in this brain region. Full synaptic results from the nucleus accumbens and other brain areas such as the amygdala, will be presented. Figure 1



Disclosures: **E. Repo:** None. **N. Landry:** None. **T. Bent:** None. **A. Stillar:** None. **M. Saari:** None. **A.C. Weeks:** None.

Poster

076. Addictive Drugs and Development

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Program#/Poster#: 76.11/AAA13

Topic: G.08. Drugs of Abuse and Addiction

Title: Permanent disruptions of hippocampal GABAergic neurotransmission in adult rats following perinatal Δ^9-THC exposure.

Authors: *S. BEGGIATO[1,2], M. C. TOMASINI[3], A. C. BORELLI[3], T. ANTONELLI[3], L. FERRARO[3], S. TANGANELLI[3];
[1]Psychiatry, Univ. of Maryland Baltimore, Baltimore, MD; [2]Life Sci. and Biotech., [3]Univ. of Ferrara, Ferrara, Italy

Abstract: Cannabis or its psychoactive component delta^9-tetrahydrocannabinol (Δ^9-THC) is the most commonly abused illicit drug by pregnant women. Despite its wide use, at present, the long-lasting effects on the adult brain of the offspring are still unclear. It has already been showed that prenatal cannabinoids exposure induces learning and memory disruption in rat adult offspring, associated with permanent alterations of cortical glutamatergic neurotransmission (1) and cognitive deficits (2). In the present study, the risk of long-term consequences induced by perinatal exposure to cannabinoids (CBs) on hippocampal GABAergic system of the male offspring, has been explored. To this purpose, pregnant rats were treated daily with Δ^9-THC (5 mg/kg by gavage) or its vehicle. Litters from both groups were then assigned to non-exposed mothers whose pups were born on the same day. Perinatal exposure to Δ^9-THC induced a significant reduction ($p < 0.05$) in basal and K^+-evoked [^{3}H]-GABA outflow from 90-day old rat hippocampal slices. Furthermore, by using this preparation, we observed that the pharmacological challenge with either Δ^9-THC (0.1 μM) or WIN 55,212-2 (2 μM), significantly reduced K^+-evoked [^{3}H]-GABA outflow ($p < 0.05$). The reduction was significantly blocked by adding the CB_1 antagonist SR141716A. Perinatal exposure to Δ^9-THC induced a significant reduction on CB_1 receptor binding in hippocampus of rats and a significant reduction on CB_1 receptor mRNA expression ($p < 0.01$). Finally, in adult rats born from Δ^9-THC exposed dams were observed a significant decrease of hippocampal [^{3}H]-GABA uptake compared to vehicle exposed groups and a significant reduction of GAT-1 mRNA expression ($p < 0.01$). These findings suggest that perinatal exposure to Δ^9-THC induces long-term alterations of hippocampal GABAergic system. Interestingly, previous behavioural studies demonstrated that, under the same experimental conditions as in the present study, perinatal CBs exposure induced cognitive impairments in adult rats (1,3) Thus, although it is difficult and sometimes misleading to extrapolate findings obtained from animal models to humans, the possibility that an alteration of hippocampus aminoacidergic transmission might underlie, at least in part, some of the cognitive deficits affecting the offspring of marijuana users, is supported. *References* 1) Ferraro et al., J

Neural Transm (Vienna) (2009): 116(8):1017-27. 2) Campolongo et al., Addict Biol. (2007): 12(3-4):485-95. 3) Mereu et al., Proc Natl Acad Sci USA (2003): 100(8):4915-20.

Disclosures: **S. Beggiato:** None. **M.C. Tomasini:** None. **A.C. Borelli:** None. **T. Antonelli:** None. **L. Ferraro:** None. **S. Tanganelli:** None.

Poster

076. Addictive Drugs and Development

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Topic: F.03. Neuroendocrine Processes

Support: NSF OISE-1545803

Title: Nandrolone during adolescence increases the response to cocaine in adulthood.

Authors: ***A. C. SEGARRA**[1], C. J. RIVERO QUILES[2], J. A. FREIRE ARVELO[2], S. RIVERA BERMUDEZ[3], I. SANTIAGO MARRERO[4], J. DIAZ RIVERA[3];
[1]Physiol Dept, Univ. of Puerto Rico, San Juan, PR; [2]Physiol., Univ. of Puerto Rico, Med. Sci. Campus, San Juan, PR; [3]Biol., Univ. of Puerto Rico, Rio Piedras Campus, Rio Piedras, PR; [4]Biol., Univ. of Puerto Rico, Humacao Campus, Humacao, PR

Abstract: The use of anabolic androgenic steroids (AAS) by adolescents is rising. Adolescents seek AAS because of their anabolic properties unaware of the effects these might have on the developing reproductive and nervous system. Previous studies show that AAS cross-sensitize with other drugs of abuse such as cocaine. Whether these effects persist through adulthood remains unknown. For 10 consecutive days, starting on day 28, adolescence male rats received daily injections of nandrolone decanoate (20mg/kg/sc) or of vehicle. When they reached 64 days of age, Starting on day 64, they were tested for locomotor sensitization to cocaine. From days 1-5 and at days 13 and 23 rats received an injection of cocaine (15 mg/kg/ip). Their locomotor response to cocaine was measured at days 1, 5, 13 and 23. Nandrolone increased the locomotor response to cocaine on day 1, but did not affect the response thereafter. In addition, spermatogenesis, as well as testes and seminiferus tubule size, was decreased. These data show that AAS have long lasting effects on cocaine-induced locomotor activity and the reproductive system.

Disclosures: **A.C. Segarra:** None. **C.J. Rivero Quiles:** None. **J.A. Freire Arvelo:** None. **S. Rivera Bermudez:** None. **I. Santiago Marrero:** None. **J. Diaz Rivera:** None.

Poster

076. Addictive Drugs and Development

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH-NIGMS SC2GM109811

NIH-NIGMS R25GM100829

Title: Fluoxetine exposure during adolescence, in female C57bl/6 mice, does not influence Morris water maze performance in adulthood

Authors: *J. B. ALIPIO[1], L. M. RIGGS[1], S. D. IÑIGUEZ[2];
[1]Neurosci., Univ. of Maryland Baltimore, Baltimore, MD; [2]Psychology, Univ. of Texas at El Paso, El Paso, TX

Abstract: Mood-related disorders such as major depression are commonly diagnosed in children and adolescents, with a higher occurrence among females. The increasing prevalence of adolescent depression has resulted in parallel increases in the prescription rate of fluoxetine (FLX), the only antidepressant currently approved by the FDA for treatment of clinical depression within the juvenile population. Although treatment can last for years, very little is known about the consequences of antidepressant exposure during early developmental periods on memory performance in adulthood. Recently, in male rodents, it has been reported that juvenile FLX exposure results in a long-lasting altered memory-related behavioral phenotype - however, whether such memory-related alterations are observed in female mice has not been examined. Thus, we exposed adolescent (postnatal day [PD]35) c57bl/6 female mice to FLX (0 or 20 mg/kg/day) for 15 consecutive days. We then assessed animals' behavioral performance on the Morris water maze spatial memory task, three weeks after antidepressant exposure (PD70+). Specifically, mice were trained to find the location of a submerged escape platform on a single day task of 8 training trials, and memory for the platform location was tested after a 24 hour delay (distance traveled and velocity). To increase the demands of the spatial task, the mice returned to the maze, 48 hours after training, and completed a probe trial (escape platform absent). We found that adolescent FLX exposure did not influence spatial memory acquisition on the training day. Additionally, no differences between the groups were observed when spatial memory was examined 24 hours (test day) or 48 hours after training (probe trial). Together, our results suggest no long-lasting spatial memory deficiencies become apparent in female c57bl/6 mice exposed to FLX during adolescence.

Disclosures: **J.B. Alipio:** None. **L.M. Riggs:** None. **S.D. Iñiguez:** None.

Poster

076. Addictive Drugs and Development

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Program#/Poster#: 76.14/AAA16

Topic: F.04. Stress and the Brain

Support: DA033358

Title: Effects of chronic caffeine exposure on rat brain serotonergic systems

Authors: *M. ARNOLD[1], T. M. SMITH[1], S. R. ARCHULETA[2], P. H. WILLIAMS[1], J. A. MCARTHUR[1], C. E. O'NEILL[2], C. A. LOWRY[1], R. K. BACHTELL[2];
[1]Integrative Physiol., [2]Psychology, Univ. of Colorado Boulder, Boulder, CO

Abstract: Chronic caffeine exposure during adolescence has been shown to induce persistent maladaptive anxiety-like behavioral responses that are evident during adulthood in rats. It is possible that these maladaptive responses are mediated by the serotonergic system. In this study, we investigated the effects of chronic adolescent caffeine exposure on rat brain serotonin (5-hydroxytryptamine; 5-HT) systems. Specifically, we analyzed serotonergic neuron gene expression and activation in subregions of the dorsal raphe nucleus (DRN), a brainstem region with abundant serotonergic neurons. After a week of acclimatization, rats were randomly divided into four groups in a two-by-two experimental design. Two groups received chronic caffeine (CC) administration in drinking water (0.3 g/L) from postnatal day 28 to postnatal day 56 while the other two groups received drinking water (NC) alone during the same developmental time period. After 28 days of caffeine or control treatment and a 24-hour washout period, rats received an i.p. injection of either 30 mg/kg caffeine (C) or 0.9% sterile saline (S) vehicle, were then replaced in their home cages, and were euthanized 90 minutes following treatment for immunohistochemistry experiments and 4 hours following treatment for in situ hybridization histochemistry experiments. This was a 2 x 2 design with four treatment groups, NCS, NCC, CCS, and CCC. In situ hybridization histochemistry analysis revealed that acute caffeine injection, in rats that chronically consumed either drinking water or caffeinated drinking water, induced widespread decreases in the expression of *tph2*, encoding tryptophan hydroxylase 2, the rate-limiting enzyme in neuronal serotonin biosynthesis. Similar effects of acute caffeine treatment were observed on *slc22a3* mRNA expression, encoding organic cation transporter 3 (OCT3). Rats that received chronic caffeine, relative to controls, and were subsequently treated with saline, also responded with decreased *tph2* and *slc22a3* mRNA expression, indicating persistent effects of caffeine on serotonergic systems. Using a double immunostaining technique we quantified the immunoreactivity for the acute activation marker c-Fos and tryptophan hydroxylase 2 (Tph2) as a marker of serotonergic neurons. NCC rats, relative to NCS and CCC groups, had higher activation of 5-HT neurons in a number of DRN subregions, supporting

persistent effects of caffeine exposure on anxiety-related serotonergic systems These data are consistent with the hypothesis that the DRN is a key structure in promoting the adult pro-anxiety behavioral phenotype following adolescent caffeine exposure.

Disclosures: **M. Arnold:** None. **T.M. Smith:** None. **S.R. Archuleta:** None. **P.H. Williams:** None. **J.A. Mcarthur:** None. **C.E. O'neill:** None. **C.A. Lowry:** None. **R.K. Bachtell:** None.

Poster

077. Cannabinoids: Neural Mechanisms and Addiction

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Program#/Poster#: 77.01/AAA17

Topic: G.08. Drugs of Abuse and Addiction

Support: Ministerio de Economía y Competitividad, Instituto de Salud Carlos III PI14/00438

Title: Cannabidiol mediated regulation of alcohol consumption

Authors: *M. GARCÍA-GUTIÉRREZ, SR[1], A. VIUDEZ-MARTÍNEZ[2], C. NAVARRON[2], M. MORALES-CALERO[3], F. NAVARRETE[2], A. TORRES-SUÁREZ[3], J. MANZANARES[2]; [1]Inst. De Neurociencias, Univ. Miguel Her, San Juan De Alicante, Spain; [2]Inst. de Neurociencias, Univ. Miguel Hernandez, San Juan de Alicante, Spain; [3]Facultad de Farmacia, Univ. Complutense, Madrid, Spain

Abstract: The aim of this study was to evaluate the effects of cannabidiol (CBD) in alcohol reinforcement, motivation and relapse in C57BL/6J mice. To this purpose, the effects of CBD (60 mg/kg i.p.) and ethanol on rectal temperature (3 g/kg p.o.), ethanol-handling-induced convulsions (HIC) (4 g/kg i.p.) and blood ethanol concentration (BEC) (3 g/kg p.o.) were studied. The two-bottle choice test was performed to evaluate the effect of CBD (30, 60 and 120 mg/kg/day, i.p.) on ethanol intake and preference. In addition, an oral ethanol self-administration experiment was carried out to evaluate the effect of CBD (poly-ε-caprolactone spherical microparticles with small pores which provided a CBD continuous controlled release (30 mg/kg, s.c.)) on the reinforcement and motivation for ethanol. A further oral ethanol self-administration was performed to evaluate the effects of CBD (60 and 120 mg/kg/day, i.p.) on ethanol-induced relapse. Gene expression analyses of tyrosine hydroxylase (TH) in ventral tegmental area (VTA) and μ-opioid receptor in nucleus accumbens (NAcc) were carried out by Rt-PCR.
The results revealed that a single dose of CBD (60 mg/kg, i.p.) reduced the ethanol-induced hypothermia and handling-induced convulsions (Two-way RM ANOVA; $p<0.001$). CBD failed to modify blood ethanol levels. In addition, CBD (30, 60 and 120 mg/kg/day, i.p.) reduced ethanol consumption (Two-way RM ANOVA; $p<0.001$) and ethanol preference (Two-way RM

ANOVA; $p<0.001$) in the two-bottle choice experiment. Interestingly, CBD (microparticle formulation (30 mg/kg, s.c.)) significantly decreased the ethanol intake and the number of active lever presses in the ethanol oral self-administration (Two-way RM ANOVA; $p<0.05$). Furthermore, CBD (60 and 120 mg/kg/day, i.p.) significantly reduced ethanol-induced relapse (Two-way RM ANOVA; $p<0.05$). In the ethanol self-administration study, CBD significantly reduced TH gene expression in the VTA (40%) (Student's t-test, $p<0.05$) and μ-opioid receptor gene expression (35%) in the NAcc (Student's t-test, $p< 0.05$).
In conclusion, these results reveal that the administration of CBD reduced the reinforcing properties, motivation and relapse for ethanol. These findings strongly suggest that CBD may result useful for the treatment of alcohol use disorders.

Disclosures: **M. García-Gutiérrez:** None. **A. Viudez-Martínez:** None. **C. Navarron:** None. **M. Morales-Calero:** None. **F. Navarrete:** None. **A. Torres-Suárez:** None. **J. Manzanares:** None.

Poster

077. Cannabinoids: Neural Mechanisms and Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Support: Ministerio de Economía y Competitividad, Instituto de Salud Carlos III PI14/00438

Title: Cannabidiol a drug lacking reinforcing activity

Authors: ***J. MANZANARES**, J. MEDRANO-RELINQUE, A. VIUDEZ-MARTÍNEZ, C. NAVARRÓN, A. ARACIL-FERNÁNDEZ, F. NAVARRETE, M. GARCÍA-GUTIÉRREZ; Inst. De Neurociencias, Univ. Miguel Hernandez-Csic, San Juan de Alicante, Spain

Abstract: Cannabidiol, one of the main compounds present in the plant Cannabis sativa, has recently emerged as a potential drug for the treatment of different psychiatric disorders, based on its anxiolytic, antidepressant and antipsychotic properties. However, some controversy regarding its non-psychoactive properties significantly hampers the development of basic and clinical studies. Indeed, CBD is currently classified in the Schedule 1 according to the Drug Enforcement Administration (DEA) suggesting high potential for abuse.
In order to shed light on this subject, the aim of this study was to evaluate the potential reinforcing properties of CBD. Hence, three behavioral assays were performed using C57BL/6J male mice. Reinforcing properties of CBD (15, 30 and 60 mg/kg, i.p.) were evaluated in the conditioned place preference (CPP). Spontaneous withdrawal symptoms and motor activity were

evaluated in the open field (OF) on day 8 after the administration of CBD (30 mg/kg/12h, i.p., 7 days). In addition, the CBD consumption was evaluated using an oral self-administration paradigm (60 mg/kg; CBD water soluble).
The results revealed that CBD failed to produce CPP (Student t-test, p= 0.31). Furthermore, the administration of CBD did not alter motor behavior (Student t-test, p= 0.12) nor induced withdrawal symptoms after cessation of treatment. Interestingly, there was no differences between CBD and water in the oral self-administration paradigm.
In conclusion, these results demonstrate that CBD did not present reinforcing activity in any of the different evaluated behavioral assays (CPP, spontaneous withdrawal and self-administration) suggesting that the inclusion of CBD in the Schedule I should be questioned.

Disclosures: J. Manzanares: None. **J. Medrano-Relinque:** None. **A. Viudez-Martínez:** None. **C. Navarrón:** None. **A. Aracil-Fernández:** None. **F. Navarrete:** None. **M. García-Gutiérrez:** None.

Poster

077. Cannabinoids: Neural Mechanisms and Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Support: DGAPA-UNAM Grant IN218316 to OPG

DGAPA-UNAM Grant IA207416 to MMD

DGAPA-UNAM Grant IN219516 to AERC

Title: Social environment prevents alcohol intake and modifies dopaminergic and endocannabinoid systems in maternal care deprived rats

Authors: *O. AMANCIO-BELMONT[1], A. BECERRIL-MELÉNDEZ[1], M. MÉNDEZ-DÍAZ[1], A. E. RUIZ-CONTRERAS[2], Ó. PROSPÉRO-GARCÍA[1];
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Abstract: Experimental evidence suggests that natural rewards, such as a social interaction, enriched environment or physical exercise devaluate drugs of abuse, therefore the practice of these activities prevent from drug abuse and drug addiction. On the other hand, a considerable amount of experimental studies have shown that maternal care deprivation (MCD) is associated with drug abuse and drug addiction. Dopaminergic and endocannabinoid system have been implicated in reward processes, including drug intake and drug addiction. MCD causes changes

in the dopaminergic and endocannabinoid system in the prefrontal cortex (PFC) and nucleus accumbens (NAcc) that seem to facilitate alcohol consumption. This study used a rodent model to test whether exposure to a social environment could protect MCD and non-MCD adult rats from alcohol intake. Also to evaluate dopaminergic and endocannabinoid system potential changes in the PFC and NAcc.
Pregnant Wistar rats were obtained at gestational day 14-17 from our facilities (Facultad de Medicina, UNAM). The day of birth was designated as PND 0. MCD was performed from PND 2 to PND 16, between 9:00 and 12:00 h daily. Rats were weaned on PND 21. From PND 24 to PND 60 male rats were reared into two housing conditions: a social condition (10 rats/cage) and a nonsocial condition (singly housed). Once the rats became adults (PND 60), all groups (n = 10 each group) were submitted to a voluntary alcohol (10% v/v) protocol for 10 days. Different groups of rats (n = 10 each group) were sacrificed when adult but with no exposition to alcohol whatsoever to dissect PFC, NAcc and hippocampus to analyze the expression of cannabinoid (CB1R and CB2R) and dopaminergic (D1R, D2R and D3R) receptors.
Social environment reduces alcohol intake in non-MCD and MCD rats and modifies the expression of these receptors. These results indicate that socialization prevents rats from drinking alcohol and induces changes in the expression of these receptors

Disclosures: O. Amancio-Belmont: None. **A. Becerril-Meléndez:** None. **M. Méndez-Díaz:** None. **A.E. Ruiz-Contreras:** None. **Ó. Prospéro-García:** None.

Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH R21-DA032821

Title: Behavioral and neural markers of craving regulation in marijuana-dependent adolescents

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Abstract: Adolescence is a neurodevelopmental period in which cortico-limbic circuitry has not yet reached maturation, likely resulting in greater difficulty with regulation of appetitive behavior. Marijuana use is increasing among adolescents, and behavioral strategies that help with

regulation of craving for marijuana may be helpful for reducing its use yet remain underexplored. The current fMRI study uses behavioral and brain measures to examine craving regulation in adolescent heavy marijuana users (smoking at least 5 times per week, >1 gram per day). Adolescents (13-19 years old) abstinent from marijuana for 48 hours underwent fMRI scanning while performing a reappraisal-based craving regulation task, which involved proximal/distal perspective taking while viewing images containing content reflecting marijuana use (e.g., pipes, buds) or matched neutral images. Prior to viewing images, participants were instructed to either imagine themselves immersed in the scene depicted in the image, allowing themselves to experience any sensations that arose ("close", i.e., reactivity), or to imagine themselves at a distance from the scene ("far", i.e., regulation), making factual, objective observations of the content of the scene (e.g., indoors/outdoors). Participants rated their craving after each image presentation. Behavioral results indicated a main effect of cue-type, with marijuana images eliciting greater craving than neutral images, and an interaction of proximity (close/far), and image type (marijuana/neutral images) on craving - lower craving ratings were given following the "far" versus "close" instructions, and no effect of proximity was found for the neutral cues. The contrast of marijuana vs. neutral images (index of cue-reactivity) showed activation in ventromedial prefrontal cortex and ventral striatum; where as, for the far vs. close contrast (index of regulation), fMRI activation was found in fronto-parietal regions. Whole-brain correlation analyses of craving ratings and the fMRI index of regulation revealed that degree of "regulation success" in behavior (difference in craving ratings after "close" and "far" instructions) was positively correlated with rostral anterior cingulate and mid-insula. These preliminary results suggest that reappraisal strategies have an impact on cue-elicited self-reported craving for marijuana and recruit brain regions important for cognitive control among adolescents.

Disclosures: D.G. Ghahremani: None. **A. Eliaz:** None. **K. Kato:** None. **A. Dean:** None. **M. Huestis:** None. **E. London:** None.

Poster

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Program#/Poster#: 77.05/AAA21

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA IRP

Title: Preclinical evaluation of the potential utility of neutral cb1 receptor antagonists and negative allosteric cb1 receptor modulators in treatment of drug abuse and addiction

Authors: *Z. XI[1], G.-H. BI[1], X.-H. HE[1], G. THAKUR[2], A. MAKRIYANNIS[3], H. H. SELTZMAN[4], E. GARDNER[1];
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Abstract: Recent studies show that cannabinoid CB1 receptors are importantly involved in drug reward and addiction. Accordingly, brain CB1 receptors have been thought to be potential targets in medication development for treatment of drug abuse and addiction. However, rimonabent-based medication development was terminated in 2008 worldwide due to significantly unwanted side-effects such as depression and suicide intendancy. Since rimonaben is a CB1 receptor antagonist and also an inverse CB1 receptor agonist, it has been proposed and demonstrated that the inverse agonist property of rimonabent may contribute to those unwanted side-effects. Based on these findings, it has been recently proposed that neutral CB1 receptor antagonists (i.e., the drugs themselves have no intrinsic activity) or negative allosteric modulators (which bind to transmembrane allosteric bindings sites, not extracellular orthesteric ligand binding domain) may have therapeutic anti-addiction potential without such unwanted effects as rimonabent does. To test this hypothesis, we systemically evaluate the effects of these three types of CB1 receptor ligands in animal models of drug addiction. We found that 1) the inverse CB1R agonist rimobent (0, 3, 10 mg/kg, i.p.) significantly inhibited cocaine (or heroin, nicotine) self-administration and cocaine-enhanced electrical brain-stimulation reward, while rimonabent itself produced significant aversive effects in electrical brain-stimulation reward; 2) the CB1 neutral antagonists AM4113 and PIMSR1 (0, 3, 10 mg/kg) are similarly effective in attenuation of cocaine (nicotine, heroin) self-administration and cocaine-enhanced brain-stimulation reward, while both compounds themselves have no effect in brain reward function as assessed by electrical brain-stimulation reward; 3) In contrast, the negative allosteric modulators (NAMs) GAT358 and GAT369 (0, 10, 20 mg/kg) neither altered nicotine-enhanced brain-stimulation reward nor altered brain reward function by themselves. Taken together, all these (preliminary) findings suggest that neutral CB1R antagonists appear to be more promising than CB1R NAMs in medication development for treatment of drug abuse and addiction.

Disclosures: Z. Xi: None. **G. Bi:** None. **X. He:** None. **G. Thakur:** None. **A. Makriyannis:** None. **H.H. Seltzman:** None. **E. Gardner:** None.

Poster

077. Cannabinoids: Neural Mechanisms and Addiction

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Program#/Poster#: 77.06/AAA22

Topic: G.08. Drugs of Abuse and Addiction

Support: Stuit Fellowship to JHF

Title: Adolescent cannabinoid agonist exposure impairs learned timing in adult rats

Authors: *J. H. FREEMAN[1], M. ELKIN[2], B. DE CORTE[2];
[1]Psychological and Brain Sci., [2]Univ. of Iowa, Iowa City, IA

Abstract: Cannabinoid receptors are highly expressed within the cerebellar cortex and systemic administration of cannabinoid agonists impairs cerebellum-dependent eyeblink conditioning in adult animals and humans. Eyeblink conditioning is an associative learning task that includes a conditional stimulus (CS) that is followed after 400 ms by an unconditional stimulus (US) which causes eyelid closure before training. With repeated training trials the subject will produce an eyelid closure conditional response (CR) that is timed to the onset time of the US. Recent findings indicate that learned timing at longer intervals (12 s) may also depend on the cerebellum. Although there have been several studies of cannabinoid effects on cerebellar learning in adults, the effects of cannabinoid exposure during adolescence on subsequent cerebellar learning in adults have not been examined. The current study examined the effects of systemic injections of the cannabinoid agonist WIN55,212-2 or vehicle during adolescence in rats (postnatal days 35-45) on eyeblink conditioning and interval timing tasks on PD65. Rats given adolescent exposure to WIN55,212-2 showed deficits in the amplitude, area, and timing of the CR. During CS-alone extinction testing, the group given WIN55,212-2 showed deficits in the frequency, amplitude, area, and timing of the CR. Interval timing was examined in a 12 s fixed interval task using a touchscreen apparatus. The rats were trained to touch a stimulus presented on a touchscreen after a 12 s interval to obtain a food reward. Rats given WIN55,212-2 showed slower acquisition of temporal modulation of response rate across sessions and their responses were less precisely timed to the end of the interval within sessions. The results indicate that adolescent exposure to cannabinoid agonists has persistent effects on learned timing mechanisms.

Disclosures: **J.H. Freeman:** None. **M. Elkin:** None. **B. De Corte:** None.

Poster

077. Cannabinoids: Neural Mechanisms and Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Support: Principal's Initiative Grant, The University of the West Indies, Mona Campus

Title: A comparison between delta-9-tetrahydrocannabinol and a Jamaican marijuana tea extract in an animal model of addiction

Authors: *L. E. YOUNG, K. P. CHIN-QUEE;
The Univ. of the West Indies, Kingston 7, Jamaica

Abstract: Marijuana is the most widely used illicit drug world-wide because of its psychoactive compound, Δ^9-tetrahydrocannabinol (THC). The plant, *Cannabis sativa,* however contains a myriad of other non-psychoactive cannabinoids. In Jamaica, it is smoked, drank or eaten for recreational and medicinal purposes. Rodents do not voluntarily consume or self-administer cannabis due to the anhedonic properties associated with THC and often exhibit conditioned place aversion (CPA), inconsistent with many known addictive drugs. Like ethanol and phencyclidine, modifications of classic animal models of addiction to show reward have been demonstrated. We hypothesize that drug-seeking behavior or reward to cannabis can be achieved at a low dose by modifications to the conditioned place preference (CPP) paradigm that involves a decrease or elimination of the dysphoria associated with the THC, which could significantly impact cue-associated reward. Sprague-Dawley rats were taken through a biased CPP paradigm utilizing an 8-day schedule. The pre-conditioning phase consisted of 3 trials of 15 mins each and the conditioning phase lasted either 40 or 18 mins. A decrease in the spontaneous motor activity was assessed to determine the 18 min confinement period and dosage of the marijuana tea extract (MTE) that was compared with THC (0.05 - 2 mg/kg). Time spent in the non-preferred (white) chamber on the test day over a 15 mins period was compared with the pre-conditioning time. The MTE was prepared by boiling the dried leaves and stems of the marijuana plant under reflux for 6 hours followed by vacuum filtering, freeze-drying and reconstituting with saline for intra-peritoneal injection (*i.p*). The effect of sensitization to a low dose (3 mg/kg, *i.p.)* of amphetamine (AMP) given 14 days prior to conditioning was also assessed. Data were analyzed using Student's paired t-test. The 40-min confinement period resulted in significant CPA to the drug-paired chamber (MTE, $p<0.01$); whereas the 18-min confinement period did not (MTE, $p>0.05$). AMP sensitization also increased MTE place preference for the drug-paired chamber ($p = 0.09$). Significant CPP ($p<0.001$) was achieved with the low dose of 0.05 mg/kg THC using an 18-min confinement period in the conditioning phase. CPP can thus be achieved at very low doses of THC if attention is paid to decreasing the dysphoric or anhedonic cue-associated effects due mainly to the psychoactive cannabinoid, THC, either separately or when part of the bio-material of an extract of the whole cannabis plant.

Disclosures: L.E. Young: None. **K.P. Chin-Quee:** None.

Poster

077. Cannabinoids: Neural Mechanisms and Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Support: R03DA038714

R15AR066806

U54GM104942

Title: Delta^9-THC withdrawal induces somatic and emotionality-related behaviors in mice

Authors: *S. G. KINSEY, K. R. TREXLER, S. R. NASS, M. S. CROWE, A. W. MCKITRICK;
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Abstract: Cannabis dependence can be inferred from tolerance and withdrawal symptoms following repeated exposure. In rodents, cannabinoid withdrawal is commonly quantified using somatic signs, for example frequency of paw tremors and head twitches. Two critiques of the validity of these models are (1) these symptoms are absent during human cannabis use disorder, which is characterized by drug craving, sleep disturbances, and altered emotional processing; and (2) the reliance of a CB_1 selective antagonist to precipitate withdrawal symptoms. Thus, behavioral assays that exploit emotionality-related measures of withdrawal may more closely mimic symptoms experienced in humans. In the present study, two models of anxiety-like behavior, marble burying test and light/dark box, and a model of depressive-like behavior, the tail suspension test, were used to evaluate withdrawal induced by 5.5 days of tetrahydrocannabinol(THC; 10 or 50 mg/kg, s.c.) administration compared to vehicle-treated control animals. Withdrawal was precipitated using rimonabant (3 mg/kg, i.p.) in some instances, however, spontaneous withdrawal was also evaluated to address critiques associated with precipitated withdrawal. We hypothesized that THC withdrawal-induced increases and anxiety-like and depressive-like behaviors would be attenuated by pretreatment with a FAAH or MAGL inhibitor. Surprisingly, precipitated THC withdrawal significantly suppressed both marble burying and immobility in the tail suspension test. Neither effect was altered by pretreatment with the FAAH inhibitor PF-3845 (10 mg/kg, i.p.), the MAGL inhibitor JZL184 (8 or 40 mg/kg, i.p.), or propranolol (10 mg/kg, i.p.). However, as published previously, JZL184 did significantly attenuate paw tremors and head twitches induced by precipitated THC withdrawal, indicating differential effects of MAGL inhibition on withdrawal behaviors. Of particular interest, spontaneous THC withdrawal also significantly altered marble burying behavior. The results of these experiments suggest that marble burying and tail suspension may be appropriate to include

in test batteries of cannabinoid withdrawal with traditional tests of somatic withdrawal signs in order to increase the overall predictive validity of potential pharmacological treatments for cannabis dependence.

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Poster

077. Cannabinoids: Neural Mechanisms and Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Support: DA 020129

Title: Diacylglycerol lipase-α expression increases in the coeruleo-cortical pathway in dopamine-β-hydroxylase knockout mice as well as rats treated with DSP-4

Authors: *M. URQUHART[1], B. A. S. REYES[1], S. A. THOMAS[2], K. MACKIE[3], E. J. VAN BOCKSTAELE[1];
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Abstract: Endocannabinoids are involved in the regulation of many physiological processes including behavioral responses to stress. Endocannabinoids modulate norepinephrine (NE) signaling primarily via involvement of CB1 cannabinoid receptors (CB1r). Our previous studies have shown that acute and repeated administration of a CB1r agonist increases multiple indices of noradrenergic activity involving the locus coeruleus (LC)-frontal cortex (FC) pathway. Diacylglycerol lipase-α (DGL-α), a key enzyme in the biosynthesis of the endocannabinoid 2-arachidonoylglycerol (2-AG) is localized to both the FC and the LC. Using electron microscopy, we have recently shown that in the rat FC DGL-α is localized in postsynaptic profiles that are targeted by dopamine-β-hydroxylase (DβH), the enzyme that converts dopamine to norepinephrine and represents a marker of noradrenergic neurons (Hartman et al., 1972). In this study, we also described interactions between DGL-α, CB1r and DβH in the FC using confocal microscopy. In the present study, we investigated expression levels of DGL-α under two conditions of NE deletion: in a rat model using a systemic injection of saporin conjugated with antibody against DβH (DSP-4) and in a genetically engineered mouse that lacked the enzyme DβH (DβH-knockout, KO). We compared expression levels of DGL-α to either control rats or

wild type (WT) mice using Western blot analysis. Protein extracts from micropunches of FC and LC were obtained and probed for DGL-α. Results showed that DGL-α expression was significantly increased in FC ($P < 0.05$) of both DSP-4 treated rats and DβHKO mice when compared to WT mice. DGL-α expression was also significantly increased in the LC ($P < 0.05$) of DβHKO when compared to WT mice. These data add to the accumulating evidence that dysregulation of NE transmission results in significant adaptations in the brain endocannabinoid system.

Disclosures: M. Urquhart: None. **B.A.S. Reyes:** None. **S.A. Thomas:** None. **K. Mackie:** None. **E.J. Van Bockstaele:** None.

Poster

077. Cannabinoids: Neural Mechanisms and Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Support: USPHS grant DA035482

USPHS grant DA041967

Title: Sex differences in delta-9-Tetrahydrocannabinol-induced hypothermia and hypolocomotion in rats

Authors: *M. JAVADI PAYDAR[1], J. D. NGUYEN[1], Y. GRANT[1], S. A. VANDEWATER[1], M. COLE[2], M. A. TAFFE[1];
[1]Committee on the Neurobio. of Addictive Disorders, The Scripps Res. Inst., San Diego, CA; [2]La Jolla Alcohol Res. Inc,, La Jolla, CA

Abstract: Backgrounds:
Previous studies suggest that there are sex differences in response to exogenous cannabinoids. Unfortunately data from animal and clinical studies comparing cannabinoid effects between males and females, under controlled laboratory conditions, are limited. This study was undertaken to determine any sex differences in thermoregulatory and locomotor responses to the inhalation of THC in rats.
Methods:
Male and female Sprague-Dawley rats were exposed to vapor of either a propylene glycol vehicle (PG) or THC (12.5-100 mg/mL in PG, 4 puffs every 5 minutes) for a duration of 30 minutes. Body temperature and locomotor responses were evaluated post-inhalation using a

radiotelemetry system.
Results:
Experiments determined that THC reduced the body temperature of both sexes in a dose-dependent manner. Temperature was reduced following 50 and 100 mg/ml THC inhalation with significant effects observed up to 90 min post-exposure for males and up to 120 min post-exposure for females. THC-treated male rats showed a significant dose-dependent reduction of locomotor activity for the first 30 min after THC exposure, but female locomotor activity did not change significantly. Additional experiments show that the inhalation of THC vapor attenuates nociception using a tail-flick assay.
Discussion:
THC vapor produced a robust and lasting decrement in the body temperature of both sexes. Unlike the males, female rats did not show hypolocomotion following exposure to THC vapor. THC inhalation also decreased tail-flick latency, demonstrating anti-nociceptive efficacy. The vapor inhalation model is therefore validated for use in both sexes of rat.

Disclosures: **M. Javadi Paydar:** None. **J.D. Nguyen:** None. **Y. Grant:** None. **S.A. Vandewater:** None. **M. Cole:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); La Jolla Alcohol Research Inc, La Jolla, CA, USA.. **M.A. Taffe:** None.

Poster

077. Cannabinoids: Neural Mechanisms and Addiction

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 77.11/AAA26

Topic: G.08. Drugs of Abuse and Addiction

Title: EEG resting state analysis of cannabis users with and without rTMS

Authors: *S. PRASHAD, E. DEDRICK, T. RHINEHARDT, W. T. TO, J. EROH, S. VANNESTE, J. HART, Jr., F. FILBEY;
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Abstract: Cannabis is the most widely used illicit drug in the United States. With recent policy changes towards cannabis, it is important to investigate deficits associated with cannabis use. Although various therapies and medications have been used to treat and manage addiction, many individuals are left untreated, revealing a need for novel interventions and strategies. Repetitive transcranial magnetic stimulation (rTMS) has a neuromodulatory effect on cortical networks and has been used to relieve symptoms for many neurological and psychiatric disorders. Previous studies investigating rTMS in addiction suggest that active stimulation reduces craving; however,

it is still not known how rTMS affects the resting state of individuals that are regular cannabis users. In the current study, we analyzed resting state EEG (eyes closed condition) in ten users of cannabis compared to ten non-using controls in the theta (4-7Hz), alpha (8-12Hz), beta (13-30Hz), and gamma (30-50Hz) frequency bands. All participants attended two data collection sessions and underwent rTMS targeting the posterior cingulate cortex using a double cone coil prior to the EEG data collection. Half of the participants received rTMS at 1Hz (Low frequency; LF) in the first session and the other half received rTMS at 10Hz (High frequency; HF). These stimulation frequencies were reversed in the second session. We found that users of cannabis exhibited lower theta, alpha, and beta power, but higher gamma power compared to non-using controls in the LF condition. All participants exhibited greater power for all frequency bands after the HF rTMS condition compared to the LF rTMS condition, except for the gamma band in cannabis users in which power did not change after HF rTMS. However, non-using controls continued to exhibit greater power across the frequency bands compared to cannabis users after HF rTMS. These results are consistent with previous studies that have reported impaired memory, attention, and cognitive processing in users of cannabis as well as neuroimaging studies that suggest changes in brain networks. The increase in power after HF rTMS could suggest its potential effectiveness in reducing the impairments in cognition and that rTMS may be viable for intervention and treatment strategies for cannabis addiction.

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Poster

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Mayo Clinic Addiction Research Center

Mayo Clinic Department of Biochemistry and Molecular Biology

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Title: Endocannabinoid signaling as a modifier of zebrafish stress responses

Authors: *R. G. KRUG, M. O. PETERSEN, K. J. CLARK;
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Abstract: The number of annual cannabis users exceeds 100,000,000 globally and an estimated 9% of these individuals will suffer from dependency, but a dearth of knowledge exists about the potential consequences on public health. However, the psychoactive constituents of cannabis are known to affect the endocannabinoid (eCB) system, and disrupt features of vertebrate physiology and behavior. Our central hypothesis is that disruptions in the eCB signaling system have pathological consequences on vertebrate behavior and physiology, including dysregulation of the stress response system. Herein, we use a preclinical zebrafish model to clarify the ramifications of disturbances in the eCB signaling system. Using qRT-PCR and *in situ* hybridization we show that the genes encoding enzymes that synthesize (*abhd4*, *gde1*, *napepld)*, enzymes that degrade (*faah*, *faah2a*, *faah2b*), and receptors that bind (*cnr1*, *cnr2*, *gpr55-like*) eCBs are expressed throughout development. We show that disruptions of this system via exogenous cannabinoid administration results in altered behavior and physiology, including increased secretion of glucocorticoids in our stress response reporter line. We are developing a zebrafish eCB signaling mutant library using TALENs and show that disruption of *faah2a* alters stress-associated behavior. Collectively, these results establish zebrafish as a viable model for studying eCB signaling, and lay a foundation for informing a better understanding of the toxicological and therapeutic potential of the eCB system.

Disclosures: R.G. Krug: None. **M.O. Petersen:** None. **K.J. Clark:** None.

Poster

077. Cannabinoids: Neural Mechanisms and Addiction

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Topic: G.08. Drugs of Abuse and Addiction

Support: DA 020129

DA09082

Title: Colocalization of estrogen receptor alpha and cannabinoid receptor type 1 in the medial prefrontal cortex of female rats

Authors: *J. ZHANG, B. A. S. REYES, R. D. SHADE, E. J. VAN BOCKSTAELE;
Pharmacol. and Physiol., Col. of Medicine, Drexel Univ., Philadelphia, PA

Abstract: Mood disorders including anxiety and depression are the most common mental illnesses in the United States. Women are twice as likely as men to suffer from these psychopathologies. Estrogen has been reported to be strongly involved in the observed sex differences associated with these disorders. However, the underlying mechanism regarding how estrogen affects these non-reproductive brain functions remains unclear. Estrogen exerts its actions via binding to the estrogen receptor alpha (ERα), beta (ERβ), and GPR30. The endocannabinoid (eCB) system functions as "stress buffer" ubiquitously distributed throughout the brain and, modulates neurotransmitter release via the activation of CB type 1 (CB1r) and type 2 (CB2r) receptors. The eCB system may influence the development of anxiety and other stress related psychiatric disorders via dysregulation in stress-integrative neural circuits. Sex differences exist in the expression levels of CB receptors in many different brain regions including the prefrontal cortex, which is has been strongly implicated in the etiology of mood disorders. While functional sex differences in the eCB system have been reported, it is still not clear how these sex differences manifest physiologically. Recent studies have shown that estrogen is able to modulate eCB tone and consequently suppress gamma amino butyric acid (GABA) release in a sex specific manner, via involvement of ERα. Multiple studies have reported that ERα is mainly localized to axons and axon terminals in the medial prefrontal cortex (mPFC) where CB1r is predominantly localized. Taken together, we hypothesize that CB1 and ERα may colocalize in the same neuronal profiles in the mPFC and functionally interact, which may contribute to the observed sex differences in the eCB system. The present study utilized dual immunogold electron microscopy to investigate the cellular substrates for interactions between ERα and CB1r. Forty-micron thick tissue sections of female Sprague Dawley rats were collected from mPFC and processed for immunocytochemical detection of ERα and CB1r. Our preliminary data show that CB1r is predominantly located in axon terminals that also express ERα. CB1r was also found colocalized with ERα-labeled cell bodies and dendrites. Occasionally, CB1r-labeled axon terminals contacted ERα-labeled dendrites. These results indicate that CB1r may affect the neuronal activity of ERα-responsive neurons in the mPFC. ERα may also affect the neurotransmitter release via interacting with CB1r in the mPFC.

Disclosures: J. Zhang: None. **B.A.S. Reyes:** None. **R.D. Shade:** None. **E.J. Van Bockstaele:** None.

Poster

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Topic: G.08. Drugs of Abuse and Addiction

Title: Acute delta-9-tetrahydrocannabinol reduces loss sensitivity in a binary choice task.

Authors: *S. WONG, S. R. RANDOLPH, V. E. IVAN, A. J. GRUBER;
Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: We have previously shown that lose-shift responding, wherein animals tend to switch actions following reward omission, is disrupted in rats by infusion of D-amphetamine into the dorsolateral striatum or by systemic injection of D-amphetamine. If this behavioral effect is due to the drug's ability to elevate the level of dopamine in the striatum, then other drugs that elevate tonic brain dopamine levels should also reduce lose-shift responding. Here we tested if intraperitoneal injection of Δ^9-Tetrahydrocannabinol (THC; 0.5-2 mg/kg) prior to the task would reduce lose-shift responding. THC did evoke a dose-dependent reduction of lose-shift responding, providing further evidence that lose-shift responding is driven by the brief pauses in activity of dopaminergic neurons proposed to signal negative reward prediction error.

Disclosures: S. Wong: None. **S.R. Randolph:** None. **V.E. Ivan:** None. **A.J. Gruber:** None.

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Topic: G.08. Drugs of Abuse and Addiction

Title: On the role of TRPV1 receptors within the brain in anxiety elicited by cocaine cues

Authors: *W. NORZE[1], A. LOYOLA[2], E. TORRES[2], C. MALDONADO-VLAAR[2];
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Abstract: Transient Receptor potential Vanilloid (TRPV1) is activated in peripheral terminals of nociceptive fibers by noxious heat, low pH and natural product such as capsaicin, and is expressed also in the brain where it seems to be involved in antinociception, locomotor control and regulation of affective behaviors. Capsazepine is a competitive antagonist of the vanilloid receptor. We investigated the effects of blockade of TRPV1 receptors in eliciting anxiolytic responses following exposure to cocaine related cues in rats. Male Sprague Dawley rats received daily intraperitoneal injections of cocaine (10 mg/kg) for five consecutive (D1-D5) prior to being placed in activity chambers. During the daily 90 min sessions, rats paired visual and olfactory cues with the cocaine treatment. Following one day of abstinence, animals were divided into two groups which received either vehicle or capsazepine (10 ug/kg, ip) and returned to the activity

chambers followed by Elevated Plus Maze (EPM) testing. Results showed that cocaine significantly increased locomotor activity and produced behavioral sensitization within the first five days. Animals treated with capsazepine showed no significant difference in locomotor behavior on Day 7 when compared to vehicle treated group ($p<0.05$, T-test). In the EPM, the capsazepine dose had no effect on the total time spent on open and close arms during 5 minutes of testing. Future studies are needed to test several doses of capsazepine in other to characterize a possible therapeutical profile of the drug.

Disclosures: W. Norze: None. **A. Loyola:** None. **E. Torres:** None. **C. Maldonado-Vlaar:** None.

Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Intramural Research Program

Title: Cocaine-induced endocannabinoid mobilization disinhibits mouse midbrain dopamine neurons

Authors: *D. I. DRYANOVSKI, C. R. LUPICA;
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Abstract: Endogenous cannabinoids (eCB) are lipid molecules released from postsynaptic neurons to activate cannabinoid CB1 receptors (CB1R) on presynaptic axon terminals to inhibit neurotransmitter release. 2-arachidonoylglycerol (2-AG) is an eCB closely associated with activity dependent modulation of synapses, and its synthesis can be triggered by activation of $G\alpha_{/11}$-coupled GPCRs via their coupling to phospholipase C-β (PLCβ). PLCβ can hydrolyze phosphatidylinositol 4,5-bisphosphate to form 1,2-dacylglycerol (DAG) and inositol triphosphate. DAG is then hydrolyzed to form 2-AG via diacylglycerol lipase-α (DGLα). 2-AG can then be degraded to inactive metabolites by monoacylglycerol lipase (MAGL). We found that retrograde eCB signaling plays a crucial role in modulating synapses on midbrain dopamine (DA) neurons located in the mouse ventral tegmental area (VTA), using electrophysiological recordings of synaptic GABA release ($GABA_B$ IPSCs). Antagonism of CB1Rs with AM251 increased GABAergic inhibition of VTA DA neurons, and this was not observed in transgenic mice lacking CB1. We also found that cocaine mobilized 2-AG in mouse VTA, causing inhibition of $GABA_B$ IPSCs via activation of presynaptic CB1Rs. Extracellular bath application

of THL, a DGLα inhibitor, significantly reduced cocaine inhibition of $GABA_B$ IPSCs. JZL184, an inhibitor of MAGL, increased inhibition of $GABA_B$ IPSCs by cocaine. The disinhibition of DA neurons by cocaine represents a plausible mechanism to explain interactions between this drug and the eCB system observed in reward and addiction studies. The establishment of this interaction in the mouse VTA permits a more thorough molecular dissection of the relevant pathways using transgenic approaches.

Disclosures: D.I. Dryanovski: None. **C.R. Lupica:** None.

Poster

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Topic: G.08. Drugs of Abuse and Addiction

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DA016511

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Title: From LTD to LTP and back again - Changes in synaptic metaplasticity after extinction from THC self-administration and cue induced relapse

Authors: *D. NEUHOFER, S. SPENCER, P. KALIVAS;
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Abstract: Synaptic metaplasticity is a form of higher order plasticity that is not necessarily expressed as a change in the efficacy of synaptic transmission but as a change in the direction or degree of synaptic plasticity that will be induced by a distinct stimulation pattern. While there is ample evidence that drug induced alternations of glutamatergic synaptic transmission in the nucleus accumbens (NAc) might be a cellular substrate for drug seeking, the mechanisms and consequences of changes in metaplasticity after drug abuse have been less well studied, and this especially holds true for neuroadaptations induced by the chronic use of the psychoactive ingredient of marijuana, delta-9-tetrahydrocannabidiol (THC). To evaluate THC-induced metaplasticity, we trained rats to self-administer THC and cannabidiol (CBD) in a 10:1 ratio. Synaptic metaplasticity after drug extinction and cue induced reinstatement was evaluated via whole cell patch clamp recordings in the NAc core, using a pairing protocol that induces

NMDA-dependent LTD in control animals. After extinction from THC+CBD self-administration we observed a switch in the valence of synaptic plasticity from LTD to LTP. Surprisingly these distinct changes in metaplasticity were fully reversed and LTD reestablished when slices were made from animals that underwent 15 min of cue-induced reinstatement. We will discuss experiments underway to target the molecular substrates that might mediate these drug-seeking induced changes in metaplasticity.

Disclosures: D. Neuhofer: None. **S. Spencer:** None. **P. Kalivas:** None.

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Title: Alterations in glutamatergic transmission after cannabinoid self-administration: parallels and distinctions with cocaine

Authors: *S. M. SPENCER, D. NEUHOFER, C. GARCIA-KELLER, M. D. SCOFIELD, D. SCHWARTZ, P. W. KALIVAS;
Neurosci., Med. Univ. of South Carolina, Charleston, SC

Abstract: A preponderance of evidence indicates that addiction to drugs of abuse produces enduring changes in brain synaptic physiology that contribute to the vulnerability to relapse. Chief among these, it has been shown that chronic cocaine, nicotine, heroin, and ethanol administration leads to dysregulated glutamatergic transmission in the nucleus accumbens core (NAcore). For example, following self-administration and extinction of each of these drugs there is a decrease in the expression and activity of GLT-1 (glial glutamate transporter). However, it was unknown whether chronic THC administration produces similar effects. Here we performed a glutamate uptake assay to assess GLT-1 function after THC self-administration and extinction,

but were surprised to discover that there was no difference in sodium-dependent glutamate uptake between vehicle and THC self-administering rats. Next we went on to assess whether there might be alterations in glutamatergic transmission measurable at the electrophysiological level using the whole-cell patch clamp techniques to assess the ratio of AMPA to NMDA currents (A/N), measure glutamate spillover via NMDA current decay, and evaluate NMDA-dependent LTD. In contrast to cocaine that produces a potentiation of A/N, after THC self-administration and extinction there was no change in AMPA/NMDA ratio. However, akin to cocaine we observed a loss of LTD in NAcore suggesting drug-induced metaplasticity. Moreover, preliminary data suggests that there is an increase in glutamate spillover in THC-extinguished rats, implying alterations in the elimination of glutamate although undetected by the glutamate uptake assay. In summary, these data illustrate that THC self-administration and extinction results in enduring changes in glutamatergic transmission in the NAcore and suggests both distinct and overlapping mechanisms between marijuana and cocaine may prime glutamate transmission to support relapse.

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Topic: G.08. Drugs of Abuse and Addiction

Title: The phytocannabinoid THC mimics effects of chronic mild stress to reduce dendritic spine density in the vocal learning-essential brain region Area X of zebra finch striatum

Authors: *T. L. HOLLAND, K. SODERSTROM;
East Carolina Univ., Greenville, NC

Abstract: Zebra finches learn a complex song during a developmental sensitive period through a process of sensorimotor integration and auditory feedback, which shares features with language development in humans. Chronic treatment with the synthetic cannabinoid full agonist WIN 55,212-2 during this developmental period persistently alters song quality, suggesting that exogenous cannabinoid agonist exposure alters normal late-postnatal brain development. Presently, we are studying the persistent effects of psychological stress on vocal development and adult song patterns. An acute stressor activates the hypothalamic-pituitary-adrenal (HPA) axis, causing increased release of the stress hormone corticosterone. This stimulates endocannabinoid release as part of a mechanism of feedback inhibition on HPA activity. We

hypothesize that enhanced endocannabinoid signaling following psychological stress may alter song learning in a manner similar to that caused by exogenous agonist exposure.
Developing (50 days old) and adult (>100 days old) male zebra finches (n=4) were administered vehicle or THC (3 mg/kg) treatments under concurrent no stress or stress conditions daily for 25 days. Injections occurred at 11 AM, and stress treatments were administered at 2-7 PM using a chronic mild unpredictable stress paradigm. 2-3 stressors were randomly chosen per day, and possible stressors included restraint stress (30 min), white noise (1 h), rubber snake (1 h), bright light (1 h), or food and water deprivation (1 h). Following the 25 day treatment period, zebra finches received no treatment for >25 days, until developing animals had matured. Vocalizations were recorded for 24 hours, and brains were collected for Golgi-Cox staining.
Area X is a striatal song region that is necessary for vocal learning as part of a basal ganglia-thalamocortical circuit. Both THC + No stress and Vehicle + Stress groups had similarly reduced dendritic spine densities relative to Vehicle + No stress controls ($p<0.05$, two-way ANOVA, Student-Newman-Keuls post-test). In contrast, spine densities following adult treatments with THC or Vehicle + Stress did not differ.
In light of the significance of Area X to vocal learning, we hypothesize that chronic THC treatment or stress during development, but not during adulthood, will persistently alter song quality. This is currently being tested via analyses of song recordings. Results will illuminate persistent effects of chronic THC or stress exposure on late postnatal brain development and may suggest mechanistic commonalties.

Disclosures: T.L. Holland: None. **K. Soderstrom:** None.

Poster

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA IRP

Title: Identification of cannabinoid CB_1 receptors in midbrain dopamine neurons

Authors: *H. ZHANG[1], X. HAN[1], G.-H. BI[1], Q.-R. LIU[1], E. S. ONAIVI[2], E. L. GARNDER[1], Z.-X. XI[1];
[1]Natl. Inst. on Drug Abuse Intramural Res. Program, Baltimore, MD; [2]Dept. of Biol., William Paterson Univ., Wayne, NY

Abstract: Δ^9-tetrahydrocannabinol (THC) is the principal psychoactive constituent of marijuana or cannabis. The major effect of THC is mainly mediated by cannabinoid CB_1 receptors (CB_1Rs). THC produces both rewarding and aversive effects in experimental animals. However, the receptor mechanisms underlying such opposite effects are unknown. It is generally believed that CB_1Rs are mainly expressed in brain glutamate and GABA terminals, but not in ventral tegmental area (VTA) dopamine (DA) neurons. However, conclusive evidence regarding the cellular distributions of CB_1Rs in the VTA is lacking. It is also unclear how CB_1Rs located in different cell populations modulate striatal DA release. In this study, we first used classical immunohistochemistry (IHC) assay to detect CB_1 expression in rat midbrain slices. We found, under low magnification, high densities of CB_1R immunostaining in the substantia nigra pars reticulata (SNr) and low densities of CB_1R in the VTA and substantia nigra pars compacta (SNc). Under high magnification, CB_1R immunostaining was found mainly in nerve fibers or terminals within the VTA and no clear CB_1R immunostaining was found in the cell bodies of VTA DA neurons. Due to the limitation of IHC in distinguishing CB_1R expression in VTA DA neurons and the fibers or projection terminals, we next used highly sensitive RNAscope *in situ* hybridization assays to detect CB_1 mRNA expression in rat midbrain slices. We found clear CB_1 mRNA expression in ~ 20% VTA DA neurons and ~40% SNc DA neurons. To confirm the CB_1 mRNA signal specificity, we crossed CB_1-floxed mice and DAT-cre mice to generate conditional CB_1-knockout mice (CB_1-cKO) in brain DA neurons. We detected similar CB_1 mRNA signaling in midbrain DA neurons only in WT mice, but not in CB_1-cKO mice. We also used similar strategies to detect CB_2 mRNA expression in midbrain DA neurons. We detected low densities of CB_2 mRNA in almost all VTA DA neurons in WT mice, but not in CB_2-cKO mice. In addition, we found CB_1 and CB_2 mRNA co-localization in VTA/SNc DA neurons. CB_1 mRNAs were also found in VTA glutamate neurons ($vGluT2^+$), and VTA glutamate-DA dual neurons ($vGluT2^+/TH^+$), and VTA GABA neurons ($GAD1^+$). Taken together, these findings, for the first time, provide convincing evidence that CB_1Rs are expressed in a small subpopulation of midbrain DA neurons. The functional significance of CB_1Rs in DA neurons is unknown. Further studies are needed to address this issue.

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA IRP

Title: Activation of GPR55 receptor attenuates nicotine self-administration in rodents

Authors: Y. HE[1], H.-Y. ZHANG[1], J.-T. GAO[2], G.-H. BI[1], *E. L. GARDNER[1], Z.-X. XI[1]; [1]NIDA/IRP, Baltimore, MD; [2]Jilin Med. Collage, Jilin, China

Abstract: Cannabinoid CB1 and CB2 receptors are involved in drug reward and addiction, and therefore, have been considered as important potential therapeutic targets for treatment of substance dependence. Interestingly, growing evidence indicates that many CB1 and CB2 receptor agonists or antagonists, such as Δ9-tetrahydrocannabinol, AM251, rimonabant, anandamide and CP55940 exhibit affinity for the GPR55 receptor, a potential cannabinoid-related orphan receptor. However, little is known as to whether the GPR55 receptor is also involved in drug reward and addiction and in other pharmacological actions produced by cannabinoids. Recent studies suggest that brain GPR55 receptors may modulate excitatory synaptic transmission, in a manner opposite to the CB1 receptor. Thus, we hypothesized that the GPR55 receptor may be involved in drug reward and dependence in a manner similar to the CB2 receptors. To test this hypothesis, we used a pharmacological approach for evaluating the role of the GPR55 receptor in a rodent self-administration paradigm. Specifically, O-1602, a potent GPR55-selective agonist, and CID 16020046, a selective GPR55 antagonist, were used as pharmacological. We found that: 1) O-1602 (10, 20mg/kg, i.p.) dose-dependently reduced intravenous nicotine self-administration in alcohol-preferring P rats under fixed-ratio 1 and progressive-ratio reinforcement schedules, and that these effects can be blocked by CID 16020046; 2) mice receiving the same doses of O-1602 displayed a similar reduction in nicotine self-administration, but not oral sucrose self-administration, suggesting a specific effect on nicotine self-administration, not due to non-specific sedation or locomotor impairment after O-1602 administration; 3) in vivo microdialysis demonstrated that systemic or local administration of O-1602 into the nucleus accumbens (NAc) had no effect on extracellular dopamine in the NAc, suggesting that non-dopaminergic mechanisms involved; finally, 4) immunohistochemistry assay detected significant GRP55 receptor expression in the prefrontal cortex (PFC), but not in the ventral tegmental area (VTA) in wild-type mice, but not in GPR55-KO mice, suggesting that the GPR55 receptor in the PFC may be the target of O-1602. Taken together, the present findings suggest that GPR55 may be involved in nicotine reward and addiction, and therefore deserves further studies as a promising therapeutic target for treatment of nicotine dependence.

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Title: Darylureas as allosteric modulators of the cannabinoid CB1 receptor

Authors: *Y. ZHANG[1], T. NGUYEN[1], A. DECKER[1], J.-X. LI[2], B. THOMAS[1], J. WILEY[1], T. KEN AKIN[3];
[1]Res. Triangle Inst., Research Triangle Park, NC; [2]Univ. at Buffalo, Buffalo, NY; [3]Univ. of North Carolina, Chapel Hill, NC

Abstract: Several classes of allosteric modulators of the CB1 receptor have recently been discovered which display unique pharmacological properties than the orthosteric ligands and may offer a much needed alternative strategy to modulate CB1 signaling for therapeutic benefits. We recently reported the first structure-activity relationship studies on one such modulator PSNCBAM-1. Here we will describe a series of diarylureas based on this scaffold with high CB1 potency and binding affinities.
All the compounds were characterized by MS, NMR and HPLC, and then evaluated in calcium-dependent functional assays in RD-HGA16 (Molecular Devices) cell lines stably expressing the CB1 receptor. These modulators reduced the Emax of CB1 receptor agonists (e.g. CP55940), as expected with negative allosteric modulators (NAMs), with low nanomolar IC50 values. In the binding assays, they increased the binding affinity of CB1 agonist CP55940, consistent with PSNCBAM-1 and other CB1 allosteric modulators such as Org27569. SAR studies suggest that the urea functionality is required for CB1 receptor activity. These compounds showed high selectivity against the CB2 receptor. These results will facilitate the development of potent and selective CB1 receptor modulators as potential medications for the treatment of drug addiction and related conditions.

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WPUNJ

Title: Cannabinoid type 2 receptors in brain dopamine neurons modulates anxiety-like and psychostimulant behaviors in floxed DAT-Cnr2 mouse model

Authors: *E. S. ONAIVI[1,1], H. ZHANG[2], E. L. GARDNER[2], H. ISHIGURO[3], Z.-X. XI[2], Q.-R. LIU[1];
[1]William Paterson Univ., Wayne, NJ; [2]NIDA-IRP/NIH, Baltimore, MD; [3]Univ. of Yamanashi, Yamanashi, Japan

Abstract: The functional neuronal expression of CB2 cannabinoid receptors has been a subject of controversy and debate and had been referred to as a "sphinx" wrapped in a mystery with "identity crisis", but no more. This is because research activities from our lab and those of others have found and reported that CB_2Rs are expressed in the mammalian brain and functionally involved in several dopamine (DA)-related and other CNS disorders including drug addiction in rodent models. Therefore, manipulation of CB_2R in mouse models is of critical importance in characterizing the molecular basis of CB_2R neuronal signaling mechanisms. The two available CB2R gene knockout mice contain partial *Cnr2* gene deletion at C- and N- terminal amino acid sequences and residues of CB2R activities might remain. Furthermore, these germline knockout mice in which the CB_2R function could be compromised by developmental compensation are not suitable for tissue- and cell-type specific studies at molecular, pharmacological and behavioral levels. Therefore, we have generated *Cnr2*-floxed mice that were crossed with DAT-*Cre* mice, in which the Cre recombinase expression is under DAT (dopamine transporter) gene promoter control, to generate conditional CB2-KO mice in midbrain DA neurons in DAT-*Cre*-*Cnr2*-Lox transgenic mice. By using a novel highly-sensitive RNAscope *in situ* hybridization method, we detected clear CB2 mRNA expression in VTA DA neurons in Dat-heterozygous and wildtype control mice, but not in conditional CB2-KO mice, suggesting neuronal CB2 gene expression in VTA DA neurons. The performance of the conditional DAT-*Cnr2* mutant mice were determined in motor function and emotionality tests in comparison to wild type controls. We report that in the motor function test using the spontaneous wheel running monitors, DAT-*Cre*-*Cnr2* homozygous mice were more responsive to cocaine induced motor activity than heterozygous

and wild type mice. In the plus maze test of aversive behavior, DAT-*Cre-Cnr2* homozygous mice were less aversive to the open arms of the maze than the heterozygous and the wild type mice. We conclude that CB2R in dopaminergic neurons plays a role in modulating anxiety-like and psychostimulant motor behaviors in mice.

Disclosures: E.S. Onaivi: None. **H. Zhang:** None. **E.L. Gardner:** None. **H. Ishiguro:** None. **Z. Xi:** None. **Q. Liu:** None.

Poster

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Topic: G.03. Emotion

Support: DFG Grant CRC-TRR 58 A04

Title: Distinctive sufficiency of cannabinoid CB1 receptor functions: contributions of GABAergic and glutamatergic components of the endocannabinoid system

Authors: *F. REMMERS[1], V. ENK[1], M. HÄRING[1], M. D. LANGE[2], S. RUEHLE[1], G. MARSICANO[3,4], H.-C. PAPE[2], B. LUTZ[1];
[1]Inst. of Physiological Chemistry, UMC Mainz, Mainz, Germany; [2]Inst. of Physiol. I, Westfaelische Wilhelms-University, Muenster, Germany; [3]Neurocentre Magendie INSERM U1215, Bordeaux, France; [4]Univ. of Bordeaux, Bordeaux, France

Abstract: The cannabinoid CB1 receptor is expressed in different neuronal subpopulations, including glutamatergic and GABAergic neurons. Via retrograde suppression of neurotransmission, it modulates a plethora of functions. The extent to which the CB1 receptor in these neurons is *necessary* for these functions has been addressed in studies using conditional knockout mice lacking the receptor in these neurons. However, little is known about the *sufficient role* of different CB1 receptor subpopulations.
To address *sufficiency* of the CB1 receptor subpopulations expressed in glutamatergic and GABAergic neurons, we used a conditional Cre-mediated cell-type-specific CB1 rescue strategy in a CB1-null background. Several behavioral domains that are modulated by the endocannabinoid system were analyzed: exploration, social behavior, fear and anxiety, metabolism, and seizure susceptibility.
Sufficiency of the dorsal telencephalic glutamatergic CB1 receptor subpopulation was found in the domains of social behavior, exploration, anxiety, metabolism, and seizure susceptibility, whereas the forebrain GABAergic subpopulation showed sufficient effects in the domains of

social behavior, exploration, seizures, and fear and anxiety. No sufficiency could be detected for the CB1 receptor in either neuronal population for the analgesic and cataleptic effects of exogenous stimulation of the endocannabinoid system. Although more sufficient effects were found after a glutamatergic rescue, on a few occasions GABAergic rescue resulted in additional effects that were opposite to normal endocannabinoid action. Including these opposite effects, both subsets of the CB1 receptor seem to have similar degrees of importance in the behavioral domains investigated.
This study revealed cell-type specificity of the actions of the endocannabinoid system and underlined the relevance of assessing not only necessary but also sufficient CB1 receptor functions, in order to understand the roles of this receptor in the context of the complexity of the brain.

Disclosures: F. Remmers: None. **V. Enk:** None. **M. Häring:** None. **M.D. Lange:** None. **S. Ruehle:** None. **G. Marsicano:** None. **H. Pape:** None. **B. Lutz:** None.

Poster

077. Cannabinoids: Neural Mechanisms and Addiction

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 77.25/BBB14

Topic: B.05. Transporters

Support: NIH/NIMH 1R01MH104488-01A1

Title: Macro extracellular vesicles, a novel transport system in the human brain

Authors: *H. PANTAZOPOULOS[1], M. ARDELT[2], A. BOYER-BOITEAU[2], S. A. MAUNEY[3], T.-U. W. WOO[1], M. MARKOTA[2], C. BERCIU[2], S. BERRETTA[1];
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Abstract: Growing evidence indicates molecules such growth and transcription factors, and miRNAs can travel long distances across the brain parenchyma, and from the choroid plexus (ChP) to specific regions deep into the brain. How can these molecules reach their targets across the dense and tortuous brain parenchyma without being diluted and/or catabolized? To answer this question we focused on OTX2, a non-cell autonomous transcription factor involved in developmental and adult brain functions. In adult rodents, OTX2 is detectable, but not synthesized in several brain regions, while the ChP may represent a main source of OTX2. Thus, OTX2 travels unidirectionally from the ChP to deep brain targets, transported by a yet unidentified mechanism. On the basis of preliminary findings of large OTX2-positive vesicles in

proximity of the lateral ventricles in the human brain, we tested the hypothesis that OTX2 is secreted by the ChP into vesicles that transport OTX2 through the cerebrospinal fluid (CSF) into the brain.
To test this hypothesis, we used a combination of in situ hybridization, immunohistochemistry, laser-capture micro-dissection, QRT-PCR, dual-fluorescence confocal and electron microscopy on human postmortem brain tissue and cerebrospinal fluid (CSF). Human ChP epithelial cell cultures were used to support observations made in postmortem tissue.
OTX2 protein was detected in all regions examined, amygdala, entorhinal cortex, prefrontal cortex, superior temporal gyrus, and visual cortex. Distinct populations of neurons and glia were immunoreactive (IR) for OTX2. In addition, we observed OTX2-IR, DAPI negative vesicles, ranging in size from 1-20 μm in diameter, which we termed 'macro extracellular vesicles' (MEVs). The largest MEVs were concentrated in areas corresponding to CSF flow, around the lateral ventricles and pial surfaces, and decreased in size deeper in the brain parenchyma. MEVs were detected in CSF and conditioned media from human ChP epithelial cells. Similarly, the human ChP contained labeled OTX2-IR epithelial cells and OTX2-IR MEVs. In contrast, none of the brain regions containing OTX2 protein expressed OTX2 mRNA. Analysis of specific cell populations confirmed that OTX2 mRNA is not expressed in pyramidal cells, PVB-IR neurons, or glia. Notably, OTX2 mRNA was detected robustly in the ChP.
Our result show a novel extracellular transport system, MEVs, that may carry molecules across long distances, in this case from the ChP, via CSF flow, to specific cell targets in the brain. This system has the potential to systemically administer factors that target specific cell populations in the brain, for therapeutic and research purposes.

Disclosures: H. Pantazopoulos: None. **M. Ardelt:** None. **A. Boyer-Boiteau:** None. **S.A. Mauney:** None. **T.W. Woo:** None. **M. Markota:** None. **C. Berciu:** None. **S. Berretta:** None.

Poster

078. Opioid Abuse

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 78.01/BBB15

Topic: G.08. Drugs of Abuse and Addiction

Support: IRP/NIDA/NIH/DHHS

Title: Context-induced relapse to the prescription opioid oxycodone in rats

Authors: *J. M. BOSSERT[1], S. ADHIKARY[1], H.-L. WANG[2], M. MORALES[2], Y. SHAHAM[1];
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Abstract: Background and objective: High relapse rates are a core feature in prescription opioid addiction, which drives the current drug overdose epidemic in the US. This public health problem is not the focus of current basic research on drug relapse, which primarily focuses on illicit drugs like heroin and cocaine. Here, we present a rat model, based on our previous studies with illicit drugs, to study context-induced reinstatement to oxycodone seeking after repeated cycles of drug self-administration and extinction.
Methods: We trained rats to self-administer oxycodone (0.1 and 0.05 mg/kg/infusion; 8-10 days per dose, 6-h/d) in Context A; lever pressing was paired with a discrete cue. Next, we extinguished lever pressing in the presence of the discrete cue in Context B for 9-11 days (6-h/d). We then tested them in both Contexts A and B under extinction conditions (1-h/day). Following this, we retrained the rats to self-administer oxycodone in Context A, re-extinguished lever pressing in Context B, and retested rats in Contexts A and B. We also tested the effect of the preferential mu opioid receptor antagonist, naltrexone (0, 0.5 or 1.0 mg/kg, s.c.) on context-induced reinstatement.
Results: Rats re-exposed to the oxycodone (Context A), but not the extinction (Context B) context, reinstated lever pressing; pretreatment with 1.0, but not 0.05 mg/kg naltrexone, decreased this reinstatement.
Conclusions: We modified an established rat model of relapse to illicit drugs to study relapse to oxycodone seeking after repeated cycles of drug self-administration and abstinence. We also showed a role of mu opioid receptors in context-induced relapse to oxycodone seeking.

Disclosures: J.M. Bossert: None. **S. Adhikary:** None. **H. Wang:** None. **M. Morales:** None. **Y. Shaham:** None.

Poster

078. Opioid Abuse

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 78.02/BBB16

Topic: G.08. Drugs of Abuse and Addiction

Support: US Public Health Service (USPHS) grants R01 MH92412

Title: Attenuated conditioned place preference for buprenorphine in stress-exposed mice.

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[2]Psychiatry, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Ongoing clinical and preclinical trials have highlighted the considerable antidepressant potential for low doses of the mixed opioid analgesic buprenorphine (BPN), an FDA approved medication for opioid addiction and chronic pain. Despite these promising results, the abuse liability associated with the potent partial agonist activity of BPN at mu opioid receptors (μ-ORs) may impede repurposing BPN as a therapeutic for major depressive disorder (MDD). Utilizing a rodent model of depression, unpredictable chronic mild stress (UCMS), to induce a robust depressive phenotype, these studies evaluated the effects of BPN (0.25 mg/kg) in stressed and non-stressed (NS) mice in a condition place preference (CPP) paradigm. UCMS consisted of exposure to three unpredictable mild stressors per day for 14 days and resulted in diminished bodyweight gain ($p<0.001$) and significant anhedonia, as measured by reductions in sucrose preference compared to NS controls ($p<0.001$). Mice were randomized into four unbiased experimental groups for CPP based on pretest scores established on day 0: NS/Vehicle, NS/BPN, UCMS/Vehicle and UCMS/BPN. All mice received 8 conditioning days of 30 min exposures, alternating vehicle (day 1, 3, 5 and 7) and drug (day 2, 4, 6 and 8). On day 12, the time spent by each mouse in both chambers of the apparatus was recorded. NS mice exhibited robust preference for BPN ($p<0.001$) compared to vehicle-paired NS mice. This effect was significantly attenuated in UCMS-exposed mice ($p<0.01$). In addition, sucrose preference tested on day 13, revealed that BPN normalized the anhedonia in UCMS mice compared to the UCMS/Vehicle treated group ($p<0.05$). These results indicate that the rewarding effects of BPN are blunted in mice exposed to chronic stress, just as chronic stress has been shown to blunt other forms of reward. Moreover, BPN administration reversed the behavioral deficits induced by UCMS in these animals, possibly due to its antidepressant effects. These data in animals support the need for clinical studies to evaluate the abuse potential of low doses of BPN in depressed patients separately from populations of opioid abusers or normal volunteers.

Disclosures: **C.A. Browne:** None. **R. Nussbaum:** None. **W. Palmer:** None. **I. Lucki:** None.

Poster

078. Opioid Abuse

Location: Halls B-H

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Program#/Poster#: 78.03/BBB17

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA DA033373

Title: Modulating remifentanil choice

Authors: *J. J. CHOW, J. S. BECKMANN;
Psychology, Univ. of Kentucky, Lexington, KY

Abstract: Drugs of abuse, such as opioids, are a significant public health problem. However, the mechanisms that drive opioid-associated choice are unknown. Alternative reinforcers have been shown to compete with drugs of abuse. Herein, we assessed how an alternative reinforcer (palatable food pellet) can modulate the choice for an opioid reward (remifentanil), using a dependent schedule and free choice procedure. Male Sprague Dawley rats were first magazine shaped and then trained to lever press for sucrose pellets on a FR1 to FR5 schedule. Following FR5 lever training, an orienting response was trained into the response chain, where the onset of the houselight signaled a contingent head entry (orienting response) into the magazine, which turned off the houselight and extended the response levers. Rats were then catheterized. Following catheterization recovery, rats were then trained on an FR1 to FR5 schedule for 10 μg/kg/infusion of remifentanil on a single lever. Next, rats were allowed to earn five sucrose pellet reinforcers and five remifentanil reinforcers (10 μg/kg/infusion) on a FR5, where presentation of the associated lever (remifentanil or food) was randomly presented alone. Finally, rats were placed on a dependent schedule or free choice procedure. Both choice procedures consisted of 5 blocks, where remifentanil dose increased as a function of block (0, 0.32, 1.0, 3.2, and 10 μg/kg/infusion) on one alternative, while the other alternative consistently offered a single 45-mg palatable food pellet across all blocks. The free choice procedure consisted of 2 sample trials per reinforcer and 6 choice trials per block, where choice of one option forwent the other option. The dependent schedule consisted of 6 trials (3 remifentanil and 3 food) per block, where allocation of reinforcement across alternatives was randomized on each trial to maintain equal experience with each option across blocks. Upon stability, food restriction, removal of drug infusion cues, and removal of the orienting response were manipulated to determine the effects on remifentanil versus food choice. Moreover, a matching analysis was used to compare changes to relative magnitude sensitivity and choice bias. Overall, the results indicate that remifentanil preference is dose-dependent. Additionally, food restriction and removal of drug infusion cues shifted preference towards food, while removal of the head entry shifted preference towards remifentanil. Collectively, the results herein indicate that remifentanil value is relative and can be modulated by an alternative. Future use of drug choice procedures will help to isolate variables of importance in drug-associated decision making.

Disclosures: **J.J. Chow:** None. **J.S. Beckmann:** None.

Poster

078. Opioid Abuse

Location: Halls B-H

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Program#/Poster#: 78.04/BBB18

Topic: G.08. Drugs of Abuse and Addiction

Support: NSERC

CIHR

Title: Modulation of traumatic memory acquisition and recall via prefrontal cortical dopamine d4 and d1 receptor transmission differentially controls opiate reward sensitivity: implications for addiction comorbidity in post traumatic stress disorder

Authors: ***J. LI**, J. RENARD, H. SZKUDLAREK, S. LAVIOLETTE;
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Abstract: PTSD and opiate addiction share strong co-morbidity and the inability to suppress obtrusive memory recall related to either stressful or rewarding experiences may be an underlying neuropsychological feature triggering PTSD and/or addiction. Our previous research has shown that dopamine (DA) transmission in the prefrontal cortex (PFC) strongly modulates emotional memory formation: activation of the DA D4 receptor (D4R) strongly potentiates the emotional salience of normally non-salient fear memories whereas DA D1 receptor (D1R) activation blocks the behavioural recall of fear memory. Thus, while intra-PFC D4 transmission strongly controls the acquisition of emotional memory, D1 transmission is selectively involved in the recall phase of emotional memory processing. Using a pre-clinical model of PTSD in rats, we examined if recall of associative fear memory would increase subjects' sensitivity and vulnerability to morphine addiction. We also examined if blocking traumatic memory recall with PFC D1R stimulation may block this effect and if artificially creating a fear memory with PFC D4R stimulation would increase morphine reward sensitivity. Using an olfactory fear conditioning paradigm, we conditioned salient or non-salient associative fear memories by delivering supra-threshold (0.8 mA) vs. sub-threshold (0.4 mA) foot shock conditioning cues, and tested if recalling these memories increased sensitivity to morphine's rewarding properties, measured in a conditioned place preference (CPP) paradigm. We then examined the effects of intra-PFC DA D1R/D4R activation on expression and acquisition phases of associative fear memories and the subsequent influence on morphine reward sensitivity. Rats receiving supra-threshold fear conditioning showed strong associative fear memories and strongly potentiated morphine reward sensitivity. PFC activation of D1 receptor transmission with SKF 81297 (10-100 ng), dose-dependently blocked the recall of fear memory and similarly blocked the potentiation of morphine reward CPP through a cyclic AMP-dependent molecular pathway. In contrast, PFC D4 activation with PD-168077 (50 ng) during memory acquisition, created false fear memories in rats receiving sub-threshold foot shock. Remarkably, D4-mediated potentiation of normally non-salient fear memories also caused a dramatic potentiation in morphine reward sensitivity. Our findings have important implications for the role of the PFC DA receptor transmission in PTSD-related traumatic memory acquisition and recall and suggest that dysregulation of PFC DA transmission may underlie co-morbidity between PTSD and opiate addiction.

Disclosures: **J. Li:** None. **J. Renard:** None. **H. Szkudlarek:** None. **S. Laviolette:** None.

Poster

078. Opioid Abuse

Location: Halls B-H

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Program#/Poster#: 78.05/BBB19

Topic: G.08. Drugs of Abuse and Addiction

Title: The effects of orexin-1 receptor antagonist into the ventral tegmental area on forced swim stress-induced reinstatement of morphine in rats

Authors: *R. AZIZBEIGI[1], M. MORADI[2], A. HAGHPARAST[2];
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Abstract: It has been shown that the hypothalamic neuropeptide orexin (hypocretin) is not only involved in conditioned reward processes, but also in cue induced reinstatement of morphine. Regarding the effect of stress on reinstatement of morphine, it is shown that the ventral tegmental area (VTA) is one of the most important area involved which receive massive orexinergic inputs. Thus, in the present study, we investigated the role of orexin-1 receptor in the VTA in stress-induced reinstatement of morphine. The conditioned place preference (CPP) paradigm was done in adult male Wistar rats weighing 220 - 280 g, and conditioning score and locomotor activities were recorded by Ethovision software. After pre-test (day1), animals received morphine (5 mg/kg) during the 3-day conditioning phase and the next day (post-test; day 5) tested for CPP scores. In the extinction phase, rats were daily put in the CPP box for 30 min until the CPP score in two continuous days become similar to those on the pre-test; extinction happened on day13. On reinstatement day (day14), animals were microinjected bilaterally into the VTA by different doses of orexine-1 receptor antagonist, SB334867 (0.3, 1 , 3 nM/0.3 µl DMSO per side), then exposed to forced swim stress (FSS) for 6 min before subcutaneous injection of ineffective dose of morphine (0.5 mg/kg) to facilitate the reinstatement of extinguished morphine-CPP. The results revealed that FSS induced reinstatement of extinguished morphine-CPP and SB334867 deceased the FSS-induced reinstatement at the higher doses (1 and 3 nM/0.3 µl DMSO per side). Findings shows that intra-VTA orexin receptors have a role in reinstatement of morphine and it seems that stress partially exerts its effects on the reinstatement of morphine via orexin receptors in the VTA.

Disclosures: R. Azizbeigi: None. **M. Moradi:** None. **A. Haghparast:** None.

Poster

078. Opioid Abuse

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA R21 DA037728

Minneapolis Medical Research Foundation: Translational Addiction Research Program

Minneapolis Medical Research Foundation: Career Development Award

Title: Relationship between locomotor activity and individual differences in morphine self-administration in rats

Authors: *A. C. HARRIS[1], P. MUELKEN[1], M. G. LESAGE[1], J. C. GEWIRTZ[2];
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Abstract: Understanding factors contributing to individual differences in opioid addiction vulnerability is essential for developing more effective preventions and treatments. Locomotor activity in a novel environment predicts individual differences in self-administration (SA) of several drugs of abuse (e.g., cocaine, amphetamine) in rodents, but the relationship between this variable and individual vulnerability in opiate SA has not been well established. The goal of the current study was to evaluate the ability of locomotor activity to predict individual differences in morphine SA in rats. The reinforcing efficacy of morphine was evaluated using a behavioral economic approach. This framework has been useful for studying individual differences in addiction vulnerability in both humans and animals, but has not previously been applied to morphine SA in rodents. Following an initial locomotor activity screen, animals were allowed to acquire morphine SA at a unit dose of 0.5 mg/kg/infusion in 4 hour/day sessions (Experiment 1) or 0.2 mg/kg/infusion in 2 hour/day sessions (Experiment 2) until infusion rates were stable. Unit price was subsequently manipulated via progressive reductions in unit dose (Experiment 1) or increases in response requirement per infusion (Experiment 2). Activity levels were not associated with acquisition of MSA or elasticity of demand (reinforcing efficacy) in either Experiment. Our findings indicate that locomotor activity in a novel environment did not predict individual differences in morphine SA in rats. These data contrast with findings using other drugs of abuse, and suggest that unique factors may contribute to individual differences in opioid addiction vulnerability.

Disclosures: **A.C. Harris:** None. **P. Muelken:** None. **M.G. LeSage:** None. **J.C. Gewirtz:** None.

Poster

078. Opioid Abuse

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Topic: G.08. Drugs of Abuse and Addiction

Support: 14182MFDS979

NRF-2013R1A1A2062362

NRF-2013R1A6A3A01027711

Title: Inhibitory effects of SB366791, a selective TRPV1 antagonist, on morphine self-administration and anxiety-like behavior in rats after withdrawal of morphine

Authors: ***S. MA**[1], S.-Y. LEE[2], C.-G. JANG[2];
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Abstract: TRPV1, the archetypal member of the vanilloid TRP family, was initially identified as the receptor for capsaicin, the pungent ingredient in hot chili peppers. We previously demonstrated that TRPV1 in the dorsal striatum significantly contributes to morphine reward using the conditioned place preference (CPP) paradigm in mice, but it is unknown whether TRPV1 has the same effect in other reward models. In this study, we investigated the role of TRPV1 in morphine reward using a self-administration paradigm in rats. We found that treatment with a selective TRPV1 antagonist, SB366791, significantly decreased morphine self-administration on a fixed-ratio 1 schedule or a progressive ratio schedule of reinforcement. Furthermore, administration of SB366791 decreased an anxiolytic-like effect during the morphine abstinence period. Moreover, treatment with SB366791 significantly decreased morphine-priming reinstatement. Taken together, our findings suggest that blockade of TRPV1 receptors could provide an approach to limiting morphine addiction.

Disclosures: **S. Ma:** None. **S. Lee:** None. **C. Jang:** None.

Poster

078. Opioid Abuse

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Topic: G.08. Drugs of Abuse and Addiction

Support: USU Intramural Grant

Center for the Study of Traumatic Stress

Title: Cerebral glucose utilization (^{18}F-FDG-PET) following intravenous morphine self-administration in rats

Authors: *K. CHOI[1], T. PARK[1], K. NISHIDA[1], C. WILSON[2], S. JAISWAL[2], J. SCOTT[2], A. HOY[3], R. SELWYN[3], B. DARDZINSKI[3];
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Abstract: Biological mechanisms by which opioid drugs impair brain energy utilization, and thus contribute to opiate addiction, remain unclear. Using computed tomography (CT), ^{18}fluoro-deoxyglucose positron emission tomography (^{18}F-FDG-PET), and intravenous morphine self-administration (MSA), we investigated the effects of voluntary morphine intake and different ^{18}F-FDG uptake conditions on cerebral glucose utilization in rodents. Jugular vein cannulated male Sprague-Dawley rats self-administered intravenous saline or morphine (0.5 mg/kg/infusion, 4hrs/day) for 12 days. The animals were scanned at a baseline (pre-MSA) and 2 days after chronic MSA. One batch of animals (morphine and saline group) was anesthetized with isoflurane and the other (morphine and saline group) was kept awake during the ^{18}F-FDG uptake period. PET/CT images were analyzed using a 3D rat brain atlas for volume of interest and a statistical parametric mapping (SPM) for voxel-based analysis. The MSA group exhibited locomotor hyperactivity, constipation, and delayed weight gain during the self-administration period as compared with the saline controls. Isoflurane anesthesia reduced glucose utilization mainly in the cortical regions while increasing glucose utilization in the subcortical regions including midbrain and hypothalamus. Spontaneous withdrawal from chronic morphine increased glucose utilization in the basal ganglia only in isoflurane anesthetized ^{18}F-FDG uptake. This suggests that *in vivo* regional glucose utilization is different between isoflurane anesthetized and awake ^{18}F-FDG uptake conditions. Increased glucose utilization in the basal ganglia following chronic MSA may be associated with the development of opiate addiction. This study demonstrates the utility of combining a non-invasive brain imaging technology with an intravenous drug self-administration paradigm to enhance our understanding of the biological mechanisms of opiate addiction.

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Poster

078. Opioid Abuse

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Topic: G.08. Drugs of Abuse and Addiction

Support: DA033526 (EC)

Brooking Fellowship (MM)

Title: Oxycodone self-administration in male and female rats

Authors: ***M. MAVRIKAKI**[1], M. PRAVETONI[2], S. PAGE[1], D. POTTER[1], E. CHARTOFF[1]; [1]Harvard Med. School, McLean Hosp., Belmont, MA; [2]Departments of Med. and Pharmacology, Univ. of Minnesota, Minneapolis, MN

Abstract: The prescription opioid oxycodone is one of the most widely prescribed painkillers in the US. However, its use is complicated by high abuse potential. Since sex differences have been described in most stages of drug addiction, the present study tests if there are sex differences in oxycodone intravenous self-administration, a rodent model of drug addiction. Male and female Sprague-Dawley rats were implanted with jugular vein catheters and trained to self-administer oxycodone (0.03mg/kg/infusion). Rate of acquisition and maintenance of self-administration behavior on fixed ratio 1 (FR1), FR2, and FR5 schedules of reinforcement were measured. In addition, sensitivity to the reinforcing effects of oxycodone (dose response), and motivation to work for oxycodone (progressive ratio) were measured. In a separate cohort of rats, distribution of oxycodone to serum and brain were measured after intravenous delivery. On an FR1 schedule of reinforcement, male rats self-administered more oxycodone than females. On FR2 and FR5 schedules, no significant sex differences in drug intake were observed, although females had significantly more inactive lever presses than males. In the dose response experiment, females tended to self-administer more oxycodone across doses, but this effect was not significant. Similarly, there was a trend for females to work harder for oxycodone in the progressive ratio experiment. No significant sex differences were observed in plasma or brain oxycodone levels, suggesting that sex differences in oxycodone self-administration behavior are not due to pharmacokinetics. Taken together, our results suggest that there are sex differences in abuse liability of oxycodone, which has ramifications for the treatment of oxycodone dependence and abuse.

Disclosures: **M. Mavrikaki:** None. **M. Pravetoni:** None. **S. Page:** None. **D. Potter:** None. **E. Chartoff:** None.

Poster

078. Opioid Abuse

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH-CSORDA DA005010

Title: Mu opioid receptors in striatal medium spiny neurons are not necessary for opiate reward but contribute to heroin and food seeking

Authors: *P. CHARBOGNE[1,2], O. GARDON[2], E. MARTÍN-GARCÍA[3], H. L. KEYWORTH[4], A. MATSUI[5], A. ROBÉ[2], L. MOQUIN[1], A. MATIFAS[2], K. BEFORT[6], C. GAVÉRIAUX-RUFF[2], A. GRATTON[1], I. KITCHEN[4], A. BAILEY[4,7], V. A. ALVAREZ[5], R. MALDONADO[3], B. L. KIEFFER[1,2];
[1]Douglas Mental Hlth. Univ. Inst., Verdun, QC, Canada; [2]CNRS/INSERM/Université de Strasbourg, Inst. de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France; [3]Departament de Ciencies Experimentals i de la Salut, Univ. Pompeu Fabra, PRBB, Barcelona, Spain; [4]Univ. of Surrey, Guildford, Fac. of Hlth. and Med. Sci., Surrey, United Kingdom; [5]Section on Neuronal Structure, Natl. Inst. on Alcohol Abuse and Alcoholism, Natl. Inst. of Hlth., Bethesda, MD; [6]CNRS, LNCA – UMR7364, Faculté de Psychologie, Neuropôle de Strasbourg – Univ. de Strasbourg, Strasbourg, France; [7]St. George's Univ. of London, Inst. of Med. & Biomed. Educ., London, United Kingdom

Abstract: The mu opioid receptor (MOR), widely expressed throughout the nervous system, mediates analgesic and addictive opiates effects. Several brain areas responsible for MOR-mediated reward have been identified by intracranial pharmacological manipulations; however underlying circuit mechanisms are unclear. In this study, we investigated whether MORs expressed in striatal medium spiny neurons (MSNs) contribute to central effects of opiates, with particular emphasis on addiction-related behaviors.
We developed a conditional knockout line (Dlx-MOR) by crossing floxed mice for the MOR gene *Oprm1* with transgenic mice expressing Cre recombinase in GABAergic forebrain neurons (Dlx5/6-Cre). First, we determined MORs distribution in these animals using qRT-PCR and autoradiographic binding. MOR mRNA expression analysis reveals inactivation of *Oprm1* in the striatum (nucleus accumbens, NAc, and dorsal striatum) only. Ventral tegmental area (VTA) slice electrophysiological recordings indicate a MOR-mediated inhibitory control over Dlx-MOR

dopamine (DA) but no GABA neurons, demonstrating that within VTA, presynaptic MSN-MORs are lacking, while MORs expressed by interneurons or other regions are intact. Second, we examined opiate effects on dopamine release and behavior. In Dlx-MOR mice, heroin fails to increase locomotor activity. Heroin reward in the conditioned place preference test (CPP), as well as heroin-induced NAc DA release measured in microdialysis experiment, is preserved in mutant mice, concordant with intact MOR expression in the VTA. We further studied heroin and palatable food self-administration (SA). In both experiments, Dlx-MOR mice improve operant performance in both fixed and progressive ratio sessions, which contrasts with CPP and microdialysis results. After extinction phase, they reinstate heroin SA upon cue exposure while control mice do not, revealing seeking behavior.
In conclusion, our targeted inactivation of *Oprm1* first demonstrates that MSN-MORs mediate locomotor effects of opiates. Second, the study shows that heroin rewarding effects are not mediated by striatal MORs, in agreement with the 2-neuron VTA model. Third, our data reveal a novel, yet unreported role of MSN-MORs in seeking behaviors for both drug and natural rewards. MORs therefore exert multiple functions throughout brain circuits of reward, which extend beyond hedonic control, and our study opens the way to circuit mapping of opioid mechanisms underlying motivation and decision-making.

Disclosures: **P. Charbogne:** None. **O. Gardon:** None. **E. Martín-García:** None. **H.L. Keyworth:** None. **A. Matsui:** None. **A. Robé:** None. **L. Moquin:** None. **A. Matifas:** None. **K. Befort:** None. **C. Gavériaux-Ruff:** None. **A. Gratton:** None. **I. Kitchen:** None. **A. Bailey:** None. **V.A. Alvarez:** None. **R. Maldonado:** None. **B.L. Kieffer:** None.

Poster

078. Opioid Abuse

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 78.11/BBB25

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH-CSORDA DA005010

Title: Mu opioid receptors in the habenula: dissecting reward and aversion in addiction

Authors: *L.-J. BOULOS[1], E. DARCQ[2], C. GAVÉRIAUX-RUFF[3], B. L. KIEFFER[2];
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Abstract: It is well-established that mu-opioid receptors (MORs) mediate the potent analgesic and rewarding effects of opiates (Matthes et al., Nature, 1996). Further, these receptors are central to reward processing for both drugs of abuse (Contet et al., Current opinion in neurobiology, 2004) and natural stimuli (Moles et al., Science, 2004), and largely contribute to the initiation of addictive behaviors. Finally, chronic MOR activation induces neuroadaptations that contribute to drug withdrawal symptoms and the negative and aversive emotional states associated to protracted abstinence (Goeldner et al., Biological Psychiatry, 2011; Lutz et al., Neuropsychopharmacology, 2014). Traditionally, the positive rewarding effects of opioids have been attributed to opioid receptors in the mesolimbic tract. Intriguingly, the medial habenula (MHb), a small epithalamic brain structure that shows the highest MOR density, has been shown to be implicated in both reward and aversive processes. Here, we created and characterized a conditional knockout mouse model that lacks MORs specifically in the MHb -using the nicotine receptor subunit ChrnB4 as a promoter- (B4-MOR mice) to dissect the role of MHb MORs in reward. Our cellular characterization of B4-MOR mice using PCR, qPCR, RNAscope and immunohistochemistry techniques proved that the conditional knockout mouse successfully shows a reduction of Hb MORs. In addition, our RNAscope results indicate that MORs are mainly expressed in glutamatergic substance P positive neurons in the MHb but they also colocalize with acetylcholine transferase in a smaller population of habenular neurons, consistent with protein distribution (Gardon et al., Neuroscience, 2014). Our first behavioral results show a reduction of withdrawal symptoms upon chronic morphine exposure in B4-MOR mice, thus indicating a role of Hb MORs in aversive processes associated to abstinence.

Disclosures: L. Boulos: None. **E. Darcq:** None. **C. Gavériaux-Ruff:** None. **B.L. Kieffer:** None.

Poster

078. Opioid Abuse

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA Grant DA009815

PA DOH, CURE funds SAP# 4100055576

Title: Pretreatment with low dose naltrexone prevents death due to heroin overdose in rats

Authors: *P. S. GRIGSON, C. B. JENNEY, P. J. MCLAUGHLIN, I. S. ZAGON, M. VARVARIS;
Dept Neural/Behav Sci., Pennsylvania State Univ. Col. of Med., Hershey, PA

Abstract: Addiction is a growing problem worldwide, with an estimated 187,000 drug-related deaths in 2013 alone. Opioids are said to account for about three quarters of these cases. In the United States, opiate overdose is now recognized as an 'epidemic' in many states and overdose has surpassed car accidents as the leading cause of accidental death. The number of opiate overdose deaths more than quadrupled in a single decade nationwide, increasing from 4,030 in 1999 to 16,651 in 2010. These numbers soared to 43,982 in 2013. Unfortunately, such deaths continue to occur despite recent approval of the opiate blocker, naloxone, for use by caregivers and first responders. Here, rather than rescue one from opiate overdose with an opiate blocker, we tested whether opiate overdose could be prevented, altogether, in rats by daily administration of a relatively inexpensive opiate blocker, low dose naltrexone (LDN). The results showed that while 53.8% of the saline pretreated rats died following infusion of the high dose of heroin, only 14.3% and 13.3% of the rats died of heroin overdose when pretreated daily with a 1.0 or a 3.0 mg/kg dose of naltrexone, respectively. This constitutes a 74.4% reduction in heroin overdose death in LDN vs. saline pretreated rats. If these findings translate to humans, thousands of opiate deaths may be prevented yearly in the United States and around the world.

Disclosures: P.S. Grigson: None. **C.B. Jenney:** None. **P.J. McLaughlin:** None. **I.S. Zagon:** None. **M. Varvaris:** None.

Poster

078. Opioid Abuse

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 78.13/CCC1

Topic: G.08. Drugs of Abuse and Addiction

Title: VK4-116, a highly selective and metabolically stable D3R antagonist, inhibits oxycodone addiction-like behaviors in rodents.

Authors: *Z.-B. YOU, G.-H. BI, V. KUMAR, E. L. GARDNER, Z.-X. XI, A. H. NEWMAN;
Mol. Targets and Medications Discovery Br., NIDA-IRP/NIH/DHHS, Baltimore, MD

Abstract: Prescription opiates such as oxycodone are highly effective analgesics used in clinical pain management. One of the adverse effects that limits opiate analgesic use is the tendency of some patients to develop dependence. Therefore, development of effective pharmacotherapeutic agents to reduce the addictive potential of opiate analgesics, facilitate detoxification in opiate

dependence and prevent drug relapse from opiate abstinence has been a major focus of opiate addiction research. In the present study, we investigated the potential utility of VK4-116, a novel highly selective and metabolically stable dopamine D3 receptor (D3R) antagonist in the treatment of oxycodone abuse and dependence in rodents. In mice, pretreatment with VK4-116 (5-15 mg/kg, i.p.) showed no effect on basal levels of locomotor activity, but dose-dependently inhibited oxycodone-induced (4 mg/kg, i.p.) locomotor activation. Repeated treatment of mice with oxycodone (4 mg/kg for 5 days) progressively increased oxycodone-induced locomotor activation while pretreatment with VK4-116 (5-15 mg/kg) dose-dependently blocked this effect. In order to evaluate the effects of VK4-116 on opiate reward, male Long-Evans rats were first allowed to self-administer oxycodone at 0.1 mg/kg/infusion (3 h per daily session) for 2 weeks under an FR-1 schedule of reinforcement, followed by 0.05 mg/kg/infusion for an additional week, and subsequently tested following pretreatment with VK4-116 (5-25 mg/kg, i.p; 15 min before testing). We found that pretreatment with VK4-116 dose-dependently inhibited oxycodone self-administration. Furthermore, pretreatment with VK4-116 (5-15 mg/kg) dose-dependently inhibited drug-seeking tested in trained rats given an extinction session in which oxycodone was replaced by saline, and blocked oxycodone-induced (1 mg/kg, i.p.) reinstatement of drug-seeking after the drug-seeking behavior was extinguished. Importantly, in oxycodone treated rats (3 mg/kg, i.p; twice daily for 7 days), VK4-116 (5-15 mg/kg) dose-dependently blocked naloxone (1 mg/kg s.c.) precipitated conditioned place aversion. Taken together, these findings suggest an important role for brain D3R in opiate reward and dependence. The novel D3R antagonist VK4-116 is effective not only in diminishing the rewarding effects of oxycodone, but also the aversive responses precipitated by blockade of opiate receptors in dependent animals. Thus, VK4-116 may serve as an effective agent for mitigating the development of opiate addiction, reducing the severity of withdrawal and preventing relapse. (*Supported by NIDA IRP*)

Disclosures: **Z. You:** A. Employment/Salary (full or part-time): Molecular Targets and Medications Development Branch, NIDA-IRP, NIH, Baltimore MD 21224. **G. Bi:** None. **V. Kumar:** None. **E.L. Gardner:** None. **Z. Xi:** None. **A.H. Newman:** None.

Poster

078. Opioid Abuse

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 78.14/CCC2

Topic: G.08. Drugs of Abuse and Addiction

Title: Environmental enrichment reduces heroin seeking; a novel approach to treat heroin relapse.

Authors: *E. J. GALAJ[1], M. MANUSZAK[2], R. RANALDI[3];
[1]City Univ. of New York, New York, NY; [3]Psychology, [2]Queens College/CUNY, Flushing, NY

Abstract: Heroin-related cues can trigger craving and relapse in addicts or heroin seeking in rats. In the present study we investigated whether environmental enrichment (EE) implemented after heroin exposure can reduce cue-induced reinstatement of heroin seeking and the expression of heroin conditioned place preference. In experiment 1, male Long Evans rats that already acquired a heroin self-administration habit, were housed in an enriched or non-enriched environment, underwent extinction and later were tested for cue-induced reinstatement of heroin seeking. In experiment 2, rats were conditioned with heroin in one compartment of a CPP apparatus and saline in the other, exposed to 30 days of enrichment/no enrichment and were later tested for heroin CPP. The results showed that exposure to EE significantly reduced responding during the reinstatement test (experiment 1) and prevented the expression of heroin CPP (experiment 2). Our findings suggest that EE can be an effective behavioral approach to diminish the effects of conditioned cues on heroin seeking.

Disclosures: E.J. Galaj: None. **M. Manuszak:** None. **R. Ranaldi:** None.

Poster

078. Opioid Abuse

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Topic: G.08. Drugs of Abuse and Addiction

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R21DA038738-01A1

Transformative Training Program in Addiction Science (Burroughs Wellcome 9550300872)

T32GM00854118

Title: Identification of a major qtl influencing oxycodone behavioral sensitivity and dependence

Authors: *L. R. GOLDBERG[1], S. KIRKPATRICK[1], N. YAZDANI[1], K. LUTTIK[1], M. MULLIGAN[2], C. BRYANT[1];
[1]Boston Univ. Sch. of Med., Boston, MA; [2]Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Opioid addiction is heritable, yet its genetic basis remains poorly understood. Mice are valuable for identifying novel genes that contribute to variation in addiction-associated phenotypes including acute psychomotor stimulation and conditioned reward. The closely related C57BL/6J and C57BL/6NJ strains exhibit limited genetic diversity, yet show significant strain differences in several addiction-associated traits, including oxycodone-induced (OXY) locomotor activity and naloxone conditioned place aversion. Quantitative Trait Locus (QTL) mapping in these substrains drastically reduces the number of segregating genetic variants from millions to thousands, accelerating the identification of the causal genetic factors. We conducted QTL mapping for oxycodone conditioned place preference (CPP, N=212), and naloxone conditioned place aversion (CPA, N=209), along with saline-treated mice as controls (SAL, N=213). We utilized a 9 day (D) CPP/CPA protocol. Mice received drug (1.25 mg/kg OXY, 4 mg/kg NAL, or SAL, i.p., suspended in SAL) on D2 and D4, and SAL on D3 and D5. Mice were assessed for drug-free CPP/CPA (D8) and drug state-dependent CPP/CPA (D9). Mice were genotyped at 96 informative markers and QTL mapping was performed in R/qtl (scanone, 1000 permutations). We identified a major genome-wide significant QTL for D2 and D4 locomotor (Chr. 1 72.43 cM, LOD= 9.79) that co-mapped to the same region as a QTL for anxiety-like opioid withdrawal behavior in the elevated plus maze (chr. 1 77.33 cM; LOD=5.33). High priority candidate genes within this locus include Rgs7 and Akt. We are currently conducting striatal transcriptome analysis via RNA-seq to aid in candidate gene identification and neurobiological mechanisms

Disclosures: L.R. Goldberg: None. **S. Kirkpatrick:** None. **N. Yazdani:** None. **K. Luttik:** None. **M. Mulligan:** None. **C. Bryant:** None.

Poster

078. Opioid Abuse

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA, IRP

Title: Choice between delayed food and immediate oxycodone in rats.

Authors: *M. SECCI, J. FACTOR, C. W. SCHINDLER, L. V. PANLILIO;
Preclinical Pharmacol., Natl. Inst. on Drug Abuse Intramural Res. Program, Baltimore, MD

Abstract: The choice to seek immediate drug effects instead of more meaningful but delayed rewards is a defining feature of addiction. To develop a rodent model of this behavior, we

allowed rats to choose between immediate intravenous delivery of the prescription opioid oxycodone (50 μg/kg) and delayed delivery of palatable food pellets. Food was preferred at delays up to 30 s, but food and oxycodone were chosen equally at 60-s delay, and oxycodone was preferred at 120-s delay. Comparison of food-drug choice, food-only and drug-only conditions indicated that food availability decreased drug intake, but drug availability increased food intake. In the food-only condition, food was a highly effective reinforcer even when delayed by 120 s. Pre-session feeding with chow slowed acquisition of food and drug self-administration, but did not affect choice. To establish procedures for testing potential medications, noncontingent pretreatment with oxycodone or naltrexone (analogous to substitution and antagonist therapies, respectively) was tested on a baseline in which oxycodone was preferred over delayed food. Naltrexone decreased drug intake and increased food intake. Noncontingent oxycodone decreased drug intake, but also produced extended periods with no food or drug responding. Overall, our findings show that the contingencies that induce preference for drugs over more meaningful but less immediate rewards in humans can be modeled in rodents, and they suggest that the model could be useful for assessing the effects of potential therapeutic treatments and exploring the underlying behavioral and neural mechanisms.

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Poster

078. Opioid Abuse

Location: Halls B-H

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Program#/Poster#: 78.17/CCC5

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH NIDA R21DA032962

Title: Using ipsc derived human dopamine neurons from opioid dependent subjects to study dopamine dynamics

Authors: Y. LUO[1], P. TESAR[2], K. L. PRESTON[3], K. A. PHILLIPS[4], Z. LIN[5], Y. SHENG[1], *B. J. HOFFER[6];
[1]Neurolog. Surgery, [2]Genet., Case Western Reserve Univ., Cleveland, OH; [3]Intramural Res. Program, [4]Intrammural Res. Program, Natl. Inst. on Drug Abuse, Baltimore, MD; [5]Psychiatry, Harvard Univ. Mclean Hosp., Belmont, MA; [6]Scientist Emeritus, NIDA/NIH, Lyndhurst, OH

Abstract: The dopaminergic (DA) system plays an important role in addiction. However, human DA neurons from drug-dependent subjects were not available for study until recent development in inducible pluripotent stem cells (iPSCs) technology. In this study, we produced DA neurons

differentiated using iPSCs derived from opioid-dependent and control subjects carrying different 3' VNTR (variable number tandem repeat) polymorphisms in the human dopamine transporter (DAT, SLC6A3). We successfully generated midbrain dopamine neurons that expressed forkhead box protein A2, LIM homeobox transcription factor 1 alpha, tyrosine hydroxylase, the dopamine D_2 receptor, Nurr 1, the vesicular monoamine transporter 2, and the dopamine transporter. All iPSC lines generated dopamine neurons that released dopamine detectable by high-performance liquid chromatography. There was no apparent difference in dopamine differentiation efficiency between cell lines derived from control and opioid-dependent subjects with regard to the percentage of cells positive for tyrosine hydroxylase. We present the first evidence suggesting that the 3' VNTR polymorphism in the hDAT gene affects DAT expression level in iPSC-derived human DA neurons. There were also different expression levels of D_2 receptors in the control group's iPSC-derived dopamine neurons compared with the opioid-dependent group's iPSC-derived dopamine neurons. Specifically, our results showed lower expression levels of D_2 receptors in both opioid-dependent iPSC lines. We also examined the expression of different classes of opioid receptors. Our data showed that mu opioid receptors were not detected in our differentiated human dopamine neurons derived from iPS cells. In contrast, kappa and delta opioid receptors were detected in our human dopamine neuronal cultures from all four iPSC lines. In addition, the effects of valproic acid (VPA) exposure on iPSC-derived human DA neurons were also examined. In human DA neurons, VPA treatment altered the expression of several genes important for dopaminergic neuron function including DAT, Nurr1, and TH; this might partly explain its action in regulating addictive behaviors. VPA treatment also significantly increased DA D2 receptor (Drd2) expression, especially in the opioid-dependent iPSC cell lines. Our data suggest that human iPSC-derived DA neurons may be a useful in vitro experimental model to examine the effects of genetic variation in gene regulation, to examine the underlying mechanisms in neurological disorders including drug addiction, and to serve as a platform for therapeutic development.

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Poster

078. Opioid Abuse

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA035281

Title: Determination of kappa opioid receptor contributions to re-escalation of oxycodone self-administration under extended access conditions

Authors: *J. D. NGUYEN, D. KIRSON, F. P. VARODAYAN, S. KHOM, M. ROBERTO, M. A. TAFFE;
Scripps Res. Inst., La Jolla, CA

Abstract: Prescription opioid abuse is a significant global problem, and opioid addiction is well-characterized by compulsive drug seeking, motivational withdrawal and chronic relapse. The goal of this study was to investigate escalation of opioid intake with extended access to intravenous oxycodone self-administration and re-engagement of drug seeking (re-escalation) following detoxification. Male Wistar rats were randomly assigned to short-access (ShA, 2-h) and long-access (LgA, 12-h) groups and trained to self-administer oxycodone (0.15 mg/kg/infusion) using a fixed-ratio 1 (FR1) response contingency and evaluated on a progressive-ratio (PR) procedure incorporating dose-substitution of oxycodone (0.06-0.3 mg/kg/infusion). Both groups underwent an abstinence period (4 weeks) and were tested for re-escalation. Rats given LgA to oxycodone earned more infusions than rats trained with ShA during acquisition. PR test results demonstrated an increase in drug-intake and breakpoints in rats assigned to LgA training relative to rats assigned to ShA training. All rats exhibited high levels of drug seeking upon re-engagement; however, LgA rats failed to de-escalate drug intake following the abstinence period. Previous findings show kappa opioid receptor (KOR) mediated disruption in GABAergic signaling in the central nucleus of the amygdala (CeA) is observed after acute (24-h) withdrawal from escalated cocaine self-administration. To investigate KOR dysregulation following acute oxycodone withdrawal, electrophysiological recordings were performed in CeA slices. The selective KOR agonist U-50488 decreased GABAergic signaling in both ShA and LgA rats, and this effect was blocked by KOR antagonist nor-binaltorphimine (nor-BNI). Applied alone, norBNI increased GABAergic signaling, suggesting tonic KOR activity. These findings further support the conclusion that access duration differentially impacts the acquisition and maintenance of the self-administration of oxycodone, and suggest that KOR-mediated signaling during oxycodone dependence may be distinct from that of other drugs of abuse.

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Poster

078. Opioid Abuse

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA - IRP

R21 DA036691

R01 AA022977

R01 DA035281

R44 DA041967

Title: Evaluation of a novel "e-vape" system as a model of opioid self-administration in rats

Authors: J. C. M. VENDRUSCOLO[1], *L. F. VENDRUSCOLO[1], B. J. TUNSTALL[2], S. A. CARMACK[2], M. COLE[3], S. VANDEWATER[3], M. TAFFE[3], O. GEORGE[3], G. F. KOOB[4]; [1]Integrative Neurosci. Res. Br., [2]NIH - NIDA - IRP, Baltimore, MD; [3]TSRI, La Jolla, CA; [4]NIH - NIAAA, Rockville, MD

Abstract: Opioid misuse is a major public health issue. Rodent models have been critical for the understanding of the biological factors underlying opioid consumption and dependence. Among them, intravenous self-administration has been the “gold standard” in the drug addiction field. In this model, rodents readily learn to perform an operant response for a drug infusion and, depending on the procedure (e.g., extended drug access), exhibit somatic and motivational signs of opioid dependence. However, the use of intravenous self-administration imposes some difficulties, such as maintenance of catheter patency. Thus, the goal of the present study was to develop a model of opioid operant self-administration that does not require surgeries and can be used for long periods of time. We used an electronic vaporizer (e-vape) similar to what is used by humans for electronic cigarette smoking to vaporize the potent opioid sufentanil. Sufentanil was dissolved in distilled water and mixed with vegetable glycerin and propylene glycol. Wistar rats were trained to nosepoke the active hole to receive a 10-sec delivery of vaporized sufentanil solution in 2-h daily sessions. Each reinforced nosepoke also initiated a 60-sec timeout period signaled by cue light. Nosepokes in the inactive hole had no programmed consequences. The results indicate that rats rapidly acquired and maintained vaporized sufentanil self-administration and that responding level on the active but not the inactive lever was dose-dependent. Rats significantly increased responding after a decrease in sufentanil concentration and decreased responding after an increase in sufentanil concentration. Thus, rats partially titrate their intake to maintain an optimal level of intoxication in a manner similar to intravenous self-administration. Moreover, the rats exhibited clear visible signs of opioid intoxication following sufentanil vapor delivery (e.g., tail stiffness, abnormal ‘frozen’ posture and startle to noise). Furthermore, injection with a low dose of the preferential u-opioid receptor antagonist naloxone prior to self-administration caused a remarkable increase in responding for sufentanil. Together, the present results demonstrate that operant self-administration of vaporized sulfentanil reliably produces opioid intoxication and suggest the potential of this model as an alternative to intravenous self-administration. This model may be a useful tool for studies in some stages of development where catheterization is difficult (e.g., adolescence), longitudinal studies and drug interaction studies.

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Poster

078. Opioid Abuse

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Support: PhRMA Foundation Pre-Doctoral Fellowship in Pharmacology/Toxicology

Integrative Pharmacological Sciences Training Program: T32GM092715

PhRMA Foundation Research Starter Grant

NIDA: DA037426

Title: Examination of potential sex differences in the behavior of Rictor knockout mice

Authors: ***S. KASKA**[1], R. BRUNK[2], M. S. MAZEI-ROBISON[3];
[1]Pharmacol. and Toxicology, [2]Neurosci. Program, Michigan State Univ., East Lansing, MI;
[3]Physiol. and Neurosci. Program, Michigan Stat Univ., East Lansing, MI

Abstract: Drug addiction is a disease that poses a significant health and financial burden on society. Dysregulation of the mesolimbic dopamine reward pathway is known to play a role in drug addiction and a more complete understanding of the molecular mechanisms underlying this dysregulation may lead to improved treatment. We have previously observed that chronic opiate exposure alters ventral tegmental area (VTA) dopamine (DA) neuron morphology and activity and that these changes are dependent on mammalian target of rapamycin complex 2 (TORC2) signaling. However, since previous studies examining TORC2 effects on behavior have utilized only male mice, here we sought to characterize the effect of decreased VTA or catecholaminergic TORC2 signaling in a battery of behavioral tests in both male and female mice. Since TORC2 signaling cannot be specifically manipulated pharmacologically, we have utilized a genetic approach. Rictor is core protein of TORC2 and knockout of Rictor eliminates TORC2 signaling. We utilized two models: 1) a developmental catecholaminergic Rictor knockout through crossing floxed Rictor and tyrosine hydroxylase (TH)-Cre mice (TH-Rictor-KO) and 2) an adult VTA Rictor knockout through stereotaxic injection of AAV-Cre into the VTA of floxed Rictor mice (VTA-Rictor-KO). Consistent with prior data (Dadalko et al., 2015), we see that male TH-Rictor-KO mice have increased locomotor activity in a novel environment,

but interestingly this phenotype is absent in female TH-Rictor-KO mice. However, there were no differences between the sexes, or from littermate controls, in anxiety-like measures (center time in open field, elevated plus mice) or social interaction. Additionally, we observe an increase in consummatory behavior in male TH-Rictor-KO mice, as they drink more water, sucrose, and morphine in a voluntary two-bottle choice assay compared to littermates, but with no difference in sucrose or morphine preference. In contrast, female TH-Rictor-KO mice have normal water consumption, but exhibit significantly increased morphine intake and a trend for increased sucrose intake. We are currently examining these same behaviors in VTA-Rictor-KO mice to determine whether these effects are driven by changes in the VTA or other catecholaminergic brain regions. We also plan to examine morphine conditioned place preference in both male and female TH-Rictor-KO mice and female VTA-Rictor-KO mice, as we previously found that male VTA-Rictor-KO mice exhibit decreased morphine CPP. Together, these data may suggest a difference in the role of catecholaminergic TORC2 signaling in baseline activity and consummatory behavior of male and female mice.

Disclosures: **S. Kaska:** None. **R. Brunk:** None. **M.S. Mazei-Robison:** None.

Poster

078. Opioid Abuse

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Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA15446

Title: Chronic heroin use in humans alters striatal histone H3 hyperacetylation that regulates glutamatergic synaptic plasticity and addiction behavior

Authors: *G. EGERVARI[1,2], J. LANDRY[2], J. CALLENS[2], J. FULLARD[2], P. ROUSSOS[2], Y. L. HURD[1,2];
[1]Neurosci., Icahn Sch. of Med. At Mount Sinai, New York, NY; [2]Psychiatry, Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Opiate overdose is now the leading cause of death among drug users, and the economic costs to society are estimated to be in the billions. Heroin in particular is one of the most addictive drugs, with nearly a third of all users progressing to dependence, emphasizing the critical need for greater efforts to expand the neurobiological underpinnings of heroin use disorder. Despite a significant body of research in animal and *in vitro* models, little knowledge has directly been accrued about the molecular neurobiology of the heroin-addicted human brain.

In addition to shedding novel light about neural systems relevant to the human condition, such knowledge would provide critical 'reverse translation' perspectives to improve targeted future mechanistic studies in animal models.
In this study, we adopted a transcriptome strategy to examine gene expression in the human striatum and used biological pathway analyses to identify candidate genes relevant to heroin use disorder. The results revealed marked impairments of genes related to glutamatergic neurotransmission and chromatin remodeling in heroin abusers. These findings served to drive further molecular studies in order to dissect epigenetic mechanisms mediating drug-related changes at glutamatergic synapses. On the protein level, we found that epigenetic marks predictive of gene activation—global histone H3 acetylation (pan-AcH3) and acetylation of lysine 27 of histone H3 (H3K27ac)—correlated with history of drug use and heroin toxicology. Using chromatin immunoprecipitation, we observed a significant increase in the enrichment of pan-AcH3 and H3K27ac directly at the *GRIA1* glutamate AMPA receptor gene which was paralleled with greater open chromatin accessibility in this region as shown by the assay for transposase accessible chromatin (ATAC-seq). Importantly, we found analogous epigenetic and transcriptional impairments in heroin self-administering Long-Evans rats. Using this translational model, we showed that the small molecule bromodomain inhibitor JQ1 potently inhibits heroin self-administration as well as cue-induced drug seeking behavior.
Overall, our data suggest that heroin-related histone acetylation impairments play a crucial role in establishing glutamatergic transcriptional changes that underlie striatal synaptic plasticity and addiction behavior. The results indicate that JQ1 or other bromodomain inhibitors are promising candidates for targeted clinical interventions in heroin and potentially other substance use disorders.

Disclosures: **G. Egervari:** None. **J. Landry:** None. **J. Callens:** None. **J. Fullard:** None. **P. Roussos:** None. **Y.L. Hurd:** None.

Poster

079. Nicotine Abuse and Effects on the Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 79.01/CCC10

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant T34GM008074

NIH Grant 8UL1GM118979-02

NIH Grant 8TL4GM118980-02

NIH Grant 8RL5GM118978-02

Title: Zolmotriptan, a serotonin-1B receptor agonist, modulates the rewarding properties of nicotine in female and male adolescent rats

Authors: *K. HERNANDEZ[1,1], B. J. SALINAS[1], A. V. ABELLA[1], S. D. IÑIGUEZ[2], N. S. PENTKOWSKI[3], A. R. ZAVALA[1];
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Abstract: Activation of serotonin-1B (5-HT_{1B}) receptors has been shown to modulate the rewarding effects of cocaine and alcohol. To date, the role of 5-HT_{1B} receptors in the rewarding effects of nicotine has not been investigated. To identify if these receptors play a role in nicotine reward, the nonselective FDA approved 5-HT_{1B} agonist, Zolmitriptan, was administered to adolescent rats prior to conditioning with nicotine using the conditioned place preference (CPP) paradigm-a validated animal model of drug reward. Specifically, female and male adolescent rats were conditioned on postnatal day 29-36 with saline or nicotine (0.0, 0.0022, 0.067, or 0.2 mg/kg, subcutaneously) on alternating days over an 8-day CPP conditioning procedure. During nicotine conditioning days, rats were administered Zolmitriptan (0, 3, or 10 mg/kg, subcutaneously) 15 min prior to nicotine injections. Results indicate that activation of 5-HT_{1B} receptors with Zolmitriptan decreased the rewarding properties of nicotine in both female and male rats in a dose dependent manner. As the dose of the agonist was increased, a decrease in nicotine-induced CPP was evident. Altogether these results indicate that 5-HT_{1B} receptors play a critical role in the rewarding properties of nicotine and further suggest that 5-HT_{1B} receptors are a novel target for nicotine dependence.

Disclosures: K. Hernandez: None. **B.J. Salinas:** None. **A.V. Abella:** None. **S.D. Iñiguez:** None. **N.S. Pentkowski:** None. **A.R. Zavala:** None.

Poster

079. Nicotine Abuse and Effects on the Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 79.02/CCC11

Topic: G.08. Drugs of Abuse and Addiction

Title: Chronic exposure to nicotine during adolescence not adulthood triggers long term changes in mesolimbic expression of stress related genes

Authors: *L. F. ALCANTARA, C. BOLAÑOS-GUZMAN;
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Abstract: Individuals who smoke cigarettes often report having their initial nicotine (NIC) experience as an adolescent, escalating their use overtime. Long-term smokers have higher incidence of mood-disorders and initiation of smoking at earlier ages increases the likelihood of developing major depression later in life. This phenomenon is less pronounced in individuals whom begin smoking during adulthood suggesting that NIC exposure during different developmental stages can induce distinct biological consequences. For example, we have previously shown that adolescent, but not adult, rats exposed to chronic NIC show deficits when tested in depression- and anxiety- related tasks later in life. After 15 days of NIC exposure rats exhibit long-term decreases in latency to immobility and increased total immobility in the forced swim test (FST), suggesting increased learned helplessness. NIC pre-treated adolescents also show increased time spent in the closed arm of the elevated plus maze (EPM), indicating greater anxiety-like behavior. This effect is not seen in adult-treated rats, indicating a different NIC-induced behavioral profile. Additionally, given that these alterations are seen long after cessation of drug use and indicate more persistent biological regulation. One of the major brain circuits involved in both drug- and mood-related behaviors is the mesolimbic dopamine pathway. As one of the primary dopaminergic output sources, the ventral tegmental area (VTA) stands as the hub of this pathway, projecting mainly to the nucleus accumbens (NAc), prefrontal cortex and hippocampus. It is possible that NIC acts on this pathway to mediate depressive-like symptomology. Recent studies have implicated various signaling pathways as mediators of mood-related behaviors in the NAc and VTA. Specifically, genes related to the extra cellular-regulated kinase (Erk ½) and AKT/P13k pathways, have shown great promise in elucidating the mechanisms mediating depression. Therefore it is plausible that chronic exposure to NIC modulates these pathways differently based on age of exposure leading to divergent behavioral outcomes. To this end, Adolescent and adult male rats were injected with NIC for 15d and left undisturbed for one month. Tissue was collected from the VTA and NAc and gene expression was measured using rtPCR. We found that *Erk2, Creb, Akt* and *GSK3b* expression differed between adult and adolescent rats in both the VTA and NAc. This data demonstrates that chronic exposure to NIC alters long-term gene expression in an age-dependent manner and provides insight into the possible long-term mediators of depression-like symptoms induced by early life smoking.

Disclosures: L.F. Alcantara: None. **C. Bolaños-Guzman:** None.

Poster

079. Nicotine Abuse and Effects on the Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 79.03/CCC12

Topic: F.04. Stress and the Brain

Support: T32 DA28874

R01 DA033646

Title: Altered gene expression (RNA-Seq) and response to nicotine in F1 offspring of paternal stress exposure

Authors: *N. L. YOHN[1], M. S. BARTOLOMEI[2], J. A. BLENDY[1];
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Abstract: Objective: Evidence suggests that parental stress exposure is inherited by future generations in both humans and animals. However, no work has identified if parental stress exposure influences offspring response to drugs of abuse, including nicotine. Thus, the goal of this work is to determine if stress administered to F0 animals alters response to nicotine in future generations.
Methods: Male C57BL/6 mice underwent chronic unpredictable stress (CUS) for 2 weeks starting at 4 weeks of age. Following CUS, mice were mated with naïve partners to produce F1 offspring. A second generation of mice was created by mating F1 offspring with naïve partners. Anxiety, startle response, and response to nicotine was characterized in F1 and F2 mice between 10-14 weeks of age. Gene expression changes in the amygdala of F1 male offspring were analyzed using RNA-Seq.
Results: F1 male mice derived from fathers exposed to CUS showed blunted sensitization to chronic nicotine administration. Interestingly, this phenotype was also found in their male offspring (F2). RNA-Seq of the amygdala of F1 male offspring identified 240 genes with altered expression in mice derived from fathers exposed to CUS (log2 fold change > 0.8, FDR < 0.05, and $P < 0.05$). Of those genes, 41 had increased expression while 199 were decreased. Gene ontology (GO) functional annotation clustering (DAVIDv6.7) revealed significant enrichment of extracellular matrix and plasma membrane gene sets. Driving the gene clustering was the altered expression of collagen and metabolic signaling genes as well as genes implicated in cellular response to stess and drugs of abuse.
Summary: Multi- and trans-generational inheritance of paternal stress exposure produces blunted response to chronic nicotine exposure in male mice. In addition, differential expression of genes found in offspring may contribute to these phenotypes.

Disclosures: **N.L. Yohn:** None. **M.S. Bartolomei:** None. **J.A. Blendy:** None.

Poster

079. Nicotine Abuse and Effects on the Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 79.04/CCC13

Topic: G.08. Drugs of Abuse and Addiction

Support: DA012844

Title: A second update on susceptibility genes for nicotine dependence identified by genome-wide linkage, candidate gene association, genome-wide association, and targeted sequencing approaches

Authors: ***M. D. LI**[1,2], J. YANG[1], T. J. PAYNE[3], J. Z. MA[4];
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Abstract: Tobacco smoking is a severe health hazard worldwide, as nearly one-third of the global adult population smokes tobacco products, and these have been associated with numerous serious health problems. This high prevalence of tobacco use highlights the importance of studying the genetic determinants of nicotine dependence (ND). To identify genetic factors for ND, various approaches have been used, including genome-wide linkage, candidate gene-based association, genome-wide association (GWAS), and targeted sequencing analysis. In this study, we systematically analysed the findings from all the abovementioned approaches according to rigorous selection criteria for each included study such as sample size, statistical significance, and independent replication. Our analysis revealed 14 regions nominated by genome-wide linkage analysis and 34 significantly associated loci in 43 genes by candidate gene-based association. The GWAS and meta-GWAS revealed 11 genome-wide significant loci; however, only the loci on chromosomes 8, 15, and 19 have received independent replication. Although it is still in early stages, limited targeted sequencing studies using next-generation techniques have implicated 18 variants in the aetiology of ND. Together, we identified 14 linkage regions and 47 unique loci in 60 genes involved in the development of ND, which forms our current understanding of the susceptibility map for ND. Because almost all of these loci and genes have received replication by independent approaches in different samples, they should be considered high priorities for future functional study of ND.

Disclosures: **M.D. Li:** None. **J. Yang:** None. **T.J. Payne:** None. **J.Z. Ma:** None.

Poster

079. Nicotine Abuse and Effects on the Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 79.05/CCC14

Topic: G.08. Drugs of Abuse and Addiction

Support: NIDA-IRP

FDA-CTP Grant

Title: Adolescent and adult nicotine exposure: Precipitated withdrawal and conditioned place aversion

Authors: ***R. J. KEELEY**[1,2], T. E. MAYER[2], L.-M. HSU[2], H. LU[2], Y. YANG[2], E. A. STEIN[2]; [1]NIDA, Baltimore, MD; [2]Neuroimaging Res. Br., NIDA IRP, Baltimore, MD

Abstract: Smoking remains an economic and public health problem, with an estimated 20% of the world population engaging in regular cigarette use. Approximately 90% of smokers initiate first use before the age of 18, identifying adolescence as a development epoch sensitive to nicotine, the main addictive component of cigarettes. Preclinical studies have identified adolescents to be particularly sensitive to the rewarding effects of nicotine and less sensitive to its aversive properties. Indeed, precipitated and spontaneous withdrawal paradigms have demonstrated limited nicotine dose-dependent effect on withdrawal behavior in adolescents. Previous work from our group has observed, in adult smokers and rats exposed to nicotine, that the severity of withdrawal is negatively correlated with the strength of a resting-state functional connectivity (rsFC) between ventral striatum and dorsal anterior cingulate cortex. Here, we extend this cross-species work by first establishing a behavioral paradigm demonstrating dose-dependent withdrawal behavior in adolescent and adult Sprague-Dawley rats. In adolescents, nicotine exposure was initiated at postnatal day 33 (p33), p47 or p61 and was maintained for either 6, 4 or 2 weeks, respectively, corresponding to early, mid or late adolescence. Nicotine doses were 0, 1.2mg/kg/d or 4.8mg/kg/d delivered via osmotic minipumps, with pump replacement every 2 weeks. Adult rats were exposed to a similar paradigm for 2, 4 or 6 weeks. We trained rats to pair a precipitated withdrawal experience, through a single injection of 1.5mg/kg of mecamylamine, with a distinct context and a saline injection with another context. Precipitated withdrawal behaviors were recorded during the training. On the second day, rats were given the choice to freely explore both contexts to assess conditioned place aversion to the precipitated withdrawal. Training and testing was conducted every 2 weeks. We observed

nicotine-dependent withdrawal behaviors in adolescent rats, which persisted in rats continuously exposed to nicotine for 6 weeks. This model of nicotine withdrawal in adolescent and adult rats will next be applied in experiments examining how changes in rsFC correlate with differences in behavior in adolescent and adult rats. Determining how brain circuitry in adolescent and adult rats is differentially altered given similar exposure paradigms to nicotine will help to identify circuits in adolescence that are particularly vulnerable to the effects of nicotine, which may then serve as treatment target outcomes.

Disclosures: **R.J. Keeley:** None. **T.E. Mayer:** None. **L. Hsu:** None. **H. Lu:** None. **Y. Yang:** None. **E.A. Stein:** None.

Poster

079. Nicotine Abuse and Effects on the Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 79.06/CCC15

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant P50 AA022534

Title: Potential interactive effects of prenatal alcohol exposure combined with adolescent nicotine and/or ethanol exposure on ethanol and nicotine reward during adulthood.

Authors: K. DIXON, J. L. WAGNER, S. DAVIES, D. D. SAVAGE, *N. PENTKOWSKI; Univ. of New Mexico, Albuquerque, NM

Abstract: The use of nicotine and alcohol during adulthood is linked with drug exposure at different life stages, particularly during critical periods of prenatal and adolescent development. Prenatal alcohol exposure (PAE) is associated with birth defects, as well as cognitive, social and behavioral deficits, while adolescent drug exposure (ADE) has been associated with increased vulnerability for substance abuse during adulthood. The purpose of the present study was to examine the potential interactive effects of PAE and ADE (alcohol and/or nicotine) on drug reward for a cocktail of nicotine and alcohol during adulthood. Long-Evans dams were given daily 4-hour access to either an alcohol (5% v/v) solution or a saccharine solution starting after breeding and continuing throughout gestation. Male and female offspring from each prenatal group were aged to postnatal day (PND) 31 and then exposed to saline, alcohol (0.75 g/kg, i.p.), nicotine (0.6 mg/kg/s.c.), or a cocktail of nicotine and alcohol for 10 days. Beginning on PND 75, adult reward for the cocktail was evaluated using the conditioned place preference (CPP) procedure. Following baseline preference testing, rats were exposed to the cocktail for 10 min in their initially non-preferred side, and saline in their initially preferred side; saline and drug

sessions occurred 5 hours apart and were counterbalanced for order of presentation. The day following the last conditioning session each rat was placed into the apparatus for a 10-min test session without drug administration. We found a significant three-way interaction of sex, PAE, and ADE on adult cocktail preference (i.e., drug reward). In male rats, PAE enhanced adult preference for the cocktail, which depended on whether they were also exposed to alcohol during adolescence. A similar pattern was found in female PAE rats, although the change in preference depended on nicotine exposure in adolescence but not alcohol exposure. These results indicate that exposure to either nicotine or alcohol at critical developmental stages interacts to modulate vulnerability to drug reward during adulthood.

Disclosures: K. Dixon: None. **J.L. Wagner:** None. **S. Davies:** None. **D.D. Savage:** None. **N. Pentkowski:** None.

Poster

079. Nicotine Abuse and Effects on the Brain

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 79.07/CCC16

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH General Medical Sciences U01 GM092655

Title: Genetic variations of nicotinic receptor genes as predictors of nicotine dependence: a machine-learning approach

Authors: *S. LEE[1], W.-Y. AHN[2,1], E. S. BARRIE[1], K. HARTMANN[1], J. FRATER[1], W. SADEE[1];
[1]Ctr. for Pharmacogenomics, Col. of Med., [2]Dept. of Psychology, The Ohio State Univ., Columbus, OH

Abstract: Nicotinic acetylcholine receptors (nAChRs) are primary targets for nicotine, the pharmacological ingredient of tobacco, and play a major role in nicotine dependence (ND). Neuronal nAChRs are composed of five subunits that are encoded by eight alpha (CHRNA2-CHRNA9) and three beta (CHRNB2-CHRNB4) genes. Genome-wide association studies (GWAS) have yielded numerous variants implicated in ND, with the CHRNA5-CHRNA3-CHRNB4 gene cluster containing the most robust signals for ND. Evidence from transgenic animal studies and neuropharmacological studies indicate that additional nAChR subtypes also contribute to ND but fail to be significantly detected by genetic association studies. To overcome this gap, this study investigates the overall genetic effects on ND of multiple genetic variants from 11 nicotinic receptor genes. Using a data-driven approach, a machine learning method

called elastic net was used to identify multivariate data patterns and to make predictions that can be generalized across different study cohorts. Using smoking-related GWAS, single nucleotide polymorphism (SNP) up or downstream (± 100kb) of 11 nicotine genes were selected to predict ND measured by the Fagerstrom Test For Nicotine Dependence (FTND). Furthermore, we validated biological relevance of each classifier variant (significant predictors) using genomics databases including GTEx (Getnotype-Tissue Expression) and Braineac (Brain eQTL Almanac). The machine learning approach not only identified major signals in the CHRNA5-CHRNA3-CHRNB4 cluster including rs578776 and rs16969968 but also enabled us to detect significant genetic variations around the CHRNA9, CHRNA4 and CHRNB3 subunits, increasing the accuracy of classification predictions when assessed in combination. Taken together, our machine learning approach complements single SNP association studies to explain complex human disease conditions such as drug addiction.

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Poster

079. Nicotine Abuse and Effects on the Brain

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 79.08/CCC17

Topic: G.08. Drugs of Abuse and Addiction

Support: NIH Grant DA 040440

Title: Modulation of kappa opioid receptor activity by nicotine and ethanol in adolescent and adult male rats

Authors: *S. J. CROSS[1], J. VAN[1], A. HO[1], D. BE[1], F. M. LESLIE[1,2];
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Abstract: Concurrent use of nicotine and alcohol represents a major public health concern, and use of both substances typically begins during adolescence. Adolescence is a sensitive developmental period marked by major reorganization of brain regions involved in executive function, learning and memory, and reward processing. Previous work from our lab has demonstrated that the combination of nicotine and alcohol is reinforcing and anxiolytic in adolescent, but not in adult, male rats. A potential mediator of these age differences is the kappa opioid receptor (KOR), as pretreatment with the KOR antagonist, norbinaltorphimine (norBNI), enhances nicotine and ethanol reinforcement in adults. Adolescents, on the other hand, are not affected by norBNI pretreatment, suggesting that they do not experience the same type of KOR

activation as do adults. The current study examines whether there are age differences in KOR activation and nicotine-ethanol modulation of KOR activity, particularly in brain regions involved in reward and motivated behavior. Brain tissue from drug naïve adult and adolescent male rats was processed for agonist stimulation of [^{35}S]GTPγS binding, with or without nicotine and ethanol, to determine KOR activity. Preliminary data suggest that adolescents may be more sensitive to KOR stimulation than adults. Further, agonist-stimulated activity of KORs may be blunted by nicotine and ethanol in the nucleus accumbens and caudate putamen of both adults and adolescents. These results provide age- and region-specific understanding of kappa opioid receptor activation and the influence of combinations of nicotine and alcohol on KOR activity.

Disclosures: **S.J. Cross:** None. **J. Van:** None. **A. Ho:** None. **D. Be:** None. **F.M. Leslie:** None.

Poster

079. Nicotine Abuse and Effects on the Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 79.09/CCC18

Topic: G.08. Drugs of Abuse and Addiction

Title: Attenuating reinstatement of drug-seeking using selective kappa opioid receptor antagonists

Authors: *J. K. DASILVA[1], E. DUNN-SIMS[2], C. TYSZKIEWICZ[2], A. SAWANT-BASAK[3], Z. HUGHES[4], J. HEDDE[4], A. N. MEAD[5];
[1]Global Safety Pharmacology, Drug Safety Res. and Develop., Pfizer, Inc., Groton, CT; [2]Global Safety Pharmacology, Drug Safety Res. and Develop., Pfizer, Groton, CT; [3]Pharmacodynamics and Metabolism, Worldwide Res. and Develop., [4]Neurosci. Res. Unit, Worldwide Res. and Develop., Pfizer, Cambridge, MA; [5]Discovery Safety and Metabolism, AstraZeneca, Cambridge, United Kingdom

Abstract: Dysregulation of the brain-reward system has been associated with numerous psychiatric and neurological disorders and co-morbidity exists between mood disorders and addiction. Exposure to stress is associated with drug addiction in humans and can induce relapse and craving. Similarly, a variety of stressful stimuli can reinstate drug-seeking in animal models. Converging evidence have demonstrated that the kappa-opioid receptor (KOR) pathway plays a critical role in regulating dopamine release in areas of the brain highly associated with reward-related learning and are implicated in reward, stress and mechanisms underlying stimulus-controlled drug-seeking behavior. The objective of these studies was to investigate the dynorphin-KOR system and its involvement in stress-induced drug relapse using the novel selective KOR-antagonist, PF-04455242, and the prototype KOR-antagonist LY-2456302 in a

reinstatement model of drug-seeking. Male Sprague-Dawley rats were initially trained to self-administer nicotine or fentanyl, I.V., under a fixed ratio schedule of reinforcement using a standard 2-lever choice design, with infusion-paired cues. Following acquisition, an extinction period commenced to dissociate the act of lever pressing from delivery of the drug. Reinstatement tests were then conducted using a within-subjects design, with reinstatement induced by the pharmacological stressor, yohimbine administered alone and in combination with drug-paired (nicotine or fentanyl) cues. For reinstatement tests, animals were treated with PF-04455242 or LY-2456302, S.C., prior to each session. Congruent with the literature implicating KORs with the rewarding/reinforcing effects of drugs, and specifically the association between relapse to drugs of abuse and exposure to stress-inducing stimuli, the present findings suggest KOR-antagonists attenuate reinstatement of drug-seeking in rats and this effect appears specific to seeking induced by stress. The effect of these antagonists in blocking reinstatement of drug-seeking behavior was compared to their affinity to bind to, and occupy, the KOR. Taken together, these observations support the potential benefit of KOR-antagonism in relapse prevention.

Disclosures: **J.K. DaSilva:** A. Employment/Salary (full or part-time): Pfizer, Inc. **E. Dunn-Sims:** A. Employment/Salary (full or part-time): Pfizer, Inc. **C. Tyszkiewicz:** A. Employment/Salary (full or part-time): Pfizer, Inc. **A. Sawant-Basak:** A. Employment/Salary (full or part-time): Pfizer, Inc. **Z. Hughes:** A. Employment/Salary (full or part-time): Pfizer, Inc. **J. Hedde:** A. Employment/Salary (full or part-time): Pfizer, Inc. **A.N. Mead:** A. Employment/Salary (full or part-time): AstraZeneca.

Poster

079. Nicotine Abuse and Effects on the Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 79.10/CCC19

Topic: F.04. Stress and the Brain

Support: Fondecyt 1150194

Title: Posttreatment with cotinine alleviates symptoms in a mouse model of chronic stress

Authors: A. IARKOV[1], *V. ECHEVERRIA[3], N. PEREZ-URRUTIA[2], C. MENDOZA[2], N. ALVAREZ-RICARTES[2], F. ECHEVERRIA[2];
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Abstract: Several psychiatric symptoms such as depression, anxiety, hyperactivity and cognitive impairment appears a result of chronic stress and model PTSD symptoms. These symptoms are accompanied by hormonal changes, such as excessive activation hypothalamus pituitary- adrenal axis and the deregulation of several neurotransmitter signaling pathways, such as the sympathetic and serotonergic systems. Chronic or traumatic stress induces pathological changes in several brain regions of the fear network, including medial prefrontal cortex, medial temporal lobe system, the amygdala and hippocampus. Cotinine, considered a positive allosteric modulator of the alpha 7 nicotinic acetylcholine receptor (nAChR), when infused directly into the hippocampus, enhanced fear extinction in rats in a manner dependent on the activity of the nAChRs. Cotinine also stimulated downstream effectors of the alpha 7nAChR including the protein kinase B-Glycogen synthase kinase 3beta pathway and the extracellular signal-regulated kinases. Our hypothesis is that cotinine by these mechanisms can relieve these symptoms as a posttreatment. Thus, the positive effects of cotinine as a post-treatment on anxiety, visual recognition memory, depressive-like behavior that resulted from prolonged restrain stress. Mice were restrained 6 hours/day for 21 days. Treatment started after immobilization and extended for 14 days. Cotinine was administered via gavage (0.5 mg/kg in PBS). The impact of immobilization stress on mice´s behavior was evaluated with battery of behavioral tests (open field, Porsolt´s test, novel object recognition, light-dark box test,and elevated plus maze). Our findings suggest that cotinine can be used as a treatment option to alleviate symptoms derived of chronic stress.

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Poster

079. Nicotine Abuse and Effects on the Brain

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 79.11/CCC20

Topic: D.05. Audition

Title: Nicotine induces change in estrus cycle phase

Authors: *L. WENNING[1], R. PHILLIP[2], P. BRODERICK[1];
[1]City Col. of New York, New York, NY; [2]Bronx High Sch. of Sci., Bronx, NY

Abstract: We hypothesized that females are more likely to become affected by nicotine than males as studied by startle response, estrus cycle, NMI, and neurotransmitter studies in our laboratory in female versus male mammals (Broderick and Malave, 2014). In order to address these sex differences in nicotine, estrogen and progesterone levels of 6 female Sprague Dawley

rats were monitored by post-intraperitoneal nicotine injection at the following time intervals: pre-injection, 30 minutes post-injection, and one hour post-injection. Exfoliate cytology of the vaginal mucosa was concurrently sampled at these three interim periods, stained, and analyzed via microscopy. Nicotine was administered daily for 6 days. The number of nucleated epithelial cells, anucleated cornified cells, and leukocytes was estimated to determine estrus cycle phase: proestrus, estrus, metestrus, or diestrus. Animals injected with saline were used as a control. Before the injection, the animals were in various stages of the estrus cycle with no significant differences in the number of animals in each stage. Thirty minutes post-injection, a significantly greater number of non-prescient mammals transitioned into estrus from their original stage rather than transitioning into the subsequent stage of the cycle ($p<0.001$). One hour post-injection, these values remained significant as more animals transitioned into estrus at this time interval ($p<0.001$). Correspondingly, an overwhelming majority of the nucleated epithelial cells became anucleated cornified cells at both 30 minutes and one hour post-injection. Animals injected with saline remained in the same stage throughout administration.
Our results demonstrate that nicotine induces estrus in non-prescient mammals, regardless of cycle phase before nicotine administration. During the estrus stage, both estrogen and progesterone levels were low as confirmed by an increase in nucleated cells on vaginal smear. Since nicotine administration caused this decrease in ovarian hormone levels, a lack of estrogen and progesterone could be paramount to elucidating the sexual dimorphism of addiction.

Disclosures: **L. Wenning:** None. **R. Phillip:** None. **P. Broderick:** None.

Poster

080. Decision Making: Pharmacology and Genetics

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 80.01/CCC21

Topic: H.01. Animal Cognition and Behavior

Support: SFARI Award #304935

Title: Oxytocin flattens social order and enhances behavioral reciprocity via anterior cingulate cortex

Authors: *Y. JIANG, M. L. PLATT;
Neurosci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: The neuropeptide oxytocin (OT) is known to influence social functions in a wide array of mammals. In humans and non-human primates demonstrating complex social behaviors, OT delivered intranasally enhances trust, relaxes social vigilance, and promotes prosocial behavior.

The precise neural mechanisms underlying these effects, however, remain unclear. Here we show that treating one male macaque monkey with intranasal OT relaxed his social interaction with another male. OT treatment simultaneously suppressed the threatening behavior of dominant males and increased the boldness of submissive males, effectively flattening the pre-existing social hierarchy. Additionally, OT also enhanced the effectiveness of social communication by increasing the behavioral synchrony between the pair. Notably, OT altered the behavior of not only the treated monkey but also his non-treated partner, consistent with the idea of enhanced feedback through reciprocal social interactions. These effects were largely recapitulated when OT was injected focally into the anterior cingulate gyrus (ACCg), a brain area previously linked to empathy, self-control, and other-regarding behavior. ACCg lacks post-synaptic OT receptors, but is rich in post-synaptic vasopressin (AVP) receptors, indicating that exogenous OT shapes social behavior, in part, via nonspecific binding. These findings bear potentially important implications for the use of OT in both basic research and as a therapy for social impairments in autism, schizophrenia, and other disorders.

Disclosures: Y. Jiang: None. **M.L. Platt:** None.

Poster

080. Decision Making: Pharmacology and Genetics

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 80.02/CCC22

Topic: H.01. Animal Cognition and Behavior

Support: NIH P50 DA05312

NIDA T32 DA016176

Title: A rodent model of negative urgency predicts acquisition of amphetamine self-administration

Authors: *V. WEISS[1], M. T. BARDO[2];
[2]Psychology, [1]Univ. of Kentucky, Lexington, KY

Abstract: Negative urgency is a facet of impulsivity that can be defined as engaging in a maladaptive impulsive behavior following a negative emotional event. In humans, negative urgency predicts problematic substance use. Our laboratory has developed a rodent model of negative urgency that is a modified version of the reward omission task published by Gipson et al., 2011. The task consists of two alternating components (one Pavlovian and one operant), each component being 10 sec and 2 min long, respectively. During the Pavlovian component, a light

predicts the delivery of a food reward, whereas during the operant component, rats are required to respond for food reward on a differential reinforcement of low rate 5 sec (DRL-5) schedule. After stable performance on the task is achieved, food reward is omitted in the Pavlovian component on rare occasions and the change in responding during the subsequent operant component is measured. “Negative urgency” is defined by an increase in responding during the operant sessions immediately following omission trials compared to reward trials, which is maladaptive because impulsive responding on the DRL reduces the number of reinforcers earned. The current study investigated whether individual differences in performance on this reward omission task would predict acquisition, maintenance, and/or escalation of amphetamine (AMPH) self-administration (SA). Adult male rats were trained to SA AMPH during 1 hr daily sessions. Experiment 1 started with a 0.3 mg/kg training dose, which was then decreased 0.03 mg/kg. Experiments 2 and 3 used 0.03 mg/kg during all SA sessions; Experiment 2 examined acquisition and maintenance only, whereas Experiment 3 included extended access (6 hr) sessions to determine if individual differences in reward omission performance predicted escalation of drug intake. Results revealed that impulsive rats acquired AMPH SA more rapidly than low impulsive rats at both doses. However, there were no significant differences during maintenance or escalation. Thus, individuals showing high impulsivity in the reward omission task were more sensitive to the initial reinforcing effect of AMPH. However, after reaching stable rates of AMPH SA, these individual differences did not predict escalated use. To the extent that the reward omission task used here models negative urgency in humans, these results suggest that preventive interventions that target mood-based rash action (negative urgency) as a risk factor would be most effective during the early phase of the addiction cycle.

Disclosures: **V. Weiss:** None. **M.T. Bardo:** None.

Poster

080. Decision Making: Pharmacology and Genetics

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 80.03/CCC23

Topic: H.01. Animal Cognition and Behavior

Title: Voluntary alcohol consumption can impair reversal learning in rats in a go/no-go task that allows for multiple responses/trial

Authors: L. AURAND, M. RAY, H. FISHER, C. HARMON, *C. L. PICKENS;
Kansas State Univ., Manhattan, KS

Abstract: There is mixed evidence for an effect of alcohol exposure to impair reversal learning. In addition, previous research from our lab has shown that voluntary alcohol access may not

have an effect on reversal learning. In the current experiment, we determined whether chronic intermittent access to ethanol would cause impairments in go/no-go reversal learning in male rats if the task was made more difficult by giving more extensive discrimination training and lengthening the S- trials to make withholding responding more difficult. Rats were given 4 sessions of discrimination training with 2 levers to the right or left of the chamber. Each lever was available in alternating order with presses on one lever reinforced with food pellets ("active lever") and presses on the other lever never reinforced ("inactive lever"). Each lever-light was available for 50-sec regardless of whether lever presses were made, and presses on the active lever could earn up to 2 food pellets/trial. The rats then received 6 weeks of chronic intermittent access to 20% ethanol (24-h access, 3 times/week with water always available), or 6 weeks of access to water alone. In addition, half of each group was also given 9.1 ml/kg injections of saline at the end of each alcohol access period to determine whether injection stress would interact with alcohol to affect learning. The groups given access to alcohol with or without saline injections drank an average of ~10 g/kg/24-h of the alcohol across the 6-week alcohol access with no difference in the average drinking in the injection and no injection groups. The rats then received one discrimination reminder session 3 days after the final day of ethanol access, and then received 2 days with the lever-light contingencies switched and the previously non-reinforced lever-light compound reinforced and the previously reinforced lever-light compound non-reinforced. We found that the groups given alcohol access exhibited impaired reversal learning, specifically due to a delay in learning to press the newly reinforced lever. We found no evidence that injection stress had any effect on behavior in either the water or alcohol group. Our future research will examine the behavioral factors and neurobiological changes that occur to make our task sensitive to alcohol access.

Disclosures: **L. Aurand:** None. **M. Ray:** None. **H. Fisher:** None. **C. Harmon:** None. **C.L. Pickens:** None.

Poster

080. Decision Making: Pharmacology and Genetics

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Topic: H.01. Animal Cognition and Behavior

Support: Fundacao Bial 192/12

FCT (Foxnet)

ERC

Title: Foxp2 disruption during development but not adulthood causes deficits in model-based decision-making

Authors: *C. A. FRENCH[1], T. AKAM[1,2], M. CORREIA[1], S. E. FISHER[3,4], R. M. COSTA[1]; [1]Champalimaud Neurosci. Programme, Champalimaud Ctr. for the Unknown, Lisboa, Portugal; [2]Dept. of Exptl. Psychology, Univ. of Oxford, Oxford, United Kingdom; [3]Language and Genet. Dept., Max Planck Inst. for Psycholinguistics, Nijmegen, Netherlands; [4]Donders Inst. for Brain, Cognition and Behaviour, Radboud Univ., Nijmegen, Netherlands

Abstract: Disruptions of the *FOXP2* gene cause a rare neurodevelopmental speech and language disorder. In the KE family a heterozygous *FOXP2* mutation is dominantly inherited and affected individuals have difficulty producing the sequences of orofacial movements necessary for fluent speech. This core deficit is accompanied by other expressive and receptive language problems. Humans and other animals use multiple strategies to learn instrumental actions, which may extend to vocal communication. Model-based control learns the specific consequences of actions and evaluates choices through a process akin to search in a decision tree, while model-free control learns direct mappings from states to actions through reward prediction errors. These systems are rooted in cortico basal-ganglia circuits, where Foxp2 is expressed both during development and in adulthood. Imaging studies of the KE family have also shown changes in striatal grey-matter density as well as altered striatal activation during language-based tasks. Here we investigate whether Foxp2 modulates the balance between model-based and model-free control, which has been proposed to be disrupted in other neurodevelopmental and psychiatric disorders, including autism and obsessive compulsive disorder. A novel 'two-step' decision-making task was used to assess the learning strategies employed by mice with Foxp2 disruptions. Mice chose between two actions which led probabilistically to one of two second-step states. In each state a further action offered a possibility of reward. Both reward and state transition probabilities changed over time in blocks. We investigated developmental Foxp2 functions using the *Foxp2-S321X* line, which carries a premature stop codon and is effectively a knockout. Heterozygous *Foxp2-S321X* animals showed a reduction in both the fraction of correct choices at the end of blocks and the speed with which they adapted to reversals. Furthermore, a reinforcement learning model showed that model-based action values had a reduced influenced on the choice of *Foxp2-S321X/+* animals relative to controls and that *Foxp2-S321X/+* mice had lower learning rates in the model-based component. *Foxp2* floxed mice were crossed with a tamoxifen-inducible Cre line to disrupt *Foxp2* in adulthood. This resulted in the death of around one third of Foxp2 knockdown animals. However, surviving animals were healthy and did not show impaired performance on the two-step task. Together these data indicate that developmental Foxp2 expression is required to establish the circuits required for model-based learning.

Disclosures: **C.A. French:** None. **T. Akam:** None. **M. Correia:** None. **S.E. Fisher:** None. **R.M. Costa:** None.

Poster

080. Decision Making: Pharmacology and Genetics

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Program#/Poster#: 80.05/CCC25

Topic: H.01. Animal Cognition and Behavior

Support: Summer Undergraduate Research Experience

Title: Neural basis of ovipositional preference in fruit fly, *Drosophila melanogaster*

Authors: *L. MUSKETT[1], D. SITARAMAN[2];
[2]Dept. of Psychological Sci., [1]Univ. of San Diego, San Diego, CA

Abstract: The long-term goal of our project involves finding evolutionarily conserved neurotransmitter systems and circuits that underlie decision-making processes. Decision-making is a complex behavior that depends on organism's environment, internal state (e.g. hunger, sleep etc.) and motivation.Layers of cognitive and emotional processes make it difficult to narrow down what factors cause organisms to make the decisions that they do. In order to find true cause and effect relationships, it's necessary to study simple organisms that have simple decision-making behaviors and fewer neurons. To this end, we chose Drosophila melanogaster, fruit fly, a widely used genetic model organism because of its experimental manipulability, ease of rearing, short life cycle and ability to make simple decisions in light of competing choices.Of the many decision-making processes, we chose to study egg-laying preference of the female fly. The decision to lay eggs in specific locations depends on texture, temperature, humidity and nutrient value of the substrates to ensure the success of the progeny. For example, female flies show a strong preference for food containing ethanol. The fly integrates multiple sensory stimuli (vision, smell, touch and taste) and lays eggs in highly specialized locations but it is unclear how internal behavioral states like hunger and sleep affect this decision. We have developed a novel assay design to test the oviposition preference and will present data showing a role for conserved neurotransmitters like dopamine, serotonin and octopamine in this behavioral assay.

Disclosures: **L. Muskett:** None. **D. Sitaraman:** None.

Poster

080. Decision Making: Pharmacology and Genetics

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Program#/Poster#: 80.06/CCC26

Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant DA035443

Title: Identification of an amygdala-cortical circuit for cue-directed action.

Authors: *N. T. LICHTENBERG, V. Y. GREENFIELD, Z. T. PENNINGTON, K. M. WASSUM;
UCLA, Los Angeles, CA

Abstract: Environmental reward-predictive stimuli have the ability to trigger the recall of precise memories of their predicted reward and in doing so motivate action and guide choice. This process requires the basolateral amygdala (BLA), but little is known about how the amygdala contributes to this function within the broader circuit. The BLA shares dense and reciprocal excitatory connections with several cortical areas, including the orbitofrontal cortex (OFC), which is itself implicated in cue-directed choice. Here we examined the function of the BLA-OFC circuit in cue-directed action using the outcome-specific Pavlovian-instrumental transfer (PIT) task. Pharmacological disconnection of the two 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 unique expected reward (i.e., to express outcome-specific PIT). Direction-specific manipulations of monosynaptic projections clarified this effect. Designer receptor-mediated transient inactivation of projections from the BLA to the OFC similarly disrupted the expression of outcome-specific PIT, while inactivation of OFC projections to the BLA were without effect. These data suggest that bottom-up signaling from the BLA to OFC is vital for the cued recall of precise reward memories and the use of this information to motivate specific action plans.

Disclosures: **N.T. Lichtenberg:** None. **V.Y. Greenfield:** None. **Z.T. Pennington:** None. **K.M. Wassum:** None.

Poster

080. Decision Making: Pharmacology and Genetics

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Topic: H.01. Animal Cognition and Behavior

Support: NRSA 1F32DA038942

TNDA 5T32DA024635

R01-DA035443

Title: Glutamate released into the basolateral amygdala tracks reward value encoding and the use of this information to guide reward seeking

Authors: *M. MALVAEZ[1], H. G. MONBOUQUETTE, 90095[2], K. M. WASSUM[1]; [1]Psychology, [2]Chem. Engin., UCLA, Los Angeles, CA

Abstract: Reward-seeking decisions are heavily controlled by the value of the specific reward they attain. This value is acquired and updated through the incentive learning process that occurs when the reward is experienced in a relevant motivational state (e.g., experience with a specific food in a novel hungry state). The basolateral amygdala (BLA) participates in this learning process, but precisely how is unknown. Because the BLA is densely innervated by both cortical and thalamic glutamatergic projections, we hypothesized that glutamate released into the BLA would track changes in reward value important for value-guided reward seeking. To test this, we used electroenzymatic biosensors to make near-real time measurements of BLA glutamate concentration changes during an incentive learning experience and during a subsequent reward-seeking test. First, rats were trained mildly-food deprived to lever press for a food reward. When rats were then allowed to experience the food reward hungry for the first time, we detected transient elevations in BLA glutamate concentration that tracked the incentive learning process. Interestingly, we also detected transient glutamate elevations immediately preceding bouts of reward-seeking activity following incentive learning, but only in those rats who had been given the opportunity to learn the food reward's higher value when hungry. These data suggest that transient BLA glutamate release tracks the reward evaluation process and use of this value information for guiding reward-seeking decisions. Ongoing experiments are evaluating the source of these signals to provide information on how the BLA functions within a broader circuit to coordinate reward-seeking actions.

Disclosures: **M. Malvaez:** None. **H.G. Monbouquette:** None. **K.M. Wassum:** None.

Poster

080. Decision Making: Pharmacology and Genetics

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant DA037421

Title: Frontalstriatal BDNF overflow and cognitive control deficits following spontaneous nicotine withdrawal

Authors: *R. COLE, M. ZIMMERMAN, M. G. KUTLU, A. MATCHANOVA, T. J. GOULD, V. PARIKH;
Psychology and Neurosci., Temple Univ., Philadelphia, PA

Abstract: Nicotine addiction continues to be a leading cause of preventable death worldwide. Despite the plethora of available treatments for smoking cessation, smoking relapse after attempts to quit remains high. Loss of cognitive control may be the linchpin in the inability to maintain long-term nicotine sobriety. The present study was designed to examine the effects of nicotine withdrawal on cognitive control processes in mice using an operant strategy set-shifting task that required the animals to switch from a spatial response-driven strategy to a visual cue-based strategy to achieve rewards. Because the integrity of frontostriatal circuits is critical for executive processes, and brain-derived neurotrophic factor (BDNF) exert modulatory effects on cognitive processes and is known to play an important role in drug addiction, we also assessed the effects of nicotine withdrawal on BDNF expression in the prefrontal cortex (PFC) and striatum. Male adult C57BL/6J mice were either exposed to chronic nicotine (6.3 mg/kg/d or 18mg/kg/d) or saline using subcutaneous mini-osmotic pumps for 14 days. During the treatment duration, the animals remained on the spatial lever discrimination phase of the task. Spontaneous nicotine withdrawal was induced by removing the pumps and the animals were tested on the strategy set-shifting phase until criterion is attained. Following behavioral testing, brains were extracted for q-PCR and immunoblot analysis of BDNF mRNA and protein expression. Mice undergoing nicotine withdrawal required more trials to attain strategy switching criterion as compared to the controls. Error analysis show that animals withdrawn from both nicotine doses committed higher perseverative errors. However, animals treated with the higher nicotine dose also displayed more strategy maintenance errors. We also uncovered that both total BDNF and transcript IV BDNF mRNA expression increased in the PFC following nicotine withdrawal. Surprisingly, the levels of the mature form of BDNF protein declined in the PFC but were elevated in the striatal synaptosomes of these animals indicating that perhaps nicotine withdrawal altered the trafficking of BDNF between the PFC and striatal brain regions. Taken together, our data illustrate that loss of cognitive control during nicotine withdrawal occurs in a dose-

dependent fashion and that robust impairments in strategy switching noted in mice chronically exposed with a higher nicotine dose resulted from a generalized behavioral disinhibition. Further studies are required to determine whether nicotine withdrawal-related deficits in executive functions are causally linked to frontostriatal BDNF overflow.

Disclosures: R. Cole: None. **M. Zimmerman:** None. **M.G. Kutlu:** None. **A. Matchanova:** None. **T.J. Gould:** None. **V. Parikh:** None.

Poster

080. Decision Making: Pharmacology and Genetics

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Topic: H.01. Animal Cognition and Behavior

Support: NIDA Grant R00 DA033372 (L.A.B.)

Brain & Behavior Research Foundation NARSAD Award (L.A.B.)

Title: A sex-specific role for glutamate trafficking in reversal learning

Authors: *M. M. WICKENS, J. D. LENZ, R. D. COLE, V. PARIKH, L. A. BRIAND; Psychology, Temple Univ., Philadelphia, PA

Abstract: Cognitive deficits are a core feature of many psychiatric disorders such as schizophrenia, ADHD, Alzheimer's disease, autism, depression, and substance use disorder. Accumulating evidence point to glutamatergic dysfunction in these disorders. Much of the preclinical work in this area has focused on the role of NMDA receptors. However, the contribution of AMPA receptors in relation to cognitive deficits associated with these disorders has not been systematically studied. Therefore, the current studies aimed to examine the role of AMPA receptor trafficking in two measures of cognitive flexibility: strategy shifting and reversal learning. Further, as many of these psychiatric disorders exhibit a sex bias in prevalence, we examined both male and female mice to determine how sex might interact with glutamate trafficking to influence cognitive flexibility. To do this we utilized a transgenic mouse with a point mutation preventing PKC-dependent phosphorylation of GluA2 subunits. As this phosphorylation event leads to activity-dependent internalization of AMPA receptors containing the GluA2 subunit, this mouse model exhibits blunted AMPA receptor trafficking. In the first study, mice performed an operant analog of the Wisconsin Card Sorting Test in which they underwent a strategy shift followed by a reversal. Although no differences were seen in strategy shift performance, we found that disrupting AMPA trafficking led to enhanced reversal learning

in female mice but not male mice. Next, we examined whether these effects would be more prominent in a pure reversal task without a prior strategy shift. In contrast to our findings in the first study, we found that in the absence of the strategy shift, disrupting AMPA trafficking led to a reversal deficit in male mice without affecting female mice. Additional studies are underway to further parse apart these sex differences. Taken together, these results suggest that sex influences the role of glutamate receptor trafficking on reversal learning. The results of these studies could inform future clinical studies on glutamatergic modulators in treating psychiatric diseases and how gender might influence the treatment outcome of these disorders.

Disclosures: **M.M. Wickens:** None. **J.D. Lenz:** None. **R.D. Cole:** None. **V. Parikh:** None. **L.A. Briand:** None.

Poster

080. Decision Making: Pharmacology and Genetics

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Topic: H.01. Animal Cognition and Behavior

Support: NSERC Discovery Grant (JSS)

DFG (DS)

NARSAD (DS)

Title: Adult neurogenesis regulates delay-based decision-making

Authors: *J. S. SNYDER, R. Q. YU, D. ESPINUEVA, O. PRINCZ-LEBEL, E. CHAHLEY, S. B. FLORESCO, D. R. SEIB;
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Abstract: Neural progenitors in the hippocampus generate new neurons throughout life. In several neurological disorders the production of new neurons is interrupted, for example in depression. Behavioral traits that are altered in depression include assigning less value to future outcomes, decreased motivation to obtain rewards and increased sensitivity towards negative feedback. Previous studies have investigated the effects of reduced neurogenesis on mood but the exact nature of these disturbances is unknown. To address this we have used a novel transgenic rat (GFAP-TK) in which neurogenesis can be specifically inhibited in adulthood, and operant decision-making tests of behaviors that are commonly disrupted in depression. We first tested rats on a delay-based decision-making task, where they must choose between a low reward lever that delivers 1 sugar pellet immediately and a high reward lever that delivers 4 pellets after a

delay. Compared to intact wild types (WT), GFAP-TK rats showed a decreased preference for the high reward option with increasing delay times, indicating that adult neurogenesis increases the subjective value of future rewards.
Second, we employed a probabilistic reversal learning task where rats have to show optimal behavior in the face of an uncertain reward. Here, correct (80% rewarded) and incorrect (20% rewarded) levers switch after 8 correct consecutive trials, and the number of reversals indicates behavioral flexibility. WT and GFAP-TK rats achieved a similar number of reversals. Moreover, win-stay and lose-shift behavior was also similar, indicating that neurogenesis does not regulate reward feedback sensitivity or negative feedback sensitivity, respectively. Third, we are performing an effort-based decision-making task where the number of lever presses required to receive the high reward option increases across blocks. Since depression is often associated with reduced energy and motivation, we may expect that neurogenesis-deficient GFAP-TK rats are less willing to choose the large reward option when additional work is required. This project combines a novel rat model with complex behavioral tasks to study the role of new neurons in decision-making behaviors that are disrupted in depression. Ongoing experiments will investigate the cellular mechanisms by which new neurons promote valuation of future rewards. Identifying these mechanisms may be helpful for developing treatments for depression and other disorders characterized by impulsivity and devaluation of future rewards, such as schizophrenia, Alzheimer's disease and addiction.

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Poster

080. Decision Making: Pharmacology and Genetics

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 80.11/DDD5

Topic: H.01. Animal Cognition and Behavior

Title: Discriminative signaling of outcomes using stimuli attributed with incentive value promotes suboptimal risky choice

Authors: *A. P. SMITH, T. R. ZENTALL, J. S. BECKMANN;
Psychology, Univ. of Kentucky Dept. of Psychology, Lexington, KY

Abstract: Animal models of gambling often use risky choice tasks between probabilistic and certain alternatives where, following a response to an uncertain stimulus, the reward either occurs or is absent. With this procedure, animals generally choose adaptively to maximize reinforcement and do not tend to incur losses of reinforcement as human gamblers often do.

However, recent research has found that when discriminative conditioned stimuli (CS) attributed with incentive salience signal the outcomes (wins vs. losses), suboptimal increases in risk taking occur and have implications for other choice behavior. The present experiments tested the effects of using discriminative vs. uncertain cues in the widely used probability discounting procedure. Rats and pigeons chose between two initial link stimuli. Choice of the uncertain large (UL) alternative initially always led to a CS followed by 4 pellets of food while choice of the certain small (CS) alternative always led to a different CS and then 1 pellet. After an established preference for the UL, the UL became probabilistic across within-session blocks delivering reward only 50% of the time, then 25%, 12.5%, and 6.25%. The rate at which an individual decreases choice of the UL (or discounts its value) is the primary measure of risk taking. Importantly, for animals in the discriminative condition, choice of the UL only produced the CS when reward was to be delivered. In the nondiscriminative condition, a CS always appeared but was only sometimes followed by food making it an uncertain cue similar to traditionally used procedures. Relative to the nondiscriminative condition, the discriminative groups showed markedly reduced discounting indicative of increased risk taking. Reversing the conditions with pigeons showed the risk tendencies of individuals also reversed indicating the experimental conditions exerted strong control over individuals' level of risk taking. Additionally, increasing the ratio requirement to the UL initial link for pigeons from 1 to 2, 4, 8, 16, and 32 across blocks showed those in the discriminative condition still preferred the UL relative to the nondiscriminative group. For rats, subcutaneous administration of selective D1 and D2 dopaminergic agonists and antagonists revealed generally increased risk aversion in the nondiscriminative condition similar to previous reports but had little to no effect on the discriminative condition. Thus, the present experiments show a behavioral mechanism across species that promotes risk taking via predictive stimuli with incentive value outcompeting primary reinforcement and dissociates dopaminergic signaling between the two conditions in risky choice.

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Poster

081. Neural Networks for Executive Functioning

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Topic: H.01. Animal Cognition and Behavior

Support: Breast Cancer Research Foundation

National Cancer Institute Cancer Center Support Grant P30 CA168524

Title: Structural and functional connectivity associated with weight loss and omega-3 fatty acid supplementation in women at high-risk for breast cancer

Authors: *L. MARTIN[1], R. J. LEPPING[1], W. M. BROOKS[1], C. R. SAVAGE[3], I.-Y. CHOI[1], P. LEE[1], B. C. MCPHERSON[4,1], V. B. PAPA[1], M. G. BRUCKS[1], A. T. FOX[1], J. L. NYDEGGER[2], B. F. KIMLER[2], C. J. FABIAN[2];
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Abstract: Post-menopausal obesity is associated with increased risk of developing breast cancer Weight loss has been shown to reduce this risk by reducing inflammation. Omega-3 fatty acids have been shown to reduce inflammation and are involved in cognitive factors such as attention, learning and memory. Little is known about the association between the role of omega-3 fatty acids to enhance weight loss and the related effects on brain function. The current study examined brain function related to weight loss among individuals enrolled in a randomized clinical trial examining the effects of omega-3 fatty acid supplementation on weight loss (6-months), weight loss maintenance (12-months) and breast cancer risk factors.
Twenty-five post-menopausal obese women who were participating in the parent weight loss study enrolled in the optional magnetic resonance imaging (MRI) study. Following a 6-month weight loss intervention participants completed an MRI session including structural MRI, diffusion tensor imaging (DTI), magnetic resonance spectroscopy (MRS), resting state MRI (rsMRI) and task-based functional MRI (fMRI) examining brain activation to food images. Thus far, analyses have examined correlations between 6-month changes in body mass index (BMI) and brain structure and function indexed by DTI, rsMRI, and fMRI. Analyses are still underway for brain volume and MRS, as well as differences associated with omega-3 fatty acid supplementation.
Preliminary analyses show a negative correlation between BMI change and DTI fractional anisotropy in the right uncinate fasciculus and a positive correlation between BMI change and functional connectivity (rsMRI) between the dorsolateral prefrontal and somatosensory cortices. No significant correlations were found between changes in BMI and brain activation to food compared to non-food images. Additional analyses will be forthcoming to examine whether brain structural and functional connectivity are enhanced with omega-3 fatty acid.
Overall, preliminary results indicate that 6-month weight loss is associated with structural and functional connectivity with ventrolateral prefrontal and limbic regions associated with reward and dorsolateral prefrontal regions associated with cognitive control.

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Poster

081. Neural Networks for Executive Functioning

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FP7/2007-2013 604102 HBP

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FWO G059309

FWO G083111.10

Title: Functional MRI of macaque monkeys during task switching

Authors: *E. PREMEREUR[1], P. JANSSEN[1], W. VANDUFFEL[1,2,3];
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Abstract: The ability to switch from one task to another is central to intelligent behavior, as it allows an organism to cope efficiently with the demands of swiftly changing environments. Human functional magnetic resonance imaging (fMRI) studies have indicated the importance of prefrontal (PFC) and anterior cingulate cortex (ACC) during task switching (Liston et al., 2006; Woodward et al., 2006; Wylie et al., 2004). In addition, lesion and inactivation studies in monkeys have demonstrated impairments in task switching following both PFC (Dias et al., 1996) and ACC (Rushworth et al., 2003; Shima and Tanji, 1998) lesions. Except for set-shifting (Nakahara et al., 2002) and visual search experiments (Wardak et al., 2010), no task switching paradigms, particularly those requiring shifts in operant behavior, have been used during fMRI in monkeys. In our study, macaque monkeys (N=2) were trained to switch between saccades and arm movements. After a brief period of fixation, a blue or green target appeared. A separate go-cue (brightening of a spot) instructed the monkey to make either a saccade to the green target, or to move the arm ipsilateral to the blue target (Premereur et al., 2015). Furthermore, we used a fixation task in which the monkeys had to fixate centrally while a grey distractor appeared at the same position as the target. We performed a contrast-agent enhanced fMRI experiment (3T, 1.25 mm isotropic voxels, 2s TR). Each run contained all tasks, randomly presented in 40s blocks.

Switch trials were defined as the first trial of a block requiring another operant behavior compared to the previous block. Note that only correctly performed switch trials were included. Stay trials were defined as a correct trial (immediately following another correct trial), randomly chosen from the same blocks from which the switch trials were selected. Data were collected in 102 runs for monkey R (903 switch and stay trials, each) and 111 runs for monkey U (632 trials). We compared the activation for Switch vs Stay trials (conjunction analysis for both animals; $p<0.001$, uncorrected for multiple comparisons), and found increased activation mainly in ACC (anterior area 24), prefrontal areas 45A/B and 46v, orbitofrontal area 12, some extrastriate visual areas and ventral striatum, and to a lesser extent in parietal area LIP. Since macaque monkeys display similar performance during task switching paradigms compared to humans (Caselli and Chelazzi, 2011), they can be used as a valuable model for the investigation of this complex behavior. Our results pave the way for detailed electrophysiological and causal investigations of the cortical and subcortical network instrumental in task switching.

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Poster

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American Psychiatric Association

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Swartz Foundation

Fyssen Foundation

Title: Cortico-hippocampal contribution to the generation of contextual information

Authors: *S. BERNARDI[1,3], J. MUNUERA[2], M. RIGOTTI[4], S. FUSI[2], D. SALZMAN[2,2,3]; [1]Psychiatry, [2]Neurosci., Columbia Univ., New York, NY; [3]Psychiatry, New York State Psychiatric Inst., New York, NY; [4]IBM T.J. Watson Res. Ctr., Yorktown Heights, NY

Abstract: Identical stimuli often produce different outcomes (and thereby different emotional responses) depending upon the context in which they are encountered. One mechanism the brain

employs to infer chances of reward or punishment and to regulate emotional responses is to abstract 'cognitive contextual structures'. These contexts can be described as the set of circumstances that help us derive situationally informed meaning from the world. Previous studies have established the importance of the prefrontal cortex (PFC) in rule-guided behavior, and recent studies have shown that the amygdala, a brain structure long thought of as a key mediator of emotion, along with the PFC, also represents abstract contextual information. In particular, Saez et al. (2015) showed that the amygdala, ACC and OFC all represent abstract contexts defined by task sets; a task set is the set of CS-US contingencies in effect within a block of trials. The formation of a neural representation of task set may be created if neural signals providing a memory of a previous trial converge with neural signals representing the current trial. Thus neural signals providing information about the previous trial may be conceptualized as a providing a vital input to the formation of a representation of task set. We sought to determine whether the hippocampus (HPC) or dorsolateral prefrontal cortex (DLPFC) might provide this input by encoding the episodic memory of the previous trial. Alternatively, HPC and DLPFC might explicitly already represent task sets. We have been obtaining simultaneous electrophysiological recording from neurons in the HPC, the ACC and the DLPFC while two rhesus monkeys utilize knowledge of task sets to select an action that maximizes reward rate upon viewing an image. Using a linear decoder, we have observed that all task-relevant variables, including stimulus identity, context, planned operant action, and expected reinforcement may be decoded in each area. However, context could in principle be decoded if neural signals merely reflect a memory trace of the previous trial, since each trial type appears in only one context. We are therefore determining whether the decoding of context derives from signals that represent memory traces, a function long associated with the HPC. Alternatively, neural representations in these areas could reflect the linked trials within a task set. Assuming that neural representations of context truly reflect task sets, and not merely memory traces, a critical question concerns the extent to which these representations are created in a parallel or serial manner across brain structures that include HPC, DLPFC, ACC, OFC, and the amygdala.

Disclosures: **S. Bernardi:** A. Employment/Salary (full or part-time): NIMH T32. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution; American Psychiatric Association. **J. Munuera:** None. **M. Rigotti:** None. **S. Fusi:** None. **D. Salzman:** 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; NIMH R01-MH082017, Gatsby Foundation, Swartz Foundation, Fyssen Foundation.

Poster

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Pew Foundation

Title: Population dynamics of excitatory and inhibitory neurons in mouse parietal cortex during decision-making

Authors: ***F. NAJAFI**[1], G. F. ELSAYED[2], E. A. PNEVMATIKAKIS[3], J. P. CUNNINGHAM[4], A. K. CHURCHLAND[1];
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Abstract: Decisions are shaped by the interaction of current sensory inputs, past choices, past outcomes, and the internal state of the animal. Decision bias due to choice history and animal's internal state helps explain behavioral flexibility during the same sensory inputs. In this work we tested the hypothesis that previous choices drive a transient change in network dynamics that interacts with present evidence and biases the animal's choice in a time-dependent manner. We used two-photon calcium imaging to study population neural responses in the mouse posterior parietal cortex (PPC) during a decision-making task. To assess whether information about the previous choice and current sensory signals is carried by different subclasses of neurons, we used transgenic mice expressing tdTomato in their inhibitory neurons (GAD2-Cre crossed with Ai14 reporter line). Head-fixed mice mounted on a wheel learned to make judgements about the total rate of a series of clicks and flashes relative to an experimenter-defined category boundary. Mice reported their choices by licking to a left or right waterspout. To record neural activity, mice were injected with AAV9-Synapsin-GCaMP6f in their parietal cortex. Imaging was performed over the course of training. In each session, approximately 500 neurons were simultaneously recorded, while mice performed ~300 trials. Analysis of the behavioral data demonstrates that: 1) mice can learn the decision-making task and their performance depends on the stimulus strength (i.e., the difference between the presented rate and the learned category boundary); 2) mice have bias to repeat their previous rewarded choice; 3) this bias is time-dependent and transient. Preliminary analysis of neural population responses suggests that: 1) PPC population activity carries information about the current stimulus and the previous choice; 2) the influence of choice history on PPC population activity is time-dependent: it diminishes as the inter-trial interval increases; 3) excitatory and inhibitory neurons reflect decision and bias parameters in different

ways. Our findings demonstrate that choice history transiently modifies ongoing PPC dynamics; the modification of population activity changes the representation of incoming sensory stimuli, hence the animal's decision. Additionally, our results begin to shed light on how different neural subclasses contribute to the representation of internal and external signals during decision-making.

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Poster

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Support: Whitehall 2014-5-18

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Title: Complementary contributions of high-dimensional representation and noise correlation to cognitive processes

Authors: ***M.-R. ABOLGHASEMI-DEHAQANI**[1], A.-H. VAHABIE[1], B. NOUDOOST[2], A. SOLTANI[3];
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Abstract: Recent work in neural decoding has highlighted the importance of high-dimensional neural representations in cognitive functions. Independently, investigations into interactions between neurons have pinpointed reductions in the noise correlations between neurons as a mechanism for enhancing sensory and motor processes. However, the interactions or relative contributions of high-dimensional representations and changes in noise correlations have yet to be explored within a single population dataset. To explore the role of these coding properties during a cognitive task, we examined neural activity in populations of simultaneously recorded Frontal Eye Field (FEF) neurons in monkeys trained to perform a memory-guided saccade task. In this task, the monkeys were trained to saccade to the remembered location of a visual target, which appeared in 1 of 16 possible locations (2 eccentricities and 8 angles). Importantly, this

simple task requires multiple cognitive operations, including encoding of the location of a visual target (stimulus encoding), maintenance of this information over time (working memory), and finally generating a saccadic eye movement to the remembered location (saccadic preparation). We applied a combination of decoding and encoding methods to the simultaneously recorded neural data. We found that the information content of the neural response increased between sensory encoding and saccade preparation, but differently for the single-cell and population responses. Specifically, we found that population of FEF neurons greatly enhance their ability to encode locations far beyond their original response field, but only during saccade preparation only when population activity is taken into account. In other words, the information content of the population response has expanded across space during saccade preparation. We showed that this expansion relies on a high-dimensional neural representation. Finally, we found that a reduction in the noise correlation can further increase the information content of the population; however, this contribution can be detected only when the change in signal correlation is accounted for since the latter is much larger and affects both the single-cell and population responses. Overall, our results provide new insights into which properties of neural population codes are most relevant for encoding information and different cognitive processes.

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Poster

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Fyssen Foundation

Title: Neurophysiological mechanisms for representing abstract components of mental states

Authors: ***J. MUNUERA**[1], M. RIGOTTI[3], S. FUSI[1], C. D. SALZMAN[1,2,4];
[1]Neurosci., [2]Psychiatry, Columbia Univ., New York, NY; [3]IBM T.J. Watson Res. Ctr., Yorktown Heights, NY; [4]Psychiatry, NYPSI, New York, NY

Abstract: Contingencies between stimuli, actions, and reinforcement outcomes often differ dependent upon the situation. Serial reversal-learning is a behavioural task that can be used to explore the mechanisms underlying such context-dependent adjustments in action selection and reinforcement anticipation. In serial reversal-learning, two or more stimuli reverse their contingencies multiple times within an experimental session. Upon a reversal, agents may update independently their understanding of contingencies of each stimulus-response-outcome association, or they may learn to grasp that within a block of trials, the set of associations for all stimuli defines a task set. Knowledge of task sets allow a computationally more efficient strategy of exploiting knowledge that the association of one stimulus has changed to infer that contingencies have also switched for the other stimuli within a task set (i.e. by applying inference, a strategy recently demonstrated to be invoked by monkeys, see Saez et al., 2015). This strategy requires the formation and utilization of neural representations of task sets. We investigated the mechanisms that can underlie the formation of representations of task sets. In each of two contexts, rhesus monkeys learned to associate each of 4 images (conditioned stimuli, CSs) to an operant action (hold or release a button) and an unconditioned stimulus (US - either a drop of juice or its absence). Each context was thereby defined by the set of operant and reinforcement contingencies for the CSs, i.e. the task set. Within each context, we manipulated the temporal statistics of events such that particular trial types tended to occur in temporal proximity to each other, creating two sub-contexts. Switches between sub-contexts did not change contingencies so learning about sub-contexts could not be driven by error signals. Instead, learning about sub-contexts relied on the formation of a link between the current trial and the previous trial, a link that could be formed through a Hebbian mechanism if different interconnected neurons represent the current and most recent trial, respectively. We recorded single neuron activity in the amygdala, OFC, and ACC to determine if neural signals merely reflect a memory trace of previous trials, or if they reflect the formation of neural representations of sub-context and context. In all 3 brain areas, the neural representations reflected the linked sets of trials that form sub-contexts and contexts by virtue of the temporal statistics of events. This could underlie a process of abstraction that creates novel, abstract mental states reflecting task sets, a process vital for flexible cognitive behaviour.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Support: Whitehall Foundation 2015-12-71

Brain and Behavior Research Foundation 23017

Title: Prefrontal cortico-thalamic networks for cognitive control

Authors: *J. M. PHILLIPS[1], N. A. KAMBI[1], L. FISH[2], Y. B. SAALMANN[1];
[1]Psychology, [2]Univ. of Wisconsin-Madison, Madison, WI

Abstract: Cognitive control, our ability to flexibly adapt behavior according to goals, context and rules, is known to depend on the prefrontal cortex (PFC). The PFC is densely and reciprocally interconnected with the mediodorsal nucleus of the thalamus (MD). Although the anatomical connectivity suggests that MD may play an important role in cognitive control, this possibility remains relatively unexplored. We hypothesize that MD supports cognitive control through its ability to regulate information transmission across PFC areas. To test our hypothesis, we use diffusion MRI to map MD-PFC anatomical connectivity in macaque monkeys, which guides subsequent multi-site recordings from interconnected MD and PFC network sites in the same animals. We performed high-resolution (1.0 mm isotropic) diffusion-weighted imaging on 6 anesthetized macaque monkeys using the GE MR750 3T scanner with a 16-channel receive-only head coil (MRI Instruments). 60 diffusion directions (b=1000 s/mm^2 and NEX=14) and 112 b=0 images were acquired. FSL was used for EPI distortion, eddy current and motion correction, prior to Bayesian estimation of diffusion parameters and probabilistic tractography. Our tractography results were broadly consistent with anatomical tracer studies. Specifically, lateral PFC areas predominantly connected with lateral, likely parvocellular, MD; medial PFC connected with caudodorsal MD; and orbitofrontal cortex connected with medial, likely magnocellular, MD. However, our results further suggest a guiding principle: directly connected PFC areas are also indirectly connected via MD. This connectivity pattern is similar to that observed between another higher-order thalamic nucleus, the pulvinar, and visual cortical areas. These findings suggest that (1) there is an indirect cortico-thalamo-cortical pathway between directly connected PFC regions, a key requirement for our hypothesized role of MD in regulating information transmission between PFC areas, and (2) this may be a more generalized anatomical scheme underlying higher-order thalamo-cortical interactions. The connectivity maps for each animal allow precise targeting of MRI-compatible laminar probes to interconnected sites in lateral PFC areas 46 and 9/46 as well as their overlapping projection zones in MD, for detailed investigation of MD-PFC network dynamics.

Disclosures: **J.M. Phillips:** None. **N.A. Kambi:** None. **L. Fish:** None. **Y.B. Saalmann:** None.

Poster

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Support: Brain/Minds by MEXT

Title: A fine-timescale investigation of ventral tegmental area neuronal signalling in working memory

Authors: *V. GLYKOS, S. FUJISAWA;
RIKEN Brain Sci. Inst., Saitama, Japan

Abstract: The midbrain ventral tegmental area (VTA) is a major source of dopamine (DA) in the mammalian brain. Midbrain VTA neurons receive multiple afferent inputs to encode signals that regulate and guide behaviour via diverse efferent projections. Despite abundant classical conditioning studies demonstrating the correlative link between VTA subpopulations with reward processing, attention and aversive conditioning, little is known about the midbrain neuronal signaling in goal-directed behaviour such as working memory (WM). Recent evidence revealed a gradual increase in striatal DA levels and prefrontal cortical-VTA coordinated activity while rodents performed a WM task. However, the fine-timescale activity of VTA neurons during WM remains unknown. We performed *in vivo* electrophysiological recordings of VTA neurons while mice (n=4) performed a visual-cued version of a WM behavioural task on a Tmaze apparatus. We found that VTA neurons adjust their firing pattern to a prolonged firing state, instead of the transient phasic one, in situations where information about previously encountered stimuli is required to be held on-line, and manipulated in order to guide behaviour. Accordingly, the greatest proportion of VTA neurons (76% of 219 units) exhibited a pronounced elevated activity in the final parts of the trial (i.e. WM, reward delivery and consumption). Moreover, during the WM-component, 15% of the single units significantly differentiated their firing activity, consistent with the animal's future choices (i.e. choice-predicting units). These results suggest that VTA neurons code future action as well as reward expectation errors during WM.

Disclosures: **V. Glykos:** None. **S. Fujisawa:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: DIRP/NIMH/NIH

Title: Information coding by large scale neural ensembles from the macaque prefrontal cortex

Authors: *R. BARTOLO, R. C. SAUNDERS, P. G. BROWNING, A. R. MITZ, B. B. AVERBECK;
NIMH/NIH, Bethesda, MD

Abstract: Information processing in the brain is performed by large populations of neurons working in parallel. However, characterizing information in large populations has been difficult because it has not been possible to record large numbers or neurons (>> 10s) simultaneously from awake, behaving animals. However, multi-electrode recording techniques are making it possible to perform large scale recordings. In the present study, we recorded single and multiunit activity using 8 microelectrode arrays (96 channels per array) implanted bilaterally in the prefrontal cortex (PFC) of macaque monkeys (Macaca mulatta). In a given recording session, we were able to isolate hundreds of neurons (>500) during the execution of an oculomotor saccade task, and maintain the isolations for a large number of trials (~1500 trials). In the task the animal had to make a saccade to a target presented to the left or right of a fixation point. We were interested in estimating information scaling with population size, as many theoretical models have suggested that information saturates in large neuronal populations. Therefore we selected multiple random subsets of neurons at a range of population sizes within a recording session (25-500) and estimated information in each subset. Spike counts (0-500ms after target presentation) and saccade direction from 90% of the trials (randomized) were used to train a generalized linear model regularized with early stopping. From the remaining 10% of trials, half were used determine when to stop the training and the remaining trials were used for estimating decoding performance. We further projected population activity on the linear decision boundary, and used the distribution of projected data to estimate d-prime. We found that decoding accuracy rapidly increased as a function of the sample size, and was nearly perfect for samples with more than 150 neurons. On the other hand, d-prime continued to increase with ensemble size. We have also compared information coding in the original data with information coding in data in which the correlations between neurons were destroyed by shuffling trials between neurons, within condition. For ensembles greater than about 200 neurons, information was larger in the shuffled ensembles than it was in the original ensembles. Additional analyses will further allow us to characterize these effects.

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Poster

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Support: JSPS KAKENHI Grant Number 15K18355

Title: Fast dopaminergic modulation of prefrontal neuronal circuit activity

Authors: ***K. TAO**, S. FUJISAWA;
RIKEN Brain Sci. Inst., Wako, Saitama, Japan

Abstract: Prefrontal cortex is one of the major projecting target of dopamine neurons in the ventral tegmental area (VTA). These mesoprefrontal dopamine neurons has been considered to modulate prefrontal network activity tonically through volume transmission as well as phasically in a synaptic fashion. However, the effect of phasic firing of dopamine neurons on prefrontal circuit activity in a sub-second time frame remains elusive. Here we show that, in a classical reward conditioning paradigm using head-fixed mice, fast-spiking interneurons (FS INs) in the medial prefrontal cortex (mPFC) respond both to water rewards and reward predicting cues. Both water rewards and optogenetic stimulation of dopaminergic neurons in the VTA elicited firing of FS INs in the mPFC. Conversely, the vast majority of regular-spiking neurons were inhibited by these manipulations. Subcutaneous injection of SCH23390, a dopamine receptor D1 (D1R) antagonist, suppressed firing of FS INs induced by rewards or optogenetic stimulation. Further, we optogenetically identified the activity of dopamine transporter (DAT)-positive neurons in VTA and confirmed that their firing following reward predicting cues preceded the responses of FS INs. These results suggest that mesoprefrontal dopamine firing inhibit overall neuronal activity in the mPFC in a D1R-dependent manner, presumably in concert with glutamatergic inputs from lower order cortices or thalamic nuclei.

Disclosures: **K. Tao:** None. **S. Fujisawa:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: NSF Graduate Research Fellowship DGE-1122492

Title: Frontal ensemble dynamics signal shifts between distinct modes of sensorimotor behavior

Authors: *M. J. SINISCALCHI[1], V. PHOUMTHIPPHAVONG[2], F. ALI[2], M. LOZANO[2], A. C. KWAN[2,3];
[1]Interdepartmental Neurosci. Program, Yale Univ., New Haven, CT; [2]Psychiatry, [3]Neurobio., Yale Univ. Sch. of Med., New Haven, CT

Abstract: The ability to shift between repetitive and goal-directed actions is a hallmark of cognitive control. Previous studies have reported that adaptive shifts in behavior are accompanied by changes of neural activity in frontal cortex. However, neural and behavioral adaptations can occur at multiple time scales, and their relationship remains poorly defined. Here, we developed a novel adaptive sensorimotor decision-making task for head-fixed mice, requiring them to shift flexibly between multiple auditory-motor mappings. Two-photon calcium imaging of secondary motor cortex (M2) revealed different ensemble activity states for each mapping. Notably, when adapting to a conditional mapping, transitions in ensemble activity were abrupt and occurred before the recovery of behavioral performance. By contrast, gradual and delayed transitions accompanied shifts toward the non-conditional, repetitive mode of action selection. Moreover, muscimol inactivation of M2 selectively disrupted shifts to the conditional mode of behavior. These results demonstrate distinct ensemble signatures associated with the initiation and termination of sound-guided action selection, and suggest that M2 leads in engaging goal-directed response strategies that require sensorimotor associations.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant EY012135

Title: Frontal cortical local field potentials (LFPs) reflect working memory processing over long delays

Authors: *C. D. HOLMES[1,2], C. PAPADIMITRIOU[1], L. H. SNYDER[1,2];
[1]Neurosci., [2]Biomed. Engin., Washington Univ. in St. Louis, Saint Louis, MO

Abstract: Converging evidence shows that the dorsolateral prefrontal cortex (DLPFC) and frontal eye fields (FEF) are critical for working memory. During the memory period of a delayed response task, both DLPFC and FEF neurons code for memorized spatial locations via persistent, spatially-tuned increases in activity. While single-unit activity tells us what information is coded for by cortical networks, it fails to inform us of network-wide processing and interactions between neurons. LFPs are understood to reflect synchronized synaptic activity of local neuron populations and may offer a window into network-level processing.
We recorded LFPs in DLPFC and FEF of macaques performing a long-duration (5-15 s) memory-guided saccade task. During fixation periods, LFP power was generally inversely proportional to the frequency squared, except for a marked positive deviation in the beta-band (20-35 Hz). During target presentation, beta-band power decreased while both low-frequency (2-10 Hz) and gamma-band power (40-110 Hz) increased relative to the fixation period. All three bands had greater power for contralateral compared to ipsilateral targets. During the memory period, low-frequency power returned to fixation period levels and had no sustained tuning for target laterality. Beta-band power increased above fixation period power ~1 s after the target disappeared, then gradually decreased below fixation period power throughout the remainder of the period. Gamma-band power dropped to fixation period level immediately after the target period, then gradually decreased below throughout memory. In both DLPFC and FEF, both beta- and gamma-band power remained tuned for target position during memory. Beta-band tuning was strong for both areas while gamma-band tuning was only strong in DLPFC.
Greater power for contralateral versus ipsilateral targets is consistent with increased power arising from synchronized network activity and synchronized network activity forming a substrate for spatial memory. However, the fact that memory period power decreases below the fixation period power suggests something more complex is occurring. One possibility is that some circuits synchronize during fixation. During memory, networks globally desynchronize, dropping power below that seen during fixation. However, some circuits remain synchronized,

thereby maintaining memory. Additionally, while neurons seem to code for memories similarly between DLPFC and FEF, the areas had different spectral profiles. Thus, network-level processing between these areas may differ. These results are a step toward better understanding how memory networks process spatial information.

Disclosures: **C.D. Holmes:** None. **C. Papadimitriou:** None. **L.H. Snyder:** None.

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GRSNC

FRSQ

CFI

Title: How is neural activity in premotor and parietal cortex influenced by bottom-up and top-down information about the value of reach choices?

Authors: *A. NAKAHASHI, P. CISEK;
Dept. Neurosciences, Univ. De Montreal, Montreal, QC, Canada

Abstract: Many studies have shown that neural activity in sensorimotor regions not only represents the action plans to be executed, but also reflects the outcome values of the potential actions, indicated by a specific feature of the stimulus (e.g. color or shape). In our study, outcome value was simultaneously indicated by two types of visual features, one “bottom-up” and the other “top-down”, to investigate how different kinds of information influence value-dependent modulation of activity in sensorimotor regions. A monkey was trained in a center-out reach decision task, in which each trial is initiated after the monkey fixates and moves a cursor into the center circle. After some delay, two reach targets appear at the right and left of center. Each target’s value is indicated by two independent visual features: bottom-up (BU) cues based on luminance, and top-down (TD) cues based on a learned mapping of a line orientation. Each cue has three levels of desirability scores, and the total of the two scores determines the number of rewards given upon a successful reach to that target. After a GO signal, the monkey indicates his choice by moving the cursor to the target of his choice, receiving the corresponding number

of rewards. The monkey was trained to maintain eye fixation until he reaches a target. We are recording from dorsal premotor cortex (PMd) and area 5 of posterior parietal cortex (PPC), which have previously been shown to exhibit value-related neural modulation. Data to date show that 1) both regions contain cells whose firing rate increases within 200ms from target-onset, 2) when the decision is made based on BU information (targets differ in BU but not in TD cues), directionally-tuned cells show divergence in the firing rate that reflects the monkey's choice, which occurs earlier than when the decision is made based on TD information (targets differ in TD but not in BU cues). Additional analyses examine value-dependent modulation when only a single target is presented, and during trials in which the values of both targets are equal, but the BU and TD cues are in conflict.

Disclosures: A. Nakahashi: None. **P. Cisek:** None.

Poster

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Title: Neural constraints on the cognitive capacity of crows

Authors: *J. M. ROSE[1], D. BALAKHONOV[2];
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Abstract: The remarkable cognitive abilities of birds, especially crows and parrots, have recently attracted growing scientific interest. Mounting evidence demonstrates that these birds surpass many mammals, for instance they are capable of tool use, episodic memory, theory of mind and executive control. A core component of cognition is working memory, the ability to flexibly memorize and manipulate information over short periods of time. This neural system shows a tight limit regarding how much information can be handled simultaneously. This 'capacity' of working memory is often seen as a unit for general cognitive capacity and is a close correlate of individual fluid intelligence in humans. The present study aimed to determine working memory capacity of carrion crows. Two crows were trained on a working memory paradigm that allowed to model capacity as a function of load on working memory (a change localization task). The paradigm was adapted for crows from an experiment in monkeys in order to directly compare the working memory capacity of both species. Through the use of head-tracking we were able to test capacity for both visual hemifields independently. On all used

stimulus-sets (2, 3, 4 and 5 items), both crows performed the paradigm well above chance. As predicted, performance decreased as a function of the number of items the animals head to memorize. Overall, the performance of the crows was comparable to that of monkeys. The same holds for different estimates of capacity, including an information theoretical approach. Both species show a largely independent capacity between the two visual hemifields, such that the driving factor in performance is the number of items memorized in one visual hemifield. These results demonstrate that crows and monkeys have similar working memory capacity and possibly comparable neural strategies for working memory. In tune with the literature, or results indicate the high cognitive capacity of crows.

Disclosures: **J.M. Rose:** None. **D. Balakhonov:** None.

Poster

081. Neural Networks for Executive Functioning

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 81.15/EEE6

Topic: H.01. Animal Cognition and Behavior

Support: Human Frontier Science Program

The Uehara Memorial Foundation

NIH

Title: Functional characterization of specific interneuron subtypes in the prefrontal cortex for memory-guided behavior.

Authors: ***T. KAMIGAKI**, Y. DAN;
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Abstract: Prefrontal cortex (PFC) plays a pivotal role in maintaining the task-relevant information in short-term memory for regulating the upcoming motor responses. However, the circuit mechanisms underlying memory-guided behavior remain poorly understood. In the present study, we imaged calcium signals from the dorsomedial PFC (dmPFC) of mice performing a delayed Go/No-Go task. Each trial consisted of sample, delay, and test periods. During the sample period a target or non-target stimulus was presented, followed by the delay period during which water was inaccessible. The test period began when the water port was presented; licking in Go trials was rewarded (Hit), and licking in No-Go trials was punished (False Alarm). We found that the dmPFC showed robust activity during the delay period and different subpopulations of pyramidal neurons signaled Go and No-Go action plans. Optogenetic

manipulation of different subtypes of GABAergic interneurons in the dmPFC, even transiently during the early part of the delay period, caused dissociable effects on task performance. Moreover, cell type specific calcium imaging revealed the distinct nature of task-related activity in different interneuron subtypes. Our results suggest that the dmPFC is a crucial component of short-term memory network and different interneuron subtypes make distinct contributions to memory-guided behavior.

Disclosures: **T. Kamigaki:** None. **Y. Dan:** None.

Poster

081. Neural Networks for Executive Functioning

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Program#/Poster#: 81.16/EEE7

Topic: H.01. Animal Cognition and Behavior

Support: KAKENHI (24300143; 26112005)

Title: Distinct roles of orbitofrontal cortex in response execution and inhibition during a stop-signal task

Authors: ***J. YOSHIDA**, A. SAIKI, S. SOMA, K. YAMANAKA, S. NONOMURA, A. A. R. DAVILA, M. KAWABATA, M. KIMURA, Y. SAKAI, Y. ISOMURA;
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Abstract: Humans and animals can optimize their behavioral response adaptively by response inhibition; e.g., delaying the start of response (proactive inhibition). In a standard stop-signal task, which is often used to evaluate the response inhibition, subjects have to respond to a GO cue as quickly as possible (GO trials), but have to stop the response if a STOP cue follows the GO cue (STOP trials). The proactive inhibition will delay the GO response depending on previous experience of STOP trials. Previous studies suggest that the orbitofrontal cortex plays a critical role in the response inhibition. However, little is known about actual neural correlates with the proactive inhibition at a single neuron level in the orbitofrontal cortex. To examine the orbitofrontal neural correlates with proactive inhibition, we established a novel stop-signal task (called as Cancel-Signal task) which is suitable for precise measurements of task performance and multi-neuronal activity in head-fixed rats. In the Cancel-Signal task, rats responded to a GO cue more slowly after STOP trials than GO trials. This result means rats made proactive inhibition on a trial-by-trial basis in the Cancel-Signal task. In multi-neuronal recording during their task performance, we found some of the orbitofrontal cortex neurons showed functional spike activities in relation to task events such as the GO response and its preparation. These

activities were changed dynamically between after STOP trials and after GO trials, which suggests that the orbitofrontal cortex could encode the distinct information for response execution and inhibition.

Disclosures: **J. Yoshida:** None. **A. Saiki:** None. **S. Soma:** None. **K. Yamanaka:** None. **S. Nonomura:** None. **A.A.R. Davila:** None. **M. Kawabata:** None. **M. Kimura:** None. **Y. Sakai:** None. **Y. Isomura:** None.

Poster

081. Neural Networks for Executive Functioning

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Topic: C.06. Neurotoxicity, Inflammation, and Neuroprotection

Support: CONACYT 257092

Fellowship CONACYT 350322

Title: Thallium, a chemical warfare neurotoxic agent, is removed by metallothionein and prussian blue, without a redistribution to brain effect in rats

Authors: ***L. N. ANAYA RAMOS**[1], A. MONROY[1], S. GRACIA[2], S. GALVAN[3], A. DÍAZ[3], S. MONTES[3], C. RÍOS[3];
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Abstract: Thallium (Tl) is a neurotoxic heavy metal, included in the EPA listing of main pollutants. The metal provoked cases of accidental and intentional poisoning in human beings. It is increasingly used in industry, pointing to concern about exposure risk in animals and humans. Tl salts are absorbed by virtually all routes and show a heterogeneous distribution in all organs and tissues. Few days after intoxication the patients develop gastrointestinal and cardiac symptoms and nervous system dysfunction. The use of chelating agents as antidotes is controversial, since Tl may be redistributed from depot organs to brain, its target organ. Prussian blue (PB) has an efficiency of 70% and is the antidote of choice according to FDA. Metallothioneins I and II (MT) are endogenous metal-binding proteins with antioxidant and chelating properties. They have been proposed as neuroprotective molecules in brain diseases, and in the protection from heavy metal toxicity. The present study was designed to determine the effect of exogenously administered MT on acute thallotoxicosis. Male Wistar rats were

intoxicated with a single subletal dose of thallium acetate (16 mg/kg) and randomly allocated into 4 experimental groups: control group (C), Prussian Blue group (PB) 50mg/kg dose, metallothionein II group (MT) 100 μg and metallothionein in combination with Prussian Blue (MT+PB) group. All treatments began 24 h after thallium intoxication and were maintained for four days. Animals were killed 24 h after the last administration of antidotes to determine Tl contents in 6 organs and 6 brain regions. In all cases, $p<0.05$ was considered of statistical significance. Both PB alone or in combination with MT diminished significantly (70%) the Tl concentration in all organs and brain regions as compared to control group, treated only with Tl. The administration of MT alone showed non-significant thallium elimination in organs and brain regions compared to control group except in the kidney (60%). In addition, the combination MT+PB showed a significantly diminished Tl concentration (14%) superior than the reduction produced by PB alone. Results indicate that MT alone or in combination is able to reduce kidney Tl, without a redistribution to brain effect.

Disclosures: L.N. Anaya Ramos: None. **A. Monroy:** None. **S. Gracia:** None. **S. Galvan:** None. **A. Díaz:** None. **S. Montes:** None. **C. Ríos:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 82.01/EEE9

Topic: H.01. Animal Cognition and Behavior

Support: ONR/MURI Grant N000141310672

NIH Grant R01 EB009282

NIH Grant R01 MH099645

Title: Synaptic mechanisms of memory consolidation during NREM sleep

Authors: *Y. WEI, G. PRASHANTH, M. BAZHENOV;
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Abstract: Memory traces acquired during wakefulness and initially stored in the hippocampus are progressively transferred to the neocortex during non-rapid eye movement (NREM) sleep. Replay during sleep of temporally ordered spike sequences in cortical neurons related to memory events was proposed to be a neuronal substrate of memory consolidation. However, specific mechanisms behind memory consolidation during NREM sleep, especially stage 2 sleep, are still poorly understood. In this new study we tested how sleep spindles, one of the

electrophysiological hallmark feature of stage 2 sleep, affect the dynamics of synaptic weights in thalamocortical network. Further, we examined how the synaptic weights acquired during stage 2 sleep influence the slow oscillations during following stage 3/4 sleep. Our computational model of the thalamacortical network with spike-timing dependent synaptic plasticity (STDP) included changes in the level of the neuromodulators (reduction of acetylcholine and histamine; increase of GABA) that resulted in transition from stage 2 to stage 3/4 sleep. Spindle oscillations consisted of 7-14 Hz brief bursts of rhythmic waves that last 0.5-3 seconds, while slow oscillations (<1 Hz) were characterized by repetitive Up and Down states in all cortical neurons. Following our recent study (Wei et al., J Neurosci, 2016), we included stimulation to a small group of cortical pyramidal cells to mimic hippocampal input (sharp wave - ripple events). We found, that during stage 2 sleep, synaptic weight changes were local and asymmetrical around the stimulation sites. Such changes in synaptic weights led to the organized spatiotemporal pattern of Up state propagation during following slow oscillation, even in the absence of stimulation. This organized spatiotemporal pattern resulted in the changes of synaptic weights that extended to the entire network. When we modified synaptic connections before stage 2 activity to model memories acquired during wakefulness, we found that spindles increased these selected synaptic weights locally, resulting in a high probability of specific spike sequence replay during the following slow oscillation. Our study provides evidence that sleep spindles and sleep slow oscillation influence synaptic changes in a different way. The spatial extent of synaptic changes due to spindles was much smaller than that due to slow oscillation. Surprisingly, sleep spindles, preceding slow oscillation, had protective effect on the local synaptic changes, which would be otherwise diminished by the global synaptic dynamics during sleep slow oscillation alone.

Disclosures: **Y. Wei:** None. **G. Prashanth:** None. **M. Bazhenov:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 82.02/EEE10

Topic: H.01. Animal Cognition and Behavior

Support: MURI: N000141310672

Title: Slow-wave sleep improves learning through spike sequence replay

Authors: ***G. P. KRISHNAN**[1], M. KOMOROV[1], S. SKORHEIM[2], M. BAZHENOV[1];
[1]Univ. of California San Diego, LA Jolla, CA; [2]HRL Labs., Malibu, CA

Abstract: Sleep improves performance in cognitive tasks involving long-term memories. Such tasks often relay on learning sequences such as, e.g. finger tapping task, motor adaptation task or serial reaction time task. Replay during sleep of spike sequences associated with a previously learned task is thought to be the mechanism for synaptic reorganization underlying memory consolidation. In this new study, we used computational models to explore how replay during sleep may lead to increase in performance. We used thalamocortical network models with conductance based neurons and state dependent neuromodulators (acetylcholine, histamine and GABA) to generate sleep and waking states. STDP and homeostatic plasticity were implemented, allowing synapses to change both in awake and sleep states. To simulate sequence learning, the model was presented with multiple trials of sequential input to a group of selected cortical neurons. Performance of this task was measured by the success of the sequence completion - the percentage of a correct complete sequence recall when only the first input was presented. During initial training phase, ordered firing of neurons led to synaptic reorganization; we observed an increase in performance at the end of the training (50 sec). Two identical models were then compared. In the first model, neuromodulators were altered (reduction of acetylcholine and histamine) resulting in slow wave sleep (SWS) like activity (for 100 sec). The second (control) model remained in the awake state for the same time duration. No training was performed at that time in either of two models. Following this consolidation phase, the level of neuromodulators was changed again in the first model to bring it back to awake state. Performance of the sequence completion was then measured in both models. We found a significant increase in performance (around 20%) in the model that experienced SWS state. To reveal the neuronal mechanisms, we analyzed spike sequences of the cortical neurons during SWS state of the model and found that the learned sequences were reactivated spontaneously during the Up states of the slow waves. Synaptic weights between neurons increased in the direction corresponding to the sequence replay while reduced in the opposite direction leading to synaptic reorganization that facilitated completion of the sequence learned during initial training phase. We concluded that our model capture the critical neuronal processes involved in memory enhancement following sleep, as it has been reported in animal and human studies. It predicts that spontaneous reactivation of the learned sequences during SWS represent a key mechanism of memory consolidation.

Disclosures: G.P. Krishnan: None. **M. Komorov:** None. **S. Skorheim:** None. **M. Bazhenov:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

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Topic: H.01. Animal Cognition and Behavior

Support: ONR (MURI: N000141310672)

ONR N00014-16-1-2252

Title: Discrete-time approach to large-scale simulations of realistic brain networks.

Authors: ***N. RULKOV**[1], M. KOMAROV[1], G. P. KRISHNAN[1], I. TIMOFEEV[2], S. CHAUVETTE[2], M. BAZHENOV[1];
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Abstract: Large-scale modeling of a realistic brain network is a computationally expensive task that have to deal with multiple time scales of the neuronal dynamics. In order to simulate large-scale networks with the order of millions of neurons, different types of the reduced neuron models have been proposed but they commonly lack the ability to reproduce specific patterns of the neuronal activity. In this study, we focused on the development of discrete-time (or map-based) neuron models that are computationally efficient and designed to implement dynamics of different slow and fast intrinsic currents similar to conductance based neurons. Map-models use difference equations capturing dynamics of a neuron in discrete moments of time with relatively large intervals (~0.5 msec) that significantly reduces the overall computation time. In the past, we reported application of such models to simulate awake-like activity including cortical gamma oscillations (Bazhenov, et al., J Neurophys, 2008). Here, we discuss application of the new class of models to generating sleep slow oscillation (SO). SOs are characterized by periodic transitions between active (or Up) and silent (or Down) states in the membrane voltage of cortical and thalamic neurons. Experimental and modeling studies suggest: (i) Up-states are initiated due to progressive accumulation of spontaneous miniature EPSPs and maintained by recurrent excitatory connections and intrinsic depolarizing currents (such as persistent Na+ current), (ii) Up-states are terminated by progressive activation of the intrinsic inhibitory currents (such as Ca^{2+} dependent K^+ current), synaptic depression and synaptic inhibition. The novel map-based approach to cortical cell design allowed us to model the critical mechanisms for SO generation and closely match the activity observed in experiments and in the conductance-based models. The model included 4 discrete time variables capturing (a) the fast spike generation dynamics, (b) the slow dynamics of persistent Na^+ and Ca^{2+} dependent K^+ currents, (c) K^+ leak current allowing for modeling effects of neuromodulation. As reported in vivo, a network of map-based pyramidal cells and inhibitory interneurons revealed propagating waves of the Up state initiation and highly synchronous Up state termination. High computational efficiency of the new models makes it feasible for numerous simulations of the large-scale networks for the different connectivity configurations and parameters of intrinsic neuronal activity. Overall, our study demonstrates computational efficiency and validity of discrete-time approach in large-scale simulations of cortical networks.

Disclosures: **N. Rulkov:** None. **M. Komarov:** None. **G.P. Krishnan:** None. **I. Timofeev:** None. **S. Chauvette:** None. **M. Bazhenov:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

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Topic: H.01. Animal Cognition and Behavior

Support: ONR MURI Grant N000141310672

Title: Modeling of coordinated sequence replay in ca3 and ca1 during sharp wave-ripples

Authors: *P. MALERBA[1], A. L. FODDER[2], M. W. JONES[2], M. BAZHENOV[1];
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Abstract: During slow-wave sleep (SWS), the cortex is decoupled from external inputs, and can be devoted to consolidating previously acquired labile memories into stable memories. Reactivation of specific neural activity patterns - replay - during SWS has been observed in the hippocampus and neocortex and is believed to represent a neuronal substrate of memory consolidation. Hippocampal replay - in which ordered sequences of pyramidal cell firing recruited during behavior are reactivated during sleep - happens during sharp wave - ripple (SWR) events (150-200 Hz, lasting 60-100 ms), and is thought to be crucial for memory consolidation. Indeed, suppressing ripples disrupts memory formation and consolidation. Despite the importance of replay within the broader phenomenon of sleep-mediated memory consolidation, the neural mechanisms underlying hippocampal sequence replay are still unknown.
In this work, we build on our previous research to develop a model of hippocampal spike sequence replay during sleep. We represented CA3 and CA1 activity with a network model of synaptically coupled pyramidal and basket cells. Noise-induced activation of CA3 pyramidal cells triggered a cascade of spiking (the SW), with size controlled by the spread of recurrence in the network. SW activity resulted in strong excitatory input to area CA1, inducing a ripple. SWR occurred stochastically in the model, and their location and size were controlled by the convergence between pyramidal cells connections within CA3. Different SWRs involved distinct populations of CA1 neurons and, ultimately, led to distinct sequences of pyramidal cell spiking. In our model, a pattern of CA3-CA1 synaptic projections - Schaffer Collaterals - induced coordination between neuronal firing sites in CA1 and CA3, so that localized sharp-wave CA3 events produce consistently localized CA1 ripples. This model was then applied to study spontaneous reactivation of subsets of CA1 and CA3 pyramidal cells. We looked for the triplets showing ordered reactivation in CA1 across multiple ripples, and found that the presence of NMDA receptors at CA1-CA3 projections was critical to obtain above chance likelihood of encountering successful (“memorized”) triplets. The model predictions were tested in

simultaneous recordings of LFP and place cell activity from CA3 and CA1 in rats, including analysis of correlations between local field potential across different channels as a function of time. Our study predicted the role of CA1-CA3 interaction for hippocampal sequence replay and revealed specific role of NMDA receptors at Schaffer Collaterals in coordinating reactivation between the two regions.

Disclosures: P. Malerba: None. **A.L. Fodder:** None. **M.W. Jones:** None. **M. Bazhenov:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

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Topic: H.01. Animal Cognition and Behavior

Support: ONR (MURI: N000141310672)

Title: Precise timing of sharp wave - ripple complexes affects spatio-temporal pattern of sleep slow oscillations in a model of memory consolidation.

Authors: *P. SANDA, P. MALERBA, G. KRISHNAN, M. BAZHENOV;
Dept. of Med., UCSD, San Diego, CA

Abstract: Memory consolidation during sleep is known to depend on strength and coordination of sleep rhythms. During slow-wave sleep, cortical activity is organized in slow oscillations, in which the neuronal population switches rhythmically (< 1 Hz) between active (Up) and hyperpolarized (Down) states. Within this slow and widespread rhythm, slower oscillations are nested by other sleep rhythms - notably by sleep spindles and hippocampus sharp wave - ripples (SWRs). The interaction among different sleep rhythms enables an exchange of information between hippocampus and neocortex, and it is believed to form the neural substrate of sleep-dependent memory consolidation. The mechanisms involved in coordination of these rhythms are not fully known, and so is how the rhythm interaction can mediate memory consolidation. To address these questions, we build a computational model of thalamo-cortical-hippocampal network generating SWRs in the hippocampal CA1-CA3 network and slow oscillations in the thalamocortical system. We found that cortical network regulated the global time relationship between the SWRs and the slow waves. As reported in vivo, majority of SWRs occurred in the model near the onset of the Up states of slow oscillation. Impact of SWRs on the spatio-temporal pattern of slow waves depended on the slow oscillation phase at which SWR occurred. The SWRs arriving at the very end of an Up state or the later phase of a Down state allowed a global

change in the spatiotemporal pattern of the next Down-Up transition. Importantly, SWRs occurring in tight proximity of Down-Up state transition were capable of triggering multiple local cortical Up state initiation sites, therefore promoting ordered spiking of the cortical neurons. We then used a reduced version of our model implementing specifically crafted connectivity map between hippocampal and cortical networks. We found that a memory trace in the hippocampus, represented by a subset of cells orderly firing within a SWR event, can affect the initiation sites and the spatio-temporal pattern of the cortical Up state. We concluded that while cortical slow waves control SWR timing, SWRs can affect precise pattern of activity and the order of cell firing during Up states of slow oscillation. Our study predicts how hippocampal SWRs, presumably reflecting memory traces stored in the hippocampal network, initiate specific sequences of cortical cell firing, thus manifesting spike sequences replay during sleep.

Disclosures: **P. Sanda:** None. **P. Malerba:** None. **G. Krishnan:** None. **M. Bazhenov:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

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Program#/Poster#: 82.06/EEE14

Topic: H.01. Animal Cognition and Behavior

Support: ONR/MURI N000141310672

Title: Effect of learning cues on sleep-related memory consolidation depends on the phase of sleep slow oscillation

Authors: Y. WEI, G. PRASHANTH, *M. V. BAZHENOV;
Dept. of Med., Univ. of California San Diego, San Diego, CA

Abstract: During awake, new information is initially encoded in both hippocampus and neocortex, while during the following sleep, especially slow-wave sleep (SWS), the newly acquired memory traces are spontaneously reactivated, leading to consolidation of synaptic changes in the neocortex. A technique, called targeted memory reactivation (TMR), provides novel perspectives that auditory or olfactory cues can benefit memory reactivation during sleep. Recent empirical studies revealed that auditory cues or stimuli preferentially enhance memory consolidation when presented at a specific phase of slow oscillation during SWS. Our recent study revealed that synaptic plasticity promotes replay the spike sequences of the cortical neurons associated with recent learning (Wei, et al., J Neurosci, 2016). In this new study, we aim to understand the mechanisms behind effects of external stimulation (representing learning-related cues) on memory consolidation using a computational model of the thalamocortical

network displaying sleep slow oscillation (< 1 Hz) like activity. Synaptic weights between pyramidal cells in the cortex were regulated by spike-time dependent plasticity (STDP). We used a closed loop stimulation (lasting 100ms) of thalamic or cortical neurons at different phases of slow oscillations, similar to the experimental setup. We found that when an external stimulation was presented during the Down state (90^0-270^0) of slow oscillation, the Up state was likely to initiate around the stimulation site and it propagated towards other network sites. This was also the optimal phase of stimulation to maximize synaptic changes. The spatiotemporal pattern of slow oscillation became organized due to the external stimulation, which then determined the changes of synaptic weights between neurons. The synaptic connections in the direction of slow wave propagation were enhanced and in the opposite direction were decreased. In contrast, when the stimuli were presented during the Up state (270^0-90^0) of slow oscillations, the effect of stimulation on synaptic weights became significantly reduced. Initiation of Up states occurred independently on the stimulation site and the spike sequences were replayed less frequently. Our modeling findings are in agreement with recent experimental data that revealed that the learning-related cues would preferentially strengthen associated memories when they are delivered at the Down phase of slow oscillation. Our study proposes a possible mechanism for such phase dependence of the stimulation effects and provides insight into how the memory consolidation may be affected by sensory cues applied during sleep slow oscillation.

Disclosures: **Y. Wei:** None. **G. Prashanth:** None. **M.V. Bazhenov:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

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Program#/Poster#: 82.07/FFF1

Topic: H.01. Animal Cognition and Behavior

Support: UC MRPI (MR-15-328909)

Title: Effects of surface electrical stimulation on the cortical neurons

Authors: ***M. KOMAROV**[1], P. MALERBA[2], P. NUNEZ[3], E. HALGREN[2], M. BAZHENOV[2]; [1]Univ. of California San Diego, LA Jolla, CA; [2]Med., Univ. of California San Diego, La Jolla, CA; [3]Tulane Univ., New Orleans, LA

Abstract: Recent development of novel microelectrode arrays, capable of efficient surface electrical stimulation of the brain, opens novel avenues of interacting with brain neurons. While these new techniques continue and extend past efforts, e.g., deep brain stimulation, our understanding of how electrical stimulation affects specific types of neurons at different

locations within a cortical tissue mass remains limited. In this study we develop a method, combining current density calculations and morphological reconstructions of real cortical neurons, to estimate their activation profile in the cortex. We assumed a surface placement of the electrode grid capable of providing a predetermined current between any grid electrodes (as, e.g., in the ENIAC chip). Our method is based on a commonly accepted idea that electrical microstimulation initiates anti-dromic action potentials in axon initial segments or in nodes of Ranvier (Tehovnik et.al., J Neurophys, 2006). By calculating current density profiles for one or a few stimulation sites, we identified an area where current is strong enough to initiate anti-dromic propagation and activate a cell. Next, we used publicly available databases (Ascoli et al., J Neurosci, 2007) of the morphological reconstructions of neurons to create an "averaged" anatomy for the most frequently observed types of neurons in layers I-V. With this approach we estimated the probability that a cell of a certain type has an axonal segment in the area with above threshold current density. This probability reflects a likelihood of anti-dromic activation and, therefore, predicts the initial effect, which is caused by surface stimulation. We found that excitatory neurons in layers II-IV (pyramidal neurons in LII-LIV and spiny stellate cells in LIV) have a highest probability of activation with maximal likelihood for layer LIV. On the other hand, layer V is divided into two different groups: thin-tufted pyramidal neurons have prominent activation probability, while thick-tufted cells are very unlikely to be directly recruited by stimulation. Among different classes of inhibitory interneurons, tuft-targeting inhibitory cells (Layer I, Martinotti and Bitufted cells) have high probability of activation across all layers, while, soma- and proximal dendrite-targeting basket cells can be activated only in supragranular layers. Hence, a steep difference in basket cells activation across the layers is evident, and shapes the overall activation profile due to superficial electrical stimulation in cortex. Finally, we applied these results to develop a simplified computational model to simulate neuronal activity induced by superficial stimulation.

Disclosures: **M. Komarov:** None. **P. Malerba:** None. **P. Nunez:** None. **E. Halgren:** None. **M. Bazhenov:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

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Topic: H.01. Animal Cognition and Behavior

Support: NSERC Discovery Grant

AHFMR Polaris Award

Title: Pre-activation of neural activity patterns during REM sleep corresponds with rapid learning of a novel motor skill in rats

Authors: *M. J. ECKERT, M. TATSUNO;
Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: Neural activity during rest is hypothesized to participate in the consolidation of memory traces. Many studies have demonstrated that neural activity recorded during training on a behavioural task is replayed during subsequent rest and sleep. The majority of this evidence comes from declarative memory tasks and has shown that replay occurs primarily during slow-wave sleep following task training. Pre-play of neural activity prior to a task or experience has also been demonstrated, although support for pre-play in not consistent. Limited evidence also shows that motor skill tasks and brain-machine interface tasks are also reactivated during SWS following training. To date there is little evidence for replay or pre-play during REM sleep, with the exception of one study using a declarative memory task (Louie and Wilson, 2001). This is surprising given that behavioral studies support a role for REM sleep in the consolidation of motor skill learning in particular. Here we provide evidence that pre-activation of neural activity patterns (i.e. prior to task training) occur during REM sleep when there is rapid learning of a motor skill task, but not when learning of the task occurs gradually. We implanted 4 rats with tetrode arrays in the forelimb region of primary motor cortex and recorded single-unit activity during sleep and training on the single pellet reaching task. Daily recordings included a 3 hr pre-task sleep period, 30 minutes of training on the reaching task, and 3 hr of post-task sleep. Rats were naïve to the task at the start of recording (except to test paw preference) and daily recordings continued until they reached asymptotic levels of performance. Two of the rats showed rapid learning on the task, becoming very skilled within 5-6 days. The other two exhibited a slower and more variable gain in proficiency across 2 weeks, although they ultimately achieved similar success rates as the fast learners. Analysis of principal component reactivation of spiking activity (Peyrache et al., 2009) revealed a marked increase in pre-task activation during REM sleep in the 2 fast learning rats, specifically on days when the rapid gain in skill occurred. This increase in pre-task activation was not as evident in post-task REM sleep, nor was it evident in either pre- or post-task SWS. Furthermore, the pre-task REM activation was not present in the slower learning rats, suggesting that it was important for the rapid acquisition of the motor skill. Although our results are preliminary, they provide evidence that activation of neural ensembles during REM sleep facilitates rapid acquisition of a motor skill.

Disclosures: **M.J. Eckert:** None. **M. Tatsuno:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

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Program#/Poster#: 82.09/FFF3

Topic: H.01. Animal Cognition and Behavior

Title: Parvalbumin-expressing interneurons coordinate hippocampal oscillatory dynamics that promote network stability

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Abstract: Sleep plays a critical role in promoting various forms of memory consolidation, and it is known that activity in hippocampal area CA1 is essential for consolidating episodic memories. Using chronic electrophysiology, we find that in the hours following single-trial contextual fear conditioning (CFC), fast-spiking (FS) interneurons show greater firing coherence with sleep-specific CA1 network oscillations. Post-CFC pharmacogenetic inhibition of parvalbumin-expressing (PV+) FS interneurons blocks fear memory consolidation. This effect is associated with loss of two network changes associated with normal consolidation: 1) augmented sleep-associated delta (0.5-4 Hz), theta (4-12 Hz), and ripple (150-250 Hz) oscillations, and 2) stabilization of CA1 neurons' functional connectivity patterns. We tested whether ed oscillations in FS- interneuron firings leads to long term changes in CA1 network connectivity, by recording CA1 neurons before, during, and after rhythmic optogenetic stimulation of PV+ interneurons. In mice expressing channelrhodopsin 2 in PV+ interneurons (PV:ChR2), stimulation across a range of frequencies (2-18 Hz) led to frequency-specificrhythms in the CA1 LFP and enhanced neuronal spike-field coherence. These effects were strongest for stimulation frequencies of 4-10 Hz. Optogenticall-induced coherent firing also significantly increased the reliability (i.e., stability) of spike-timing relationships between neurons. Intriguingly, stability of network connectivity remained high relative to baseline even after optogenetic stimulation ended. A second set of experiments was carried out to test the long-term effects of PV+ interneuron-induced network coherence. Following a 30 min period of stimulation at 7 Hz, neuronal activity was continually recorded over the next 2 h. Stimulation of PV+ interneurons at 7 Hz significantly increased stability of CA1 network functional connectivity - a change that lasted through the entire post-stimulation period. Moreover, rhythmic stimulation induced a long-lasting change in the strength of connections between neurons, which also lasted for at least 2 h post-stimulation. Taken together, these data suggest that rhythmic coordination of network activity by PV+ interneurons - at frequencies that are significantly augmented following learning - has long-lasting effects on both the stability and strength of network connections.

Disclosures: **N. Ognjanovski:** None. **J. Wu:** None. **M. Zochowski:** None. **S.J. Aton:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 82.10/FFF4

Topic: H.01. Animal Cognition and Behavior

Support: NIDA IRP

Title: Hippocampal influence of anterior cingulate activity for memory consolidation

Authors: ***D. V. WANG**, S. IKEMOTO;
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Abstract: Hippocampal-cortical interaction during sleep promotes information transformation for memory storage in the cortex. In particular, hippocampal sharp-wave ripple associated neural activation is important for this transformation process during slow-wave sleep. The anterior cingulate cortex, or ACC, has been shown crucial for storage and expression of long-term memories of previous experiences. However, little is known about how ACC activity is influenced by hippocampal ripple activity during sleep. We report here that the hippocampal ripple activity triggers immediate ACC neural firings. By recording from ACC and hippocampal CA1 simultaneously in mice, we found that while almost all ACC neurons showed increased activity prior to hippocampal ripple activity, a subpopulation (20%) also showed a second greater activation immediately after ripple activity. This post-ripple activation of ACC neurons correlated positively with ripple amplitude. Moreover, the same neurons were activated upon electrical stimulation of the CA1. These results suggest that a small population of pre-activated ACC neurons is selectively influenced by hippocampal ripple activity. This post-ripple activation of ACC neurons during sleep may play a critical role in memory consolidation.

Disclosures: **D.V. Wang:** None. **S. Ikemoto:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 82.11/FFF5

Topic: H.01. Animal Cognition and Behavior

Support: ONR MURI: N000141310672

Title: Neurophysiological correlates of the influences of spatial context on hippocampal reactivation in a rodent reconsolidation paradigm

Authors: *S. NAGL[1], B. HARPER[1], P. MALERBA[2], M. BAZHENOV[2], J.-M. FELLOUS[1]; [1]Psychology, Univ. of Arizona Dept. of Psychology, Tucson, AZ; [2]Dept. of Med., UC San Diego, La Jolla, CA

Abstract: Hippocampal place cells reactivate in sharp-wave ripples (SWRs) during rest/sleep periods following a spatial task in rodents. Explained variance (EV) is a macroscopic measure of the extent to which the pattern of pairwise correlations present during a task persists in the reactivation epoch.

We used a 12-hour memory reconsolidation paradigm. The maze consisted of a circular open field with 8 equidistant peripheral feeders. Rats learned to collect rewards from 3 feeders (Set1) in context A. After a 3h consolidation period, animals learned to collect rewards from 3 other feeders (Set2) in the same or a different context. Rats were then prompted to recall Set1 in context A. The task was flanked by 2 epochs of random reward delivery on all 8 feeders ("Random" epochs). We recorded from the right dorsal distal area CA1 of the hippocampus of adult male Brown Norway rats.

We categorized cells with place fields that overlapped with either the Set1 or Set2 trajectories, but not both. We found that the degree to which Set1 cells displayed greater firing contributions within the SWRs of Post-Set2 Rest was significantly correlated with worse performance during Recall of Set1, and that this result was most pronounced in same context condition.

EV analyses indicated that reactivation was consistently more frequent after Random epochs when compared to learning epochs (Set1 or Set2). Reactivation was variable in Post-Set1 and Post-Recall Rests, but the proportion of experiments that showed significant reactivations in both conditions was comparable. In general, cells reactivated less in Post-Set2 Rests. Interestingly, in a subset of the different context experiments, EV analyses revealed there were correlations between the Pre-Set2 learning epochs, which occurred after exposure to the new context, and Set2 learning. These preliminary results suggest that the reactivation of hippocampal cell assemblies may critically depend on the context in which learning occurs, and may occur even if the task does not require explicit learning.

We next compared our results with a computational model of CA3-CA1 SWR generation during

sleep. We considered two subsets of CA1 cells to represent memories of Set1 and Set2, and represented the context by the amount of overlap between the two sets. We also considered the populations of CA3 cells projecting with NMDA connections onto Set1 and Set2 CA1 cells. The model predicts that the degree of overlap between two subsets of CA3 cells controls the degree to which Set1 cells spike in ripples in which Set2 reactivates.

Disclosures: **S. Nagl:** None. **B. Harper:** None. **P. Malerba:** None. **M. Bazhenov:** None. **J. Fellous:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

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Program#/Poster#: 82.12/FFF6

Topic: H.01. Animal Cognition and Behavior

Support: CIHR Grant MOP133444

Title: Physiological correlates of forgetting of long-term memory in the hippocampus.

Authors: *D. E. ARKELL[1], S. MARTIN[2], O. HARDT[1];
[1]CCNS, Univ. of Edinburgh, Edinburgh, United Kingdom; [2]Univ. of Dundee, Dundee, United Kingdom

Abstract: Fully consolidated long-term memories can be erased by a constitutive active decay process that involves the synaptic removal of GluA2-containing AMPA receptors (GluA2/AMPARs) (Migues et al, 2016). The precise signaling mechanisms that lead to the decreases in synaptic potentiation via AMPAR-endocytosis have not yet been fully described, but both long-term depression (LTD) and depotentiation (DP) present possible candidate mechanisms. Both lead to a sustained reduction in AMPAR expression at the post-synaptic density (PSD). Many studies have shown that blocking NMDARs promotes long-term potentiation (LTP) and long-term memory persistence, as well as inhibiting LTD, DP and the subsequent removal of synaptic AMPARs (Davis et al, 1992; Villarreal et al, 2002; Kim et al, 2007; Li et al, 2009). This indicates that NMDAR signaling could underpin the activity-dependent removal of GluA2/AMPARs, and thus DP or LTD could promote decay– like forgetting of long-term memories. Here, we thus used *in vivo* electrophysiology, in freely moving rats, to test whether a bilateral DP stimulus to CA3 accelerated decay of previously induced LTP. fEPSPs were bilaterally recorded in the CA1 to assess changes in potentiation. These showed stimulus driven decreases in amplitude, signifying synaptic DP. Using the NMDAR antagonist AP5, and the GluN2B subunit antagonist RO25-6981, we then examined

whether DP was GluN2B-dependent, while GluA2-3Y was used to determine whether DP required synaptic removal of GluA2/AMPARs. Finally we explored whether DP induction could deteriorate consolidated object location memory. In summary, our study describes physiological mechanisms that could be involved in the active decay of long-term memories in the hippocampus.

Disclosures: D.E. Arkell: None. **S. Martin:** None. **O. Hardt:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

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Topic: H.01. Animal Cognition and Behavior

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Title: The role of adult born neurons in memory consolidation during sleep-wake cycles

Authors: *D. KUMAR[1], M. HAYASHI[1], X. HAYASHI[1], G. CHANGARATHIL[1], M. WINTZER[2], T. J. MCHUGH[2], T. SAKURAI[1], M. YANAGISAWA[1], M. SAKAGUCHI[1]; [1]Univ. Tsukuba, WPI-IIIS, Tsukuba-Shi, Japan; [2]RIKEN Brain Sci. Inst., Wako, Japan

Abstract: Mammalian sleep contains rapid eye movement (REM) and non-REM sleep, both could employ different mechanisms for memory consolidation. Previous reports showed that memory-associated odor stimulation during non-REM sleep enhanced memory consolidation[1]. In addition, boosting slow oscillations or inhibiting sharp wave-ripples during non-REM sleep potentiated or interfered with memory consolidation[2,3]. On the other hand, REM sleep deprivation inhibited memory consolidation[4]. However, the memory circuit that is responsible for memory consolidation during each sleep stage has not been clearly shown. We have shown

that hippocampal adult-born neurons are incorporated in memory circuits after learning[5]. Therefore, we silence the activities of the adult-born neurons during specific stages of sleep after learning using optogenetics, which provides reversibility in intervention with higher time resolution and target specificity. The intervention reveals that the activities of the adult-born neurons is necessary for memory consolidation during specific stage of sleep.

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5. Arruda-Carvalho et al., J Neurosci, 2011, v31, p15113

Disclosures: **D. Kumar:** None. **M. Hayashi:** None. **X. Hayashi:** None. **G. Changarathil:** None. **M. Wintzer:** None. **T.J. McHugh:** None. **T. Sakurai:** None. **M. Yanagisawa:** None. **M. Sakaguchi:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 82.14/FFF8

Topic: H.01. Animal Cognition and Behavior

Title: The role of adult hippocampal neurogenesis in spatial memory reconsolidation

Authors: *M. LODS[1], G. FERREIRA[2], E. PACARY[1], A. GORON[1], N. D. ABROUS[1], S. TRONEL[1];
[1]Neurocentre Magendie, Bordeaux, France; [2]UMR1286 NutriNeuro, Bordeaux, France

Abstract: Adult neurogenesis refers to the creation of new neurons in the dentate gyrus of the adult hippocampus. A decade of research has established the role of adult neurogenesis in memory formation, in particular in spatial learning. We have recently demonstrated that, in rats, spatial learning in the water maze selects a population of neurons which is around 1 to 2 week-old, increases it survival and dendritic arborisation. However, these neurons do not participate to this particular spatial learning since too immature. Indeed, adult-born neurons participate to memory processes when they are functionally integrated into the hippocampal network (≈ 6 week-old). Our hypothesis is that, when mature, this selected population would be involved in reconsolidation. Indeed, after the learning, memory undergoes a process of consolidation to become stable. But memory can become labile again when reactivated and needs to go trough a process of reconsolidation to become stable again.

We developed a behavioural protocol in the Morris water maze to demonstrate that spatial learning undergoes reconsolidation. We then analyzed the activation of neurons born one week before spatial training, at the time of reactivation. Our results demonstrate that this selected population is activated after memory reconsolidation. In order to investigate a causal relationship between adult neurogenesis and spatial reconsolidation, the team designed a DREADD retrovirus. Injected in the dentate gyrus of rats, this retrovirus allows us to specifically activate or inhibit the learning-selected new neurons during the reconsolidation process in the water maze test. The preliminary datas show that inhibiting, at the time of reactivation, the learning-selected immature population of new-neurons impairs memory retention. Specifically activating this same population enhances memory precision. All together our results show a clear involvement of the hippocampal neurogenesis in spatial memory reconsolidation.

Disclosures: M. Lods: None. **G. Ferreira:** None. **E. Pacary:** None. **A. Goron:** None. **N.D. Abrous:** None. **S. Tronel:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Halls B-H

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Program#/Poster#: 82.15/FFF9

Topic: H.01. Animal Cognition and Behavior

Support: FAPESP grant #2015/25275-8

Title: Validation and biological relevance of a real-time ripple detection module for open-ephys

Authors: *C. L. AGUIAR[1], E. F. OLIVEIRA[3], L. R. ZACHARIAS[2], L. B. PERES[2], K. DIBA[5], D. C. SORIANO[4], J. P. LEITE[2];
[1]Neurosci., [2]Univ. of Sao Paulo, Ribeirao Preto, Brazil; [3]Federal Univ. of ABC, São Bernardo, Brazil; [4]Federal Univ. of ABC, Santo André, Brazil; [5]Univ. of Wisconsin Milwaukee, Milwaukee, WI

Abstract: Recent evidence suggests that sharp-wave ripples events (SWR), registering as 140-250 Hz “ripple” oscillations in the local field of electrodes at the CA1 pyramidal hippocampal layer, provide effective windows for the propagation of information out of the hippocampus and affect activity throughout the neocortex. Indeed, during slow wave sleep, cortical neurons fire after SWR, in concert with spindle oscillations in the local field potentials. Despite recent evidence reporting a hippocampal SWR influence on prefrontal cortex unit activity, it remains unknown whether these events are required for systems memory consolidation. Here, we sought to implement and validate a real-time ripple detection module for the Open-Ephys

(http://www.open-ephys.org/) graphical user interface (GUI) to optogenetically modulating specific circuits upon the onset of hippocampal SWR. For this, we have developed in C++ a simplified ripple detection module and added it to the Open-Ephys GUI. Briefly, the module calculates the root mean square of a 2 s sample of local field potential recording and determines its average and standard deviation (STD). The threshold for ripple detection amplitude was defined as the mean + 2*STD. Next, we have integrated our module with Pulse Pal, a module developed to control an open source device that generates precise sequences of voltage pulses (https://sites. google.com/site/pulsepalwiki/home). We first used simulated ripple data to test our algorithm for latency and accuracy (Sethi and Kemere, 2014). The round-trip latency for our simplified ripple detection module is around 35 ms. Changing the size of the Open-Ephys software buffer can reduce the latency, though it also can increase false positive and/or negative rates. The compromise between software buffer size, latency, and accuracy of SWR detection will be further investigated. Besides, ongoing experiments to evaluate the biological relevance of the module combine in vivo large-scale electrophysiological recordings in anesthetized and freely moving rats with optogenetic modulation of cortical circuits. We anticipate the tools we have developed can be useful to directly investigate some memory processes involving coordination between the hippocampus and its downstream circuits. Finally, our module will be further adjusted for online detection of fast-ripples to probe the relationship between temporal lobe epilepsy and memory impairments.

Disclosures: **C.L. Aguiar:** None. **E.F. Oliveira:** None. **L.R. Zacharias:** None. **L.B. Peres:** None. **K. Diba:** None. **D.C. Soriano:** None. **J.P. Leite:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: NIH Grant MH104384

Title: Optical stimulation of the pathway from the basolateral amygdala to the entorhinal cortex in rats impairs retention of cued response learning in a Barnes maze

Authors: *K. L. WAHLSTROM, R. T. LALUMIERE;
Behavioral and Cognitive Neurosci., Dept. of Psychological and Brain Sci., Iowa City, IA

Abstract: Previous work on multiple memory systems suggests that spatial and cued-response learning are mediated by hippocampus-based and caudate-based systems, respectively. Other

work suggests that the basolateral amygdala (BLA) modulates the consolidation for both types of learning, suggesting that BLA outputs to distinct regions mediate this ability. The medial entorhinal cortex (mEC) is a critical region in the hippocampus-based system for processing spatial information and, as an efferent target of the BLA, is the likely mechanism by which the BLA influences spatial learning. The caudate is a critical region for processing visual and cued information for response learning and, as another efferent target of the BLA, is the expected mechanism by which the BLA modulates cued-response learning. Studies, however, have suggested that these two systems compete with one another in the control and consolidation of each type of learning. That is, activation of one system may alter and impair the learning associated with the other system. Therefore, the present study examined whether optically stimulating activity in the BLA◊mEC pathway alters the consolidation of cued-response learning. To address this issue, the BLA of male Sprague-Dawley rats was bilaterally transduced to express ChR2(E123A), and fiber optic probes were implanted in the mEC to provide illumination of BLA axons. A Barnes maze was used to assess cued-response learning. The Barnes maze consisted of an open, circular platform with a series of ports along the periphery, one of which led to an escape chamber. During the training trials, the escape port was marked with a distinct cue directly above it, and the port and cue were moved in unison to a different direction for each trial to prevent the animal from using a spatial strategy to solve the task. On day 1 (training), rats were given 8 training trials on the cued-response learning task, followed immediately by 15 min of optical stimulation of the BLA◊mEC pathway in the theta-frequency range. On day 3, rats were returned to the Barnes maze for a single retention test in which the target port, together with the cue, was located in a random direction. Rats that had received optical stimulation of the pathway had impaired retention, as indicated by longer latencies to find the escape port. These findings indicate that theta-frequency stimulation of BLA◊mEC projections interfered with the consolidation of cued-response learning, consistent with the hypothesis that these two neural systems compete with one another. Ongoing experiments are examining whether stimulation of the BLA◊caudate pathway enhances the consolidation for cued-response learning.

Disclosures: **K.L. Wahlstrom:** None. **R.T. LaLumiere:** None.

Poster

082. Memory Consolidation and Reconsolidation: Neural Circuit Mechanisms

Location: Halls B-H

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Program#/Poster#: 82.17/FFF11

Topic: H.01. Animal Cognition and Behavior

Support: NHMRC

ARC

Title: Synaptic connections and electrical coupling within reverberating cell assemblies triggered by chandelier neurons in rodent amygdala

Authors: *M. BHAGAVATHI PERUMAL, R. SULLIVAN, P. STRATTON, P. SAH; Queensland Brain Insititute, Brisbane, Australia

Abstract: Chandelier neurons (ChN) are a special subset of GABA neurons that make synapses at the axon initial segment (AIS) of pyramidal neurons, the key site for generation of action potential. The ChN in the basal amygdala (BA) and cortex have been reported to excite pyramidal neurons that form a disynaptic feedback excitation to the ChN. In modified horizontal brain slice preparations we observed ChNs in the BA evoked a reverberating feedback excitation with high temporal synchrony. The reverberating feedback event followed the disynaptic component with latency approximately 4 ms and frequency 180-250Hz (n=16) and also occurred spontaneously (n=37). Pyramidal neurons received GABAergic inputs during ChN triggered reverberating feedback excitation (n=6) and spontaneous events (n=20). High frequency synchronisation requires strong synaptic connections and electrical coupling. The BA ChNs received strong excitatory inputs from local pyramidal neurons with mean amplitude of 195±101 pA (n=15). Other GABA neurons also received strong glutamatergic inputs with mean amplitude 181±100 pA (n=4). Bath application of gap junction blocker abolished reverberating events (n=4). Reverberating events were observed as sharp wave ripple complex like oscillations in the local field potential (n=6). These findings suggest BA contains reverberating cell assembly like networks that are driven by ChNs. Strong synaptic connections and non-synaptic mechanisms operate in synchrony for generation of sharp wave like network oscillations in BA.

Disclosures: M. Bhagavathi Perumal: None. **R. Sullivan:** None. **P. Stratton:** None. **P. Sah:** None.

Poster

083. Learning and Memory: Basal Forebrain Circuits

Location: Halls B-H

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Title: Activation of purinergic P2 receptors in basal forebrain promotes wakefulness by exciting basal forebrain cortically-projecting neurons

Authors: *C. YANG[1], A. KALINCHUK[1], K. A. JACOBSON[2], R. E. STRECKER[1], R. W. MCCARLEY[1], R. BASHEER[1], R. E. BROWN[1];
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Abstract: Adenosine triphosphate (ATP) serves as the cellular energy source of all organisms and is an important glio- and neuro-transmitter in the brain. While ATP-derived adenosine, acting on inhibitory purinergic P1 receptors, is an important cellular mediator of sleep homeostasis, the role of ATP, acting on purinergic P2 receptors (P2Rs), in the control of sleep and wakefulness is poorly understood. Our data presented here suggests that a P2R agonist applied into the basal forebrain (BF) increases wakefulness in vivo and excites BF putatively cortically-projecting neurons in vitro. We unilaterally infused 1 mM of a non-hydrolysable ATP analog (ATP-gamma-S) into the male mouse BF during the light (sleep) period via reverse microdialysis and found that the percentage of time spent in wakefulness increased from 33.0% to 42.5% ($p=0.0694$, $n=3$). The mean duration of wake episodes increased from 52s to 67s. Non-rapid eye movement (NREM) sleep percentage decreased from 58.2% to 48.5% ($p=0.0122$) and NREM episode duration decreased from 92s to 76s. No change in episode number or REM sleep was observed. These data suggest that activation in BF P2Rs increases wakefulness without causing sleep fragmentation. Using whole-cell recordings from coronal brain slices prepared from GAD67-GFP knock-in mice (male and female), we found that ATP-gamma-S induced large depolarizing responses in GFP-negative cholinergic neurons (20.7±4.2 mV, $n=5$) and two types of large, putative cortically-projecting GABAergic (GFP+) neurons with large or small hyperpolarization-induced inward currents (12.1±1.4 mV, $n=5$; 18.3±2.6 mV, $n=6$ respectively). The input resistance was markedly decreased suggesting an opening of cation-channels during P2R activation. These depolarizing responses were significantly decreased by a P2XR antagonist (30 μM PPADS). Taken together, these data support our hypothesis that activation of BF P2Rs induces wakefulness by exciting BF cortically-projecting neurons and suggest that pharmacological agents acting on P2Rs may be useful for combating drowsiness following short or long-term sleep deprivation.

Disclosures: **C. Yang:** None. **A. Kalinchuk:** None. **K.A. Jacobson:** None. **R.E. Strecker:** None. **R.W. McCarley:** None. **R. Basheer:** None. **R.E. Brown:** None.

Poster

083. Learning and Memory: Basal Forebrain Circuits

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Program#/Poster#: 83.02/FFF13

Topic: H.01. Animal Cognition and Behavior

Support: Intramural Research Program of the National Institute on Aging, NIH.

NARSAD Young Investigator Award

Title: Temporal backpropagation of basal forebrain neuronal activity during associative learning reveals stepwise expansion of the reward prediction model

Authors: H. MANZUR, K. VLASOV, *S.-C. LIN;
Neural Circuits and Cognition Unit, Lab. of Behavioral Neurosci., Natl. Inst. On Aging (NIA), NIH, Baltimore, MD

Abstract: Understanding how animals learn to predict reward is a central question in neuroscience. However, it has remained difficult to study the early phase of new associative learning to determine what animals have actually learned, and when. Here, by tracking the temporal evolution of a reward prediction error (RPE) signal in the basal forebrain (BF) during learning, we show that animals' internal reward prediction model undergoes stepwise expansion to incorporate new reward predictors. BF RPE signal predicts reward-seeking behavior in single trials throughout learning and temporally backpropagates from the time of reward to the reward-predicting stimulus in discrete steps. A new stimulus that objectively predicts reward is incorporated into the internal model only when BF RPE backpropagates to that stimulus. These results show that stepwise expansion of the reward prediction model through RPE backpropagation is an effective strategy to systematically explore complex decision trees to learn novel associations.

Disclosures: H. Manzur: None. **K. Vlasov:** None. **S. Lin:** None.

Poster

083. Learning and Memory: Basal Forebrain Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: Intramural Research Program of the National Institute on Aging, NIH.

NARSAD Young Investigator Award

Title: Optogenetic dissection of basal forebrain neuronal circuitry reveals GABAergic identity of salience-encoding neurons

Authors: *A. SCAGLIONE, J. LIANG, R. LAM, S.-C. LIN;
NIA-NIH-IRP, Baltimore, MD

Abstract: The survival of animals depends critically on prioritizing responses to motivationally salient stimuli that predict reward or punishment. Recent studies have identified a distinct population of neurons in the basal forebrain (BF), which will be referred to as BF bursting neurons, that encode the motivational salience of attended stimuli using robust bursting responses. Activation of BF bursting neurons generates an event-related potential in the frontal cortex and leads to faster decision speeds, while inactivation of the same neurons leads to rapid behavioral stopping. A critical question about BF bursting neurons that remains unanswered is their neurochemical identity. To address this issue, we optogenetically tagged the three major cortically-projecting BF neuronal populations (cholinergic, GABAergic and glutamatergic neurons) while recording BF neuronal activity in mice performing a reward-guided reaction time task. BF bursting neurons with properties similar to those previously descried in rats were observed in mice. BF bursting neurons were optogenetically tagged in Vgat-cre mice but not in ChAT-cre and Vglut2-cre mice, supporting their GABAergic identity. Moreover, optogenetically activating each of the three major cortically-projecting BF neurons modulates the activity of BF bursting neurons in specific ways. These results establish for the first time the GABAergic identity of BF bursting neurons, and reveal how they are under the control of other cortically-projecting BF neurons.

Disclosures: **A. Scaglione:** None. **J. Liang:** None. **R. Lam:** None. **S. Lin:** None.

Poster

083. Learning and Memory: Basal Forebrain Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: NIH NINDS RO1NS075531

Title: Forebrain cholinergic and midbrain dopaminergic neurons broadcast related but distinct signals during reinforcement learning

Authors: *J. F. STURGILL, A. KEPECS;
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Abstract: Neuromodulatory centers can dramatically reconfigure cortical circuits across widespread brain areas to influence perception, attention, and learning. In contrast to midbrain dopamine neurons and other classical neuromodulatory populations, cholinergic (ChAT) neurons in the basal forebrain (BF) project across the full extent of neocortex and are believed to underpin higher cognitive functions. Yet until recently, the technical challenge of recording ChAT neural activity in behaving animals has hampered our understanding of the principles governing their activation. In recent work from our lab, optogenetic identification of ChAT neurons in an auditory stimulus detection task revealed that ChAT neurons respond rapidly (~20ms) to reinforcers (reward, punishment) and are modulated by reinforcement expectation or surprise. A putative role in reinforcement prediction invites comparison to dopamine neurons for which a key conceptual advancement was that they compute reward prediction error (RPE): the difference between reward expectation (as informed by a predictive stimulus) and reward feedback. Here, we adopt an analogous behavioral and computational approach to understand the principles governing ChAT neuron activation. Do ChAT neurons respond only to reinforcers or also to outcome-predictive sensory cues? Are the outcome-related responses modulated by the degree of surprise, as for dopamine neurons? And computationally, do ChAT responses represent an unsigned counterpart to dopaminergic RPE? Using fiber photometry we monitor in parallel the responses of basal forebrain ChAT neurons and midbrain dopamine neurons to predictive cues and reinforcement feedback. In a classical conditioning paradigm, we pair novel odor stimuli with reward or punishment and later reverse these contingencies. To validate this approach and provide a reference point, we measure bulk GCaMP responses of dopaminergic neurons (DAT-cre) and recapitulate canonical features of RPE. We find that ChAT neurons rapidly acquire cue responses to predictive stimuli. We further demonstrate that reinforcement responses of ChAT neurons vary not only according to expectancy but also with behavioral state. Lastly, taking advantage of the ability of fiber photometry to track chronic changes in activity across a neuronal population, we delineate the temporal profile of the emergence, evolution and reversal of ChAT responses to predictive cues and reinforcers.

Disclosures: **J.F. Sturgill:** None. **A. Kepecs:** None.

Poster

083. Learning and Memory: Basal Forebrain Circuits

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Topic: H.01. Animal Cognition and Behavior

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Title: High density recording in rat basalo-cortical networks.

Authors: *P. GOMBKOTO, M. GIELOW, C. CHAVEZ, L. ZABORSZKY;
Rutgers The State Univ. of New Jersey, Newark, NJ

Abstract: Traditionally, the cortical cholinergic input system has been viewed as a nonspecific, diffuse modulatory system. However, cholinergic corticopetal neurons in the basal forebrain (BF) occupy an inhomogeneous distribution whereby dense clusters of neurons are interrupted by regions of low cellular density, in humans as well as in rodents and monkeys (Zaborszky et al., 2015). A preliminary analysis of the cortical targets of these clusters suggest that cell clusters in the BF project to cortical areas that are interconnected.
We addressed the organization of the basalo-cortical network via optogenetic stimulation to identify the cholinergic neurons in the BF, then observe the specific cortical modulation patterns in orbitofrontal (VO/VL) and visual cortex following BF spikes. We used high resolution silicon based electrode arrays to record from the BF and cortex simultaneously. We also designed 3D-printed electrode drivers housed inside a 3D-printed cap that serves to protect the high density silicon electrode arrays. An electrode array was implanted into VO/VL and another, into the visual cortex (V1/V2). These areas were presumed to receive strong projections from the BF cell clusters that we targeted with our optrodes, based upon our prior detailed analysis of the projection pattern of clusters. Additionally, EEG electrodes (surface silicon probe: size 12.96 mm2, contact distance 600 μm) were positioned in nearby non-visual cortical regions to investigate the specificity of cholinergic effects around the visual cortex probe.
We calculated BF cholinergic spike-triggered averages of the local field potentials (stLFP) and of the EEGs at specific cortical locations. We found that a specific BF cholinergic unit activation elicits a spatially distinct oscillation pattern within the visual (V2) and VO/VL cortex. In the visual cortex, theta oscillation of LFPs increased for 500 ms following a cholinergic spike, at specific electrode locations. Based on cross-correlation analysis, we also found monosynaptic connection between a specific cholinergic unit and a V2 cell. However, a neighboring non-cholinergic unit in the BF, which was connected to cholinergic neuron, did not change the stLFP/EEG patterns inside and around the visual cortex respectively. In the VO/VL beta band oscillations decreased following cholinergic activation and were also spatially localized. This

data suggest that the basal forebrain projection system is not diffuse but provides spatially segregated input.

Disclosures: **P. Gombkoto:** None. **M. Gielow:** None. **C. Chavez:** None. **L. Zaborszky:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

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Title: Connectome of the basal forebrain corticopetal system.

Authors: ***P. VARSANYI**, L. ZABORSZKY;
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Abstract: Cholinergic and non-cholinergic neurons of the basal forebrain (BF) project to the entire cortex. Despite its broad involvement in cortical activation, attention, memory, the functional details are not well understood due to the anatomical complexity of the region, where different functional systems, interdigitate with the cholinergic space. Three decades of research suggests that the BF cholinergic system integrates multiple information that leads to spatially and temporally specific release of acetylcholine in the cortex but the mechanisms by which its release is regulated in a behavioral context remains largely unknown. A critical step is to understand the input-output relations of BF neurons. Conventional tract-tracing experiments using electron microscopy (Zaborszky et al., 2015) were invaluable to define synaptic inputs to identified cholinergic neurons, however, these studies were not adequate to address the extent of specificity of input-output pattern. Therefore, constructing a brain-wide connectivity model that allow analyzing densities of different cell types and connectional data from different animals should lead to more realistic models for addressing function in behavioral studies. As compared to the cortex, there is no apparent geometrical organization in the BF that requires special tools in order to analyze data from different animals.
In more than 40 brains that received conventional retrograde or virus tracer injections in the cortex, cholinergic and non-cholinergic neurons in the BF were mapped with the Neurolucida (MicroBrightField) system. After mapping fluorescently labeled cells, sections were restained with Nissl and images of sections were aligned to existing Neurolucida maps.
We are building a single reference brain to use as a common 3D space based on gapless series of sections, in which cholinergic BF neurons were mapped from every 50 μm thick section. The

rest of the brain is reconstructed from series of sections mapped with 200 µm section distances. Interpolations are used to construct a gapless approximation of the entire brain. After an automatic registration the software allows to view together both the Nissl-images of the sections and the associated vectorial dataset. The spatial registration is carried out using mixed rigid body, affine and B-Spline based elastic transformations. This connectome model that captures the organizational principles of the basalo-cortical network will facilitate the understanding of the aberrant processing in basalo-cortical networks and may help the development of new treatment strategies to ameliorate the cognitive symptoms in Alzheimer and related degenerative diseases.

Disclosures: **P. Varsanyi:** None. **L. Zaborszky:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: NINDS Grant 5R01NS023945-24

Title: Basal forebrain cholinergic-auditory cortical network: primary versus belt auditory cortical areas.

Authors: *C. M. CHAVEZ, L. ZABORSZKY;
Ctr. for Mol. and Behavioral Neurosci., Rutgers, The State Univ. of New Jersey, Newark, NJ

Abstract: Cortical acetylcholine (ACh) release is critical for learning, memory, attention, and plasticity. The basal forebrain (BF) provides cholinergic input to the entire cerebral cortex supporting these functions. Here, we explore the cholinergic and non-cholinergic BF projections to the auditory cortex using classical retrograde and monosynaptic viral tracers deposited in electrophysiologically identified regions of the auditory cortex in Sprague Dawley and Chat::Cre transgenic rats. Cholinergic input to both primary (A1) and non-primary auditory cortical (belt) areas originate in a restricted region in the caudal BF within the globus pallidus (GP) and in the dorsal part of the substantia innominata (SId). However, there were significantly more cholinergic cells projecting to A1 than to belt areas. Monosynaptic tracing revealed that inputs to A1 projecting BF cholinergic neurons were restricted to the GP, caudate-putamen (C-P), and the medial part of the medial geniculate body (MGM), including the posterior intralaminar thalamic group. Similarly, inputs to auditory belt projecting BF cholinergic cells originate within the GP, C-P, and MGM. However, additional afferents to belt-projecting BF cholinergic neurons were

found in broader areas including the ventral secondary auditory cortex, insular cortex, secondary somatosensory cortex, and the central amygdaloid nucleus. These findings provide evidence for a differential innervation pattern between primary and secondary auditory cortical regions that may contribute to the functional differences and how ACh release is regulated in the A1 and auditory belt areas.

Disclosures: C.M. Chavez: None. **L. Zaborszky:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

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Graduate School-Newark Doctoral Fellowship

Title: Input-output organization of the cholinergic basal forebrain

Authors: *M. R. GIELOW, L. ZABORSZKY;
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Abstract: Cholinergic cells of the basal forebrain individually terminate in discrete cortical areas, and collectively innervate the entire cerebral cortex. Acetylcholine efflux is differentially controlled across different cortical areas and timescales, contributing to varied processes including sleep and waking, attention, learning, plasticity, and memory. However, the anatomical mechanism of control for this differential efflux remains mysterious, in part due to the anatomical complexity of the region. In the current anatomical tracing study we compare the relationship of inputs and outputs of the cholineric basal forebrain. The resulting maps indicate that this system is highly modular in its organization. We suggest an architecture of the basal forebrain cholinergic system by which individual groups of cholinergic cells may be individually controllable based on their anatomical properties.

Disclosures: M.R. Gielow: None. **L. Zaborszky:** None.

Poster

083. Learning and Memory: Basal Forebrain Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: NIH/NINDS, NSO23945

Title: Functional integration and segregation of magnocellular cell groups in human basal forebrain: resting state study at ultra-high field, meta-analysis, and test-retest reliability

Authors: *R. YUAN[1], B. BISWAL[1], L. ZABORSZKY[2];
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Abstract: The heterogeneous corticopetal cell groups of the basal forebrain (BFC), including cholinergic, GABAergic and glutamatergic neurons, have shown to modulate cortical activation, sensory processing, attention, learning and memory through their projection to the entire cortex. Recent advances in the ultra-high field 7T fMRI provided potentially unprecedented spatial resolution of functional MRI images to systematically study the sub-division of BFC. In this study, first, using a probabilistic 3D postmortem map of the BFC (Zaborszky 2008) and a high resolution functional connectivity dataset, the BFC was divided into functionally distinct 3 sub-divisions. Second, the overall connection of each BFC sub-division was exanimated with a test-rest study. Third, meta-analysis was used to study the related topics of each sub-division. The data-driven parcellation method gave rise to three sub-divisions along the rostral–caudal axis of the basal forebrain. The rostral subdivision is highly associated with the anterior cingulate [BA24/32], the thalamus, the hippocampus [BA28/35], the insula [BA 13], the superior [BA22] and middle temporal gyrus [BA21], the middle [BA11] and inferior frontal gyrus [BA47], the precuneus [BA31]. The functional connection of the middle subdivision are the lateral globus pallidus, putamen, caudate, the cingulate gyrus [BA24/32], the thalamus, the insula [BA13], the frontal gyrus [BA32/27], the hippocampus [BA27]. The caudal subdivision is mainly related with the putamen, lateral globus pallidus, hippocampus, thalamus, cerebellum, claustrum, insula, the precentral gyrus [BA4/6], and the medial frontal gyrus [BA6/13]. Interestingly, all three clusters have shown consistent overlaps at specific target regions, including the hippocampus, insula, the thalamus and, cingulate gyrus, contributing to distinct topographical patterns. For example, the rostral sub-division is connected with the anterior part of the thalamus, while the caudal sub-division is highly connected with the medial dorsal thalamus. Meta-analysis also suggests that the various sub-divisions are associated with specific functional topics. The rostral subdivision is related to reward, decision making, and motivation. The medial subdivision is associated with emotional regulation, anxiety and threat. The caudal subdivision relates to motor, sensorimotor and auditory process. To sum up, this study demonstrates that BFC can be

subdivided into distinct functional sub-divisions, and the connectivity pattern of each sub-division is associated with different networks and cognitive processes.
This study was supported by NIH/NINDS, NSO23945

Disclosures: **R. Yuan:** None. **B. Biswal:** None. **L. Zaborszky:** None.

Poster

083. Learning and Memory: Basal Forebrain Circuits

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Title: Optogenetic stimulation of cortically projecting basal forebrain parvalbumin neurons cause short latency arousals in mice

Authors: ***J. T. MCKENNA**[1], S. THANKACHAN[1], C. SHUKLA[1], J. M. MCNALLY[1], J. C. ZANT[1], S. WINSTON[1], K. DEISSEROTH[3], R. E. BROWN[1], R. BASHEER[1], R. W. MCCARLEY[2];
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[3]Psychiatry and Behavioral Sciences/Bioengineering, Stanford Univ., Stanford, CA

Abstract: This study focused on the specific role of basal forebrain (BF) GABAergic parvalbumin (PV) neurons in arousal. We had previously shown that BF cortically projecting neurons discharge at a high rate during wakefulness and regulate cortical gamma band (40Hz) oscillations associated with feature binding, attention and consciousness (Kim et al., 2015). These neurons are excited by acetylcholine and are involved in mediating the arousal effects of

BF cholinergic neurons (Yang et al., 2014; Zant et al., 2016). We hypothesized that the direct input of BF PV neurons to cortical PV neurons would mediate a short latency arousal when optogenetically stimulated. We also compared these latencies to those of cholinergic neuronal stimulation.

We unilaterally/bilaterally injected double-floxed adeno-associated viral vectors expressing Channelrhodopsin2 (AAV-ChR2-EYFP) into the BF of PV-Cre mice outfitted with cortical EEG & EMG electrodes for sleep-wake recordings. Optical stimulations (5s stim/min, 10ms pulses @ 40 Hz) were performed using blue laser (20mW, 473nm) for 6h (ZT2-ZT8) with simultaneous EEG/EMG recordings. For stimulations that occurred after at least 10s of NREM, latency to arousal was calculated as the time from the beginning of stim to end of the last slow wave activity leading to EEG desynchronization, and compared with that of mock-stim baseline day (BL, laser off). For comparisons, the median statistic was used because of the skewed, non-normal distribution of responses. Post-hoc immunohistochemistry was performed to confirm transduction efficiency, selectivity, and location of the optical fiber tract.

We observed that uni-/bi-lateral stimulations (N=10) significantly increased both the time spent in wakefulness compared to BL day by 22.61±3.0% ($p=<0.001$), and % transitions from NREM to wakefulness (BL day, 64.27±3.8%; Stim-day, 75.2±2.3%; $p=0.03$). The median latency for transitions from NREM to wakefulness was significantly decreased in both conditions of unilateral (BL, 16.9±2.7s vs stim, 7.18±1.5s; $p=0.02$; N=4) and bilateral (BL, 20.0±1.8s vs stim, 3.9±1.2s; $p<0.001$; N=6) stimulations.

In summary, optogenetic stimulation of BF PV neurons increased overall amounts of wakefulness. The median latency for NREM to wakefulness was shorter following optogenetic stimulation of BF-PV when compared to non-stim baseline values. These values were also shorter than those associated with optogenetic stimulation of BF cholinergic neurons (median: 10.6s). Thus, our data suggests that BF PV neuronal excitation causes rapid EEG arousal, likely due to fast cortical disinhibition elicited via direct innervation of the inhibitory interneurons.

Disclosures: **J.T. McKenna:** A. Employment/Salary (full or part-time): Merck MISP. 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; Merck MISP. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Merck MISP. **S. Thankachan:** None. **C. Shukla:** None. **J.M. McNally:** None. **J.C. Zant:** None. **S. Winston:** None. **K. Deisseroth:** None. **R.E. Brown:** None. **R. Basheer:** None. **R.W. McCarley:** None.

Poster

083. Learning and Memory: Basal Forebrain Circuits

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Title: Basal forebrain glutamate neurons studied using vGluT2-tdTomato mice: intrinsic membrane properties, cholinergic sensitivity, calcium binding protein content and projections

Authors: *R. E. BROWN[1], C. YANG[2], J. T. MCKENNA[2], J. M. MCNALLY[2], M. GAMBLE[3], A. HULVERSON[3], T. BELLIO[3], J. MCCOY[3], M. ANDERSON-CHERNISOF[2], S. WINSTON[2], K. DEISSEROTH[4], S. THANKACHAN[2], R. BASHEER[2], R. W. MCCARLEY[1];
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Abstract: Basal forebrain (BF) neurons play a crucial role in cortical activation, attention, sleep-wake behavior and reward processing. Of the three major BF neurotransmitter classes, glutamatergic neurons are the least well understood. Recent optogenetic gain-of-function experiments suggest that stimulation of BF neurons expressing the vesicular glutamate transporter type 2 (vGluT2) strongly promotes wakefulness. However, currently little is known about the subtypes of BF vGluT2 neurons and their properties which mediate this wake-promoting effect. Thus, here we have investigated the anatomy and physiology of vGluT2 neurons in a transgenic mouse model that expresses a red fluorescent protein (tdTomato) in vGluT2 neurons (vGluT2-tdTomato mice). In vitro, whole-cell recordings were made from tdTomato+ neurons (n=53) in intermediate/caudal BF regions of young (12-22d) vGluT2-tdTomato mice. Compared to cholinergic neurons, vGluT2+ neurons had a higher maximal firing rate, smaller afterhyperpolarizations and briefer action potentials. Compared to GABAergic

neurons, vGluT2+ neurons had a lower maximal firing rate and were hyperpolarized by the cholinergic agonist, carbachol, whereas GABAergic neurons were excited. A subset of vGluT2+ neurons exhibited T-type calcium currents and burst firing. However, there were marked subregional differences in hyperpolarization-activated cation currents, low-threshold calcium currents and in the inhibitory effect of carbachol. Immunohistochemical staining (n=4) revealed that major subgroups of BF vGluT2+ neurons express the calcium-binding proteins calbindin and calretinin, but not parvalbumin. Anterograde tracing using adeno-associated viral vectors expressing channelrhodopsin2-enhanced yellow fluorescent fusion proteins revealed major projections of BF vGluT2+ neurons to BF cholinergic and parvalbumin neurons as well as to extra-BF areas including frontal cortex, lateral habenula, lateral hypothalamus and ventral tegmental area (VTA). This work suggests there are several subtypes of BF vGluT2 neurons which differ in their intrinsic membrane properties, calcium binding content, cholinergic modulation and likely also their projections. Cortical projections and intra-BF connections of vGluT2+ neurons to cholinergic and GABA/parvalbumin neurons are consistent with an important role in sleep-wake control. Prominent projections of vGluT2+ neurons to lateral habenula, lateral hypothalamus and VTA suggest an additional, unexpected role in reward processing.

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Poster

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Topic: H.01. Animal Cognition and Behavior

Support: Mallinckrodt Foundation

Title: Reward-timing prediction errors in the brain

Authors: ***C. CHEN**, I. MONOSOV;
Washington Univ. In St. L, Saint Louis, MO

Abstract: Predicting the timing of important events, such as rewards, is crucial for behavioral strategy in an uncertain environment. Previous literature suggests single neurons could be timing important events through anticipatory ramping activity (Goel and Buonomano, 2014; Llyod and Dayan, 2015; Blanchard, Strait, and Hayden, 2015; Bermudez and Schultz, 2014).
We recently discovered single neurons in medial basal forebrain (mBF) and internal capsule bordering dorsal striatum (icbDS) that display anticipatory ramping activity after onset of conditioned stimuli (CSs) associated with uncertain reward delivery up to trial outcomes (White and Monosov, in review; Monosov et al., 2015). This suggests mBF and icbDS may contain mechanisms for predicting timing of events under uncertainty.
We studied mBF and icbDS uncertainty-selective ramping neurons while monkeys participated in a behavioral procedure in which reward was certain, but timing of delivery was uncertain. We conditioned monkeys with 4 CSs associated with 0.25, 0.50, 0.75, or 1.00 probability of reward delivery after 1.5 seconds (s). On trials when rewards were not delivered in 1.5 s, rewards were delivered 4.5 s after CS onset.
We found reward delivery 1.5 s after CS onset elicited a phasic excitatory response in mBF ramping neurons. The magnitude of response was inversely correlated with probability of reward. No phasic response was observed after 4.5 s. In contrast, we found reward delivery either 1.5 or 4.5 s after CS onset elicited a phasic excitatory response in many icbDS ramping neurons. The magnitude of response did not vary significantly across different CSs or times of delivery.
We tested if phasic responses in mBF and icbDS could be a reward prediction error (RPE) generated by the difference between reward and predicted value at the time of reward delivery. We recorded neurons while monkeys experienced 5 CSs associated with 0.00, 0.25, 0.50, 0.75, or 1.00 probability of reward delivery. This procedure did not introduce temporal uncertainty as timing of trial outcomes did not vary. We found neither population of mBF or icbDS neurons generated phasic responses upon reward delivery.
We propose phasic responses in mBF ramping neurons do not encode traditional RPEs, but a timing prediction error generated roughly by the difference between reward time and predicted time of delivery. We propose phasic responses in icbDS ramping neurons also do not encode RPEs, but a reward timing response generated upon resolution of trials with temporal uncertainty.
We propose a neuron model based on phasic responses in mBF and icbDS ramping neurons to demonstrate their possible usefulness for behavior in uncertain environments.

Disclosures: C. Chen: None. **I. Monosov:** None.

Poster

084. Learning and Memory: Parahippocampal and Related Cortical Circuits

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Topic: H.01. Animal Cognition and Behavior

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NSF-GRFP DGE-1444932

Title: Impaired familiarity discrimination after neonatal perirhinal lesions in rhesus macaques

Authors: *A. R. WEISS, W. GUO, J. BACHEVALIER;
Emory University/YNPRC, Atlanta, GA

Abstract: The contribution of the perirhinal cortex (PRh) to recognition memory is well characterized in adults. PRh lesions severely impair recognition memory when assessed with either Visual Paired Comparison (VPC) or Delayed Non-Match to Sample (DNMS). Similar recognition memory deficits on both tasks followed neonatal PRh lesions (Neo-PRh; VPC: Zeamer et al., 2015; DNMS: Weiss & Bachevalier, 2016), although the severity of the deficits was more pronounced in DNMS than VPC. Thus, for VPC, performance remained significantly better after Neo-PRh lesions than after adult-onset PRh lesions (Nemanic et al., 2004), suggesting that other brain areas could partially compensate. Conversely, for DNMS, recognition memory deficits were of the same magnitude after early-onset Neo-PRh lesions and adult-onset PRh lesions (Meunier et al., 1993). One procedural difference that could explain the difference in severity of memory deficits is the familiarization time with the sample objects. VPC uses a cumulative 30s familiarization, whereas in DNMS the monkeys are exposed to the sample for only the length of time it takes to displace it (3-7s). Given that PRh has been shown to play a role in familiarity judgments (Bowles et al., 2007), it is possible that better performance of Neo-PRh animals on VPC may have resulted from longer exposure with the objects. To test this possibility, we used the Constant Negative task (Browning et al., 2013), which allowed for repeated exposures to objects. The same Neo-PRh monkeys (n=6) and their controls (Neo-C, n=3) were given 60 discrimination problems. For each problem, one object was the unrewarded "constant negative" stimulus (S-) and another novel object was the rewarded stimulus (S+). Each of the 60 S- objects were presented once every daily testing session, and became familiar over several days. In contrast, the S+ objects presented with the S- were always novel. Neo-PRh and Neo-C monkeys made similar numbers of errors before reaching the learning criterion of 90% correct [$t(7)=-1.07$, $p=.32$], but the groups differed in the number of trials needed to reach criterion [$t(7)=-2.54$, $p=.04$]. Additional linear regression analyses revealed that the rate of learning significantly differed between the groups [$t(67)=5.31$,$p<.01$], with Neo-C showing faster learning (steeper slopes) than Neo-PRh. These data suggest that group Neo-PRh may

require more exposures to an object before judging it as familiar and help to account for the differential patterns of functional compensation on VPC and DNMS tasks. The results provide further support to the view that PRh is involved in familiarity judgements. This work was supported by grants MH-58846 and NSF-GRFP DGE-1444932.

Disclosures: **A.R. Weiss:** None. **W. Guo:** None. **J. Bachevalier:** None.

Poster

084. Learning and Memory: Parahippocampal and Related Cortical Circuits

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Title: Lesions of the postrhinal cortex impair extinction learning in a latent inhibition paradigm

Authors: *N. E. DEANGELI, T. P. TODD, D. J. BUCCI;
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Abstract: The postrhinal cortex (POR) is part of a network of posterior cortical regions that provide the hippocampus with processed sensory information. Consistent with this, prior lesion studies have demonstrated that POR is critically involved in hippocampal-dependent processes, such as contextual fear conditioning. However, the precise contribution of POR to learning and memory remains unclear. Based on electrophysiological findings and the connectivity of POR with attentional regions such as posterior parietal cortex, it has been postulated that POR may be involved in monitoring the environment for changes in stimuli and altering attention to those cues accordingly. To causally test this, we examined the effects of POR damage using a latent inhibition procedure. In a typical latent inhibition paradigm, prior non-reinforced exposure to a stimulus retards learning when the same stimulus is subsequently paired with a significant outcome (compared to conditioning to a novel cue). Attention-based accounts of latent inhibition posit that pre-exposure to the stimulus in the absence of reinforcement decreases the associability (attention) of that stimulus. Here, rats with either sham lesions or electrolytic lesions of the POR were first trained to lever press for food reinforcement. Half of the rats in each group rats were then assigned to the pre-exposure condition and received ten presentations of a 30-sec white noise conditioned stimulus (WN) each day for six days. Rats in the non-pre-exposure groups remained on a random-interval 60-sec schedule throughout this phase of the experiment and did

not receive presentation of the WN. During the subsequent conditioning phase, rats in all four groups received one pairing of the WN and a 0.5-mA, 0.5-sec footshock during each of six daily sessions. Then in the final phase of the experiment (extinction training), rats in all groups received four presentations of the 30-sec WN per day for ten days (no shock was delivered during the extinction phase). Suppression ratios were calculated for each WN presentation. Lesions of POR had no impact on responding during either the pre-exposure or conditioning phases. However, conditioned responding to the WN was slower to extinguish in the POR-pre-exposure group compared to the other three groups. These findings suggest that while decremental changes in associability may be intact following POR damage, the ability to increase attention when a stimulus is no longer paired with an expected outcome may be impaired when POR is dysfunctional.

Disclosures: **N.E. DeAngeli:** None. **T.P. Todd:** None. **D.J. Bucci:** None.

Poster

084. Learning and Memory: Parahippocampal and Related Cortical Circuits

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 84.03/FFF26

Topic: H.01. Animal Cognition and Behavior

Support: NIMH R01MH108729

NSF IOS-1146334

5F32MH105210-02

Title: Representations of context in the postrhinal cortex

Authors: *V. R. HEIMER-MCGINN, B. KENT, R. D. BURWELL;
Dept. of Cognitive, Linguistics and Psychological Sci., Brown Univ., Providence, RI

Abstract: The brain's ability to understand spatial context is central to many higher-order cognitive functions including episodic memory. Despite its importance to cognitive function, there are many open questions about how context is encoded and represented in the brain. The parahippocampal cortex in primates and the homologous postrhinal cortex (POR) in rodents are thought to process spatial information, whereas the perirhinal cortex (PER) is known to process object information. A prevailing view of medial temporal lobe function emphasizes that the POR provides spatial information to the hippocampus (HC) and the PER provides non-spatial information. The PER and POR each project to the HC both directly and indirectly through the lateral and medial entorhinal areas. By one view, the spatial pathway conveys both spatial and

contextual information to the HC. By another view, the HC, itself, configures spatial and object information into representations of context. Neither view takes into account the robust, multilevel connections across the so-called spatial and non-spatial pathways. For example, the PER provides a robust input to the POR. Available evidence suggests that representations of context may be formed in the POR, upstream of the hippocampus. We hypothesize that the POR combines object and spatial information to build a model of the local spatial context (including the spatial layout of objects, patterns, and features of the environment), and that it monitors the local environment in order to update the current representation when changes occur. In this study we recorded neuronal activity and local field potentials (LFPs) in the POR of rats during performance on a non-spatial biconditional discrimination task (nsBCD) designed to differentiate the neuronal correlates of objects, location, and context. In this task, dynamic floor patterns determine which object in a pair is correct. For example, one object of a pair would be correct on a striped floor and the other on a dotted floor. Automated projection of visual stimuli and online tracking of head position and direction allowed precise measurement of behavioral correlates. Nearly one-third of POR cells were significantly modulated by a combination of object identity, object location, and context (defined as floor pattern). LFP analysis revealed that ~40% of POR cells were phaselocked to theta oscillations. Also, changes in theta power predicted trial outcome independently of running speed, with lower pre-stimulus theta power associated with higher accuracy. Our findings support the hypothesis that POR forms a representation of the local environment, and signals changes to the environment as they occur.

Disclosures: V.R. Heimer-Mcginn: None. **B. Kent:** None. **R.D. Burwell:** None.

Poster

084. Learning and Memory: Parahippocampal and Related Cortical Circuits

Location: Halls B-H

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Topic: H.01. Animal Cognition and Behavior

Support: NSF GRFP 1058262 to VJE

NSF IOB-1146334 to RDB

NIMH R01MH108729 to RDB

Title: Emergence of object-location conjunctive coding in the postrhinal cortex and hippocampus

Authors: *V. J. ESTELA[1], R. D. BURWELL[2];
[1]Neurosci., [2]Cognitive, Linguistic, and Psychological Sci., Brown Univ., Providence, RI

Abstract: Episodic memory, or the recollection of autobiographical events in time and space, requires the ability to fully represent and recall context. Contexts are intricate representations that consist of places combined with objects and patterns, including the specific spatial arrangements of these features. Structures in the medial temporal lobe, specifically the perirhinal (PER) and postrhinal (POR) cortices, are crucial for contextual learning. Our guiding hypothesis is that the POR integrates object information from the PER with spatial information from the posterior parietal cortex to represent the spatial layout of objects, patterns, and features in the local environmental context. Such representations are then made available to other regions for context-guided behavior. We further hypothesize that the hippocampus (HC) relies on POR context representations for associative learning and episodic memory. Prior studies reported that both the POR and the HC exhibit object-location conjunctive coding (Furtak, Ahmed, and Burwell, 2012; Komorowski, Manns, & Eichenbaum, 2009). Such conjunctions emerge with learning in the HC, but the timing of the emergence of object-location conjunctions in the POR is unknown. One possibility is that object-location conjunctions in the POR reflect the representation of the spatial layout of objects in the local context, whereas such conjunctions in the HC reflect the binding of objects in context for episodic memory. If our hypothesis is correct, object-location conjunctions should be present in the POR before they emerge in the HC. To test this prediction, we will record simultaneously in the POR and HC during performance on the location bi-conditional (locBCD) task. In this task, the location in a bowtie-shaped maze determines which of two objects is correct. For example, if the objects to be discriminated are a star and a circle, the star might be correct on the west side of the maze and the circle would then be correct on the east. This task capitalizes on the propensity of rats to explore objects on the floor as well as available evidence that cortical input to the POR is primarily visual. We have shown that rats can learn concurrent and sequential pairs in this and similar tasks. We predict that as rats learn new discriminations, object-location conjunctive coding will be evident in the POR before it is evident in the HC. Such a finding would be consistent with the interpretation that object-location conjunctive coding in the HC relies on POR representations of context. Future studies will address this interpretation in circuit analysis experiments that combine electrophysiology and optogenetics in rats performing the locBCD task.

Disclosures: V.J. Estela: None. **R.D. Burwell:** None.

Poster

084. Learning and Memory: Parahippocampal and Related Cortical Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: NIMH R01MH108729

NSF IOS-1146334

Brown Institute for Brain Science Graduate Research Fellowship, Brown University, Providence, RI

Title: Inactivation of the lateral posterior thalamic nucleus on neuronal correlates in rat posterior parietal cortex during performance on a visuospatial attention task

Authors: *F.-C. YANG[1], R. D. BURWELL[1,2];
[1]Cog., Lin. & Psychological Sci., [2]Dept. of Neurosci., Brown Univ., Providence, RI

Abstract: The posterior parietal cortex (PPC) has been identified as important for visuospatial attention. In rats, this region receives input from visual cortex and from the lateral posterior nucleus of thalamus (LPO), the functional homolog of the primate pulvinar. The LPO and pulvinar are also implicated in attention. In primates, the pulvinar differentially projects to two subdivisions of the PPC. Recent studies showed functional differentiation of these PPC subdivisions, with one region supporting top-down attention and the other supporting bottom-up attention (Cabeza, 2008). The rodent PPC can also be subdivided into dorsal and caudal subdivisions (dPPC and cPPC). We previously reported that neuronal activity in the dPPC and cPPC is consistent with top-down and bottom-up attentional functions. Our previous work showed that neuronal activity in LPO and parietal subdivisions correlated with multiple phases of a visuospatial attention (VSA) task, including onset of the visual stimuli, decision-making, task-relevant location, and behavioral outcome. To further understand how posterior parietal activity depends on the LPO, we simultaneously recorded neuronal activity in the LPO, dPPC, and cPPC in male Long-Evans rats during performance on the VSA task. On some trials LPO activity was optogenetically inhibited. Our VSA task was adapted from the five-choice serial reaction time task for a double-sided, bowtie shaped enclosure atop the Floor Projection Maze. Trials alternated from side to side. For each trial, rats were required to attend to three locations. In one of these locations, a target stimulus was randomly and briefly illuminated. An approach to the correct target location was followed by a liquid reward. For analysis, a trial was divided into behavioral epochs including stimulus onset, selection behavior, and reward. We recorded neuronal activity of six rats, and isolated 318 cells in the dPPC, 225 cells in the cPPC, and 474 cells in the LPO. We report that LPO and PPC cells signal stimulus onset and selection behavior consistent with the interpretation that the LPO and PPC are engaged in both top-down and bottom-up visuospatial attention. We also observed that LPO and PPC cells responded to allocentric and egocentric task-relevant locations. Preliminary analyses showed that behavioral correlates of LPO and PPC neurons disappeared or changed during optogenetic inhibition of LPO activity supporting our hypothesis that the parietal activity depends on the LPO inputs when visuospatial attentional demands are high. Further analyses and detailed results will be presented.

Disclosures: F. Yang: None. **R.D. Burwell:** None.

Poster

084. Learning and Memory: Parahippocampal and Related Cortical Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: NIMH R01MH108729

NSF IOS-1146334

Title: The role of the rodent retrosplenial cortex in context-guided behavior

Authors: *E. HWANG[1], F.-C. YANG[1], R. D. BURWELL[2];
[1]CLPS, [2]CLPS, Neurosci., Brown Univ., Providence, RI

Abstract: Research in rodents and primates has addressed the role of the retrosplenial cortex (RSP) in spatial information processing, giving rise to theories of RSP function, including translating across different spatial reference frames, processing contextual associations with objects, and encoding the spatial locations of permanent landmarks. Damage to the RSP, the postrhinal cortex (POR) and RSP-POR disconnection impair contextual fear conditioning in rats, suggesting that the two regions interact in contextual learning (Burwell et al, 2004, Keene & Bucci, 2008). Anatomical studies of the rodent brain show that the RSP and the POR are interconnected (Burwell & Amaral, 1998). How these connections contribute to contextual information processing, however, is not clear. We have shown that the POR exhibits object-location and object-context conjunctive coding in simple object discrimination and context-guided object discrimination. We hypothesize that the RSP provides task-relevant spatial information to the POR for the purpose of representing context and forming contextual associations. If so, we would expect RSP neurons to show spatial correlates, but not object-location conjunctions, in a context-guided discrimination task. We recorded neuronal activity in the RSP in male Long-Evans rats during performance on the location biconditional discrimination (locBCD) task (Fig.1). This task was adapted from the conditional discrimination task from Komorowski et al. (2009) for a double-sided, bowtie shaped enclosure atop the Floor Projection Maze (Jacobson, et al. 2014). Trials alternated from the east to the west side of the maze. Fixed, distinctive floor patterns and distal cues distinguished the east side from the west side. On each trial, a pair of 2-dimensional objects was presented on the floor. For each pair of objects, one was correct in the east and the other, in the west. An approach to the correct location was rewarded. On some trials novel objects were presented in order to disambiguate location correlates from object-location conjunctions.

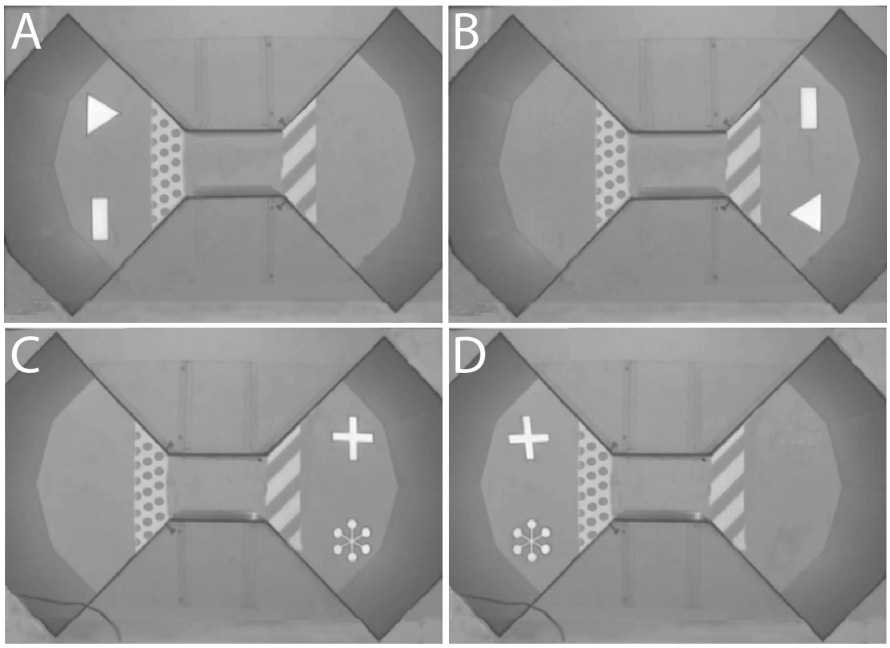

Fig 1. Location-guided biconditional discrimination task. Two object pairs are shown in A/B and C/D. Side of the maze (east or west) determines which object is correct. For example, in A the triangle would be correct and in B the rectangle would be correct.

Disclosures: **E. Hwang:** None. **F. Yang:** None. **R.D. Burwell:** None.

Poster

084. Learning and Memory: Parahippocampal and Related Cortical Circuits

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 84.07/GGG4

Topic: H.01. Animal Cognition and Behavior

Title: Encoding of episodic events by the retrosplenial cortex

Authors: ***G. FOX**, J. Z. TSIEN;
Med. Col. of Georgia, Augusta Univ., Augusta, GA

Abstract: The retrosplenial cortex (RSC), a posterior midline structure possessing vast connections with the hippocampal formation, anterior thalamic nuclei, and select areas within the cortex, has been shown to have a role in the acquisition and consolidation of spatial, contextual, and episodic memory. Of notable interest, the RSC may serve as a long-term memory storage site. However, little is known about the dynamic patterns of RSC cells during episodic events. By taking advantage of *in vivo* neural recording techniques coupled with optogenetics, we monitored the activities of various neurons in the RSC of freely behaving mice during multiple fearful events. In our behavioral paradigm, wild-type mice or optogenetically labeled mice were subjected to the following fearful events: foot shock, elevator drop, earthquake, and air puff. Large numbers of neurons were recorded and their identities were further examined by optogenetic stimulation. We demonstrated that principal cells within the RSC respond robustly to various fearful episodic events, apparently following a power-of-two-based mathematical rule. Additionally, optogenetically-identified interneurons were manipulated during fearful stimulation. These results will be valuable to understand how the RSC generates a cell-assembly level representation of fear memory engrams.

Disclosures: **G. Fox:** None. **J.Z. Tsien:** None.

Poster

084. Learning and Memory: Parahippocampal and Related Cortical Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: NSF Grant IOS1353137

NIH Grant F32MH092991

Title: Retrosplenial cortex lesions produce retrograde and anterograde context amnesia following overtraining

Authors: R. HUSZAR, M. C. EDDY, N. E. DEANGELI, D. J. BUCCI, 03755, *T. P. TODD; Psych & Brain Sci., Dartmouth Col., Hanover, NH

Abstract: The retrosplenial cortex (RSC) is positioned at the interface of primary cortical sensory areas and the parahippocampal - hippocampal memory system and is thus well-suited to contribute to learning and memory processes. Indeed, the RSC has been shown to have an important role in both spatial and contextual learning. For example, both pre- and post-training lesions of RSC attenuate contextual fear conditioning. The system that supports contextual fear

conditioning is dynamic (Fanselow, 2010). For example, pre-training lesions of the hippocampus produce deficits with "weak" training (e.g., 1 shock). However, with "overtraining" (e.g., more than 3 shocks), pre-training hippocampus lesions do not attenuate contextual fear conditioning, although post-training lesions abolish context fear. Thus, with enough training, the hippocampus is not required for context learning. The purpose of the current experiment was to examine the role of the RSC in contextual fear conditioning after overtraining. All rats first received 25 tone-shock pairings in Context A. The next day, half of the rats received sham lesions of the RSC and the other half received electrolytic lesions. Following recovery from surgery for 2 weeks, all rats were returned to Context A. We found that RSC-lesioned rats exhibited less freezing behavior compared to the sham-lesioned controls. A subsequent test of tone-specific fear in Context B revealed no differences between groups, indicating that RSC lesions produced a selective deficit on contextual fear. All rats then received retraining (i.e., 25 tone-shock pairings) in Context B. When fear to Context B was assessed, RSC-lesioned rats once again exhibited attenuated freezing behavior. The tone was then tested in Context C, and no lesion induced deficits were observed. Overall, lesions of the RSC produced both retrograde and anterograde deficits with overtraining in a contextual fear conditioning paradigm. In contrast, previous studies have demonstrated that hippocampal lesions produce retrograde, but not anterograde, amnesia following overtraining (e.g., Wiltgen, 2006). Thus, the current studies suggest that RSC has essential role in contextual fear conditioning and that other systems or pathways are unable to compensate for the loss of RSC function.

Disclosures: **R. Huszar:** None. **M.C. Eddy:** None. **N.E. DeAngeli:** None. **D.J. Bucci:** None. **T.P. Todd:** None.

Poster

084. Learning and Memory: Parahippocampal and Related Cortical Circuits

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 84.09/GGG6

Topic: H.01. Animal Cognition and Behavior

Title: Pde4d regulates spine plasticity and memory in the retrosplenial cortex.

Authors: *K. BAUMGAERTEL[1], D. WHEELER[2], A. GREEN[2], J. LAPIRA[2], D. ELOW[2], K. MARUYAMA[2], R. JOHNSON[2], R. BARIDO[2], M. PETERS[2];
[1]Neurogenetics, Dart Neurosci., San Diego, CA; [2]Dart Neurosci. LLC, San Diego, CA

Abstract: The retrosplenial cortex (RSC) has been of interest to memory researchers for over 30 years. Recently, this interest has been rekindled by reports suggesting that RSC is a storage site of spatial memory. To further elucidate this role we performed a thorough characterization of the

RSC in contextual fear memory, a task that has been extensively used to examine the hippocampus. We show that the RSC is activated during contextual memory formation, and that an intervention that blocks memory also blocks this activation. Using Pde4d downregulation as a tool, we next show that systemic inhibition, hippocampal knockdown (KD) or KD in the RSC all enhance fear memory. Both Pde4d inhibition and Pde4d KD increase the number of mature spines in RSC, a mechanism implicated in memory. This research highlights the parallels in plasticity mechanisms between RSC and hippocampus and provides the most compelling evidence for RSC as a storage site of memory to date.

Disclosures: **K. Baumgaertel:** A. Employment/Salary (full or part-time): Dart Neuroscience LLC. **D. Wheeler:** A. Employment/Salary (full or part-time): Dart Neuroscience LLC. **A. Green:** A. Employment/Salary (full or part-time): Dart Neuroscience LLC. **J. Lapira:** A. Employment/Salary (full or part-time): Dart Neuroscience LLC. **D. Elow:** A. Employment/Salary (full or part-time): Dart Neuroscience LLC. **K. Maruyama:** A. Employment/Salary (full or part-time): Dart Neuroscience LLC. **R. Johnson:** A. Employment/Salary (full or part-time): Dart Neuroscience LLC. **R. Barido:** A. Employment/Salary (full or part-time): Dart Neuroscience LLC. **M. Peters:** A. Employment/Salary (full or part-time): Dart Neuroscience LLC.

Poster

084. Learning and Memory: Parahippocampal and Related Cortical Circuits

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 84.10/GGG7

Topic: H.01. Animal Cognition and Behavior

Title: Going the distance: the effect of geometry on spatial representations

Authors: *M. V. KURUVILLA, J. A. AINGE;
Psychology & Neurosci., Univ. of St Andrews, St Andrews, United Kingdom

Abstract: Grid cells have been theorized to provide a neural metric for spatial information. However, evidence of distorted grid patterns presents some interesting questions regarding how our perception of space and distance is affected by environmental geometry. Recently, it was demonstrated that grid cell symmetry distorts when rats are placed in a trapezoid (Krupic, J., Bauza, M., Burton, S., Barry, C., & O'Keefe, J. (2015). *Nature*, *518*(7538), 232-235). Here, we assessed the impact on spatial memory and navigation when rats had to learn to consistently stop at a certain distance in either a trapezoidal or rectangular box. We predicted that rats distance perception would be altered in the trapezoid relative to the rectangle. The experiment was conducted in box that could be configured to form either a rectangle (1.9m * 0.9m) or trapezoid

(1.9m diagonals, 0.9m long parallel wall, 0.2m short parallel wall). Rats were habituated to both configurations for a total of 60 minutes before the start of the experiment. In the practice phase, rats were trained in a rectangle to run in a straight line, stop within a 0.15m zone (1.23m – 1.37m) and return back to the starter box for a food reward. In the test phase rats completed the same task across 10 trials, 5 trials each in the rectangle and trapezoid configuration. The stopping zone in the trapezoid configuration was increased by +/- 10% to account for potential changes in distance perception as a result of an alteration in geometry. Rats stopped significantly earlier in the trapezoid compared to the rectangle suggesting that their perception of space had been changed by the local geometry of the environment even though global spatial cues were available and consistent. It could be the case that rats were solving this task using either a distance or a place strategy. To assess the use of a distance strategy, rats we re-tested, with global cues concealed by a curtain. Again, rats stopped significantly earlier in the trapezoid compared to the rectangle indicating an altering of spatial representation as a result of a change to local geometry and in the absence of a global cue-dependent place strategy. Additionally, across trapezoid but not rectangle trials, rats stopped significantly earlier in the absence of global cues than in their presence. This suggests that the absence of global cues exacerbates the disruption of spatial representation caused by local geometry. We would predict that distortion of grid cell symmetry in trapezoids would be greater in the absence of global cues, which is the focus of on-going experiments.

Disclosures: M.V. Kuruvilla: None. **J.A. Ainge:** None.

Poster

084. Learning and Memory: Parahippocampal and Related Cortical Circuits

Location: Halls B-H

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Program#/Poster#: 84.11/GGG8

Topic: H.01. Animal Cognition and Behavior

Support: Henry Dryerre PhD Scholarship

Title: Object representation along the proximodistal axis of CA1

Authors: *J. A. AINGE, B. M. VANDREY;
Univ. of St Andrews, St Andrews, United Kingdom

Abstract: Models of episodic memory in the medial temporal lobe often suggest that the spatial and non-spatial content of an episode reaches the hippocampus via the medial entorhinal cortex (MEC) and lateral entorhinal cortex (LEC), respectively. However, these models are challenged by evidence that the LEC plays a critical role in binding these two types of information together

prior to the hippocampus. Further, the LEC contains neurons which are spatially tuned to objects. Notably, object-related firing in the LEC strongly resembles object-modulation of place cells in the hippocampus; neurons in both structures encode object location, respond to object displacement, and fire at locations where an object was previously located. However, the origin of object-modulation in hippocampal place cells is unknown. One possibility is that LEC input drives spatial representation of objects in the hippocampus. To explore this hypothesis, we implanted microdrives in rats (n=5), with tetrodes targeting either the proximal, bordering CA2, or distal, bordering subiculum, parts of CA1. These regions receive differential input from the entorhinal cortex, so this strategy permitted us to record from neuronal ensembles which primarily receive MEC or LEC input. Place cells were recorded during exploration in an open-field containing objects which underwent a series of spatial manipulations, including object dislocation and novel object-place recognition. Recordings from place cells receiving LEC input resulted in greater influence of objects on place cell firing, although object-modulated and non-object-modulated place cells were recorded in both regions. Object-modulation of place cells in CA1 conformed to patterns which have been described previously. These findings influence our understanding of how entorhinal-hippocampal communication supports the integration of item and location information in episodic memory.

Disclosures: J.A. Ainge: None. **B.M. Vandrey:** None.

Poster

084. Learning and Memory: Parahippocampal and Related Cortical Circuits

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Program#/Poster#: 84.12/GGG9

Topic: H.01. Animal Cognition and Behavior

Support: Henry Dryerre PhD Scholarship, Carnegie Trust

Carnegie Grant 50243

Title: Investigating the molecular organisation of superficial layers of the lateral entorhinal cortex

Authors: *B. M. VANDREY[1], D. L. F. GARDEN[2], J. A. AINGE[1], M. F. NOLAN[2];
[1]Sch. of Psychology & Neurosci., Univ. of St Andrews, St Andrews, United Kingdom; [2]Ctr. for Integrative Physiol., Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: Episodic memory relies on the hippocampus and its surrounding cortical network. The entorhinal cortex makes substantial contributions to information processing within this network.

Recent work suggests that the lateral entorhinal cortex (LEC) is critical for binding together features of an episode and processing spatial information about objects. However, the relationship between LEC circuit components and cognitive function is unclear. Further, an obstacle to progress is the lack of tools for manipulation of independent populations of neurons in the LEC. Recent work in the medial entorhinal cortex (MEC) found that MEC layer 2 (L2) contains two populations of cells that project to discrete regions of the hippocampus and are labelled by the protein markers reelin and calbindin. Cre-expressing mouse lines were identified which give genetic access to these sub-populations. Based on this work, we aimed to investigate whether L2 of the LEC is similar in organisation and molecular identity to MEC, and whether Cre driver lines used for investigation of MEC can be repurposed to give genetic access to specific cell populations within LEC L2. We find that L2 of the LEC bifurcates into two molecularly distinct sub-layers based on the distribution of reelin and calbindin. In the more superficial layer (L2a) the majority of neurons are positive for reelin, whereas in the deeper layer (L2b) the majority of neurons are positive for calbindin. To develop a strategy for targeting cells in LEC, we injected adeno-associated virus encoding a fluorescent reporter into the LEC of Sim1:Cre mice, which we found previously to label stellate cells in L2 of the MEC (Sürmeli et al. (2015), Neuron 88:1040-1053). In LEC, Cre was expressed specifically in cells in L2a that express reelin and project to the dentate gyrus (DG). We used patch-clamp recordings to further examine the properties of the labelled cells. Cre positive neurons were morphologically and electrophysiologically similar to 'fan-cells' (cf. Tahvildari and Alonso (2005), J. Comp. Neurol. 491: 123-140). In summary, we provide a novel comparison of the organisation and molecular characteristics of L2 of LEC and MEC. Further, we report that the Sim1:Cre line provides genetic access to the sub-population of LEC L2 cells which project to the DG. Our results establish a framework for investigating circuit mechanisms by which L2 of the LEC contributes to episodic-like memories.

Disclosures: **B.M. Vandrey:** None. **D.L.F. Garden:** None. **J.A. Ainge:** None. **M.F. Nolan:** None.

Poster

084. Learning and Memory: Parahippocampal and Related Cortical Circuits

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Program#/Poster#: 84.13/GGG10

Topic: H.01. Animal Cognition and Behavior

Support: MEXT/JSPS KAKENHI #15K18358

Grants-in-Aid for Scientific Research on Innovative Areas #26119502

Title: Efferent projections of the calbindin-positive entorhinal neurons in the rat: Connectional differences between the medial and lateral entorhinal cortex

Authors: *S. OHARA[1], K. ITOU[1], M. SHIRAISHI[1], M. GIANATTI[2], Y. SOTA[1], S. KABASHIMA[1], M. ONODERA[1], K.-I. TSUTSUI[1], M. WITTER[2], T. IIJIMA[1];
[1]Tohoku Univ. Grad Sch. Life Sci., Sendai, Japan; [2]NTNU, Trondheim, Norway

Abstract: The entorhinal cortex, which constitutes the major gateway between the hippocampus and the neocortex, can be subdivided into two domains: the medial and lateral entorhinal cortices (MEC and LEC). MEC is known to be involved in spatial navigation, while LEC is thought to concern with processing information of various types of sensory modalities. These functional differences are mainly attributed to the differences in the input-output connectivity of these two cortices. Although previous studies have examined the connectivity of entorhinal cortex in detail, the difference in the connectivity of specific neuron types in these two cortices has not been addressed in detail. Here we focused on the calbindin-positive neuron (CB+ neuron) located in layer II of the entorhinal cortex.
Calbindin (CB) is a calcium binding protein, which is localized in various forebrain area implicated in learning and memory. CB may play a role in synaptic plasticity, as reduced amount of CB caused impairment in spatial memory and deficit in maintaining long-term potentiation in the hippocampus. Recent studies have shown that the CB+ neurons in MEC show grid firing patterns, and are related to temporal-association memory. Interestingly, it also has been reported that there are differences in the distribution patterns of CB+ neurons between MEC and LEC (Gianatti et al., 2015). In rats, the MEC CB+ neurons intermingle with the dentate gyrus-projecting neurons (reelin-positive neurons) in layer II, while the LEC CB+ neurons localize exclusively deep to the Reelin-positive neurons in layer IIb (also referred to as layer IIIa in our previous study). In this study, we examined the projection of the CB+ neurons, and compared the connectivity of CB+ neurons in MEC and LEC. A retrograde tracer (fluorogold) was injected into either the hippocampus (dentate gyrus, CA3, CA1, subiculum), extrahippocampal regions (medial prefrontal cortex, insular cortex, piriform cortex, nucleus accumbens, amygdala), or the entorhinal cortex itself, and the distribution of the retrogradely labeled CB+ neurons were examined in the entorhinal cortex. We found that there are connectional differences between the MEC and LEC CB+ neurons. A substantial percentage of MEC CB+ neurons projected to the hippocampus and a comparable proportion projected to the LEC. In contrast, LEC CB+ neurons preferentially targeted the extrahippocampal infralimbic cortex, rather than the hippocampus. LEC CB+ neurons also showed strong intrinsic connections targeting the MEC. These anatomical differences of the MEC and LEC CB+ neurons might underlie the functional difference between the MEC and LEC.

Disclosures: **S. Ohara:** None. **K. Itou:** None. **M. Shiraishi:** None. **M. Gianatti:** None. **Y. Sota:** None. **S. Kabashima:** None. **M. Onodera:** None. **K. Tsutsui:** None. **M. Witter:** None. **T. Iijima:** None.

Poster

084. Learning and Memory: Parahippocampal and Related Cortical Circuits

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Topic: H.01. Animal Cognition and Behavior

Support: German Research Foundation (DFG-SFB1158)

German Research Foundation (DFG-SFB1134)

Helmholtz Foundation (HIGS fellowship)

Title: Cholinergic modulation of neuronal network activity in the entorhinal cortex

Authors: S. DESIKAN[1], D. E. KOSER[1,2], A. NEITZ[1], *H. MONYER[3,1];
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Abstract: For decades the medial septum and the diagonal band of broca (MS/ DBB) have been considered the pacemaker of hippocampal and entorhinal cortical theta activity. The septo-entorhinal pathway mainly comprise glutamatergic, cholinergic and GABAergic long-range projections. The functional aspects of glutamatergic and GABAergic long-range projections have been studied, while the contirbution of septal cholinergic projections to entorhinal cortex (EC) remains elusive. *In vitro* investigations using bath-application of cholinergic receptor agonists, or electrical stimulation to release endogenous acetylcholine (ACh), have shed light upon cholinergic function within EC, but questions remain because of the lack of selectivity and specificity of these techniques. On the basis of these premises, the following pressing questions arise: How do cholinergic projections from MS/ DBB contribute to the recruitment of neurons in EC? What is the mechanism by which they do so?
To investigate whether MS/ DBB cholinergic neurons innervate specific layers and cell types in the EC, we trangenically expressed mCherry and channelrhodopsin-2 in septal cholinergic neurons and utilized *in vitro* patch-clamp recordings in acute brain slices to determine postsynaptic target cells in EC. Anterograde axonal tracing from MS/ DBB revealed extensive cholinergic innervations in the superficial layers (LI-III) of medial and lateral EC. Following optogenetic stimulation of MS/ DBB axons in LI-III of EC, we observed fast monosynaptic nicotinic receptor mediated excitatory post synaptic currents and slow membrane depolarizations mediated by muscarinic receptor activation. By demonstrating functional information regarding cholinergic projections from MS/ DBB, this study will contribute to a better understanding of temporally coordinated neuronal activity within the EC.

Disclosures: **S. Desikan:** None. **D.E. Koser:** None. **A. Neitz:** None. **H. Monyer:** None.

Poster

084. Learning and Memory: Parahippocampal and Related Cortical Circuits

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 84.15/GGG12

Topic: H.01. Animal Cognition and Behavior

Support: SonderForschungBereich874

Title: Cellular evidence for the lack of contribution of CA1 and CA3 to familiarity and the selective involvement of the deep layers of the perirhinal and lateral entorhinal cortices

Authors: *E. ATUCHA TREVINO[1], A. KAREW[2], T. KITSUKAWA[3], M. SAUVAGE[1]; [1]Functional Architecture of Memory, Leibniz Inst. For Neurobio., Magdeburg, Germany; [2]Ruhr Univ., Bochum, Germany; [3]Osaka Univ., Osaka, Japan

Abstract: Recognition memory relies on two memory retrieval processes, recollection and familiarity. Recollection reflects the retrieval of specific information about events whereas familiarity is described as a vague feeling of déjà vu. While it is well-accepted that the hippocampus supports recollection, it is still unclear whether it also supports familiarity or whether the parahippocampal region, especially the perirhinal (PER) and lateral entorhinal cortices (LEC), does. One of the reason why this remains elusive is the low accessibility, in humans, to imaging techniques with spatial resolution high enough to dissociate the source of activity among adjacent brain regions. Importantly, studies in rodents have brought evidence for a functional segregation of the hippocampal subfields CA1 and CA3 along their proximodistal and longitudinal axes as well as between the PER and the LEC and their deep and superficial cell layers, but their contribution to familiarity has not been studied yet. Here, we combined an odor memory task yielding judgements based on familiarity with high resolution molecular imaging involving the detection of the immediate early gene Arc and mapped cellular activity in these areas. We report that even the ventral part of CA1 and CA3, thought to process the most odor stimuli, failed to be recruited while the deep layers of the PER and the LEC, believed to contribute to more cognitive processes than their superficial layers, were. These data provide robust cellular evidence for the lack of contribution of the hippocampus to familiarity and for the engagement of the parahippocampal region during this process.

Disclosures: **E. Atucha Trevino:** None. **A. Karew:** None. **T. Kitsukawa:** None. **M. Sauvage:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.01/GGG13

Topic: H.02. Human Cognition and Behavior

Support: NIH R01DC012947

Title: Entrainment without boundaries: thalamocortical mechanisms of parsing and grouping complex auditory patterns

Authors: *A. BARCZAK[1], M. N. O'CONNELL[1], T. MCGINNIS[1], D. ROSS[1], P. LAKATOS[1,2]; [1]Nathan S. Kline Inst. For Psychiatric Res., Orangeburg, NY; [2]Psychiatry, New York Univ. Sch. of Med., New York, NY

Abstract: Previous studies have shown that ongoing neuronal oscillations across multiple levels of the auditory pathway can entrain to rhythmically structured auditory stimuli. Since our auditory environment is composed of a continuous flow of complex, multi-timescale information (e.g. speech/species specific communication), inputs must be parsed and grouped into blocks so that they can be further processed and interpreted by lower- and higher-order regions, respectively. Our study aims to explore the neural mechanism for the perceptual parsing and grouping of repeating complex random tone sequences amid a continuous flow of auditory stimuli. Human psychophysical studies suggest that the recognition of novel auditory patterns occurs automatically about halfway through the first repetition. We hypothesized that if low frequency neuronal activity plays a role in parsing, this activity would be entrained or synchronized in frequency and phase to the repeating patterns, which would result in an increase in delta phase concentration during the first repetition. To examine pattern-related thalamocortical oscillatory effects we used linear-array multielectrodes to record cortical laminar and thalamic neuroelectric activity while subjects passively listened to a stream of rapidly presented (20 Hz) pure tones and bandpass noise bursts. At random intervals, and without any physical boundary or pause in the stimulus stream, complex auditory patterns were created by repeating 11 of the randomly presented sounds 5 times. The resulting pattern repetition rate was 1.7 Hz. We found significant delta entrainment to the patterns in most primary auditory cortical areas and some thalamic regions like the medial geniculate nucleus and the pulvinar. Interestingly, entrainment was earlier and stronger in the pulvinar and in the supragranular (rather than infragranular) layers of primary auditory cortex. Based on these observations we hypothesize that a “top-down,” pulvinar to supragranular layer projection might be responsible for the parsing and grouping of complex auditory patterns.

Disclosures: **A. Barczak:** None. **M.N. O'Connell:** None. **T. McGinnis:** None. **D. Ross:** None. **P. Lakatos:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.02/GGG14

Topic: H.02. Human Cognition and Behavior

Support: NSERC

McDonnel Foundation

Title: Beat perception induces fluctuations in motor system excitability

Authors: *D. CAMERON[1], J. EVERLING[1], T.-C. CHIANG[1], J. A. GRAHN[2,1];
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Abstract: Humans synchronize movements with the perceived, regular emphasis (the beat) in musical rhythms. Neural activity during beat perception is dynamic, time-locked, and heavily based in the motor system. Excitability in the motor system may fluctuate during beat perception, as indexed by the amplitude of motor-evoked potentials (MEPs) evoked by transcranial magnetic stimulation (TMS). In a previous study, MEP amplitude was greater when listeners heard rhythms with a strong beat vs. a weak beat, but only for MEPs elicited 100 ms before the beat, *not* for MEPs elicited at random time points relative to the beat (Cameron, et al., 2012). Thus, motor system excitability may not be uniform across the entire rhythm, but instead may fluctuate, rising at particular times on or before the beat. Here, we sought to characterize the dynamics of motor system excitability during beat perception, using MEP amplitude as our index of excitability. Participants (n=23) listened to strong beat, weak beat, and nonbeat auditory rhythms (35s each). During each rhythm, TMS was applied to left primary motor cortex at 100 time points spread across the entire the beat interval. MEP amplitudes were recorded with electromyography from the right hand (first dorsal interosseous muscle). To measure fluctuations in excitability, linear and cosine functions (the latter for frequencies corresponding to 1, 2, and, 4 times the beat rate) were fit to MEP amplitudes and compared across rhythm types. Motor system excitability dynamics differed for strong beat vs. weak beat and nonbeat rhythms: Excitability increased linearly over the beat interval (in anticipation of upcoming beat positions) in strong beat rhythms, but not in weak or nonbeat rhythms. Additionally, excitability fluctuated at the rate of individual events (the beat rate subdivided by four) to a greater extent in strong beat rhythms than in weak and nonbeat rhythms. These results suggest that during beat perception, motor system excitability 1) anticipates upcoming beats, and 2) tracks subdivisions of the beat.

Disclosures: **D. Cameron:** None. **J. Everling:** None. **T. Chiang:** None. **J.A. Grahn:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.03/GGG15

Topic: H.02. Human Cognition and Behavior

Support: Wellcome Trust Grant CQR 00430 CQ 00.1

Title: Sensory expectation of time and space is modulated by task relevance: evidence of neural and behavioral effects from model-based MEG

Authors: *R. AUKSZTULEWICZ[1,2,3], K. FRISTON[3], A. NOBRE[2];
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Abstract: Although the notion that the brain generates internal predictions to optimize behavior is now well established, it is unclear to what extent these predictions are modulated by current task demands. Here we tested whether expectations of different stimulus features (location and latency) can be independently learned in a dynamically changing environment, and whether task relevance of specific predictions modulates their encoding and updating. In the experimental paradigm, healthy participants (N=20) were asked to discriminate location or latency of visual targets which could appear in either hemifield and either early (~0.8s) or late (~1.2s) following an auditory cue. Unknown to the participants, cue features (pitch and composition) could independently predict the location and latency of the target with varying validity (90%, 70%, or 50%). Alternating blocks of task relevance (location or latency discrimination) were administered during MEG data acquisition. To analyze the effects of expectation and relevance on oscillatory neural activity, we used convolution modeling for induced responses, with regressors coding for cue, target, and response onsets as well as nuissance variables (e.g. eye movements and pupil size). Further, we modelled reaction time data using a Hierarchical Gaussian Filter (HGF) model to infer single-trial estimates of predictions and prediction errors at various levels of stimulus processing. Using these estimates of individual participants' behavior as additional regressors in the MEG analysis, we inferred the effects of predictions and prediction errors on induced activity. Sensor-level MEG analysis revealed that in both temporal and spatial tasks, gamma (30-48Hz) power induced over temporal sensors by auditory cues increased with predictability strength, independent of its relevance. Crucially, frontal alpha (8-13Hz) desynchronisation induced by visual targets increased with the strength of both spatial and temporal expectations only when they were relevant for the task, mimicking behavioral effects on discrimination accuracy. A model-based source-space analysis, using the trial-by-trial HGF regressors, indicated that target-induced gamma activity in the visual cortex reflected precision-

weighted prediction errors rather than predictions about the upcoming stimulus. These results provide novel evidence for modulatory effects of task relevance on learning and utilizing internal predictions in a dynamic context.

Disclosures: R. Auksztulewicz: None. **K. Friston:** None. **A. Nobre:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.04/GGG16

Topic: H.02. Human Cognition and Behavior

Support: BAGEP Grant, The Science Academy, Turkey

2013 Interdisciplinary Perspectives on Time Grant, University of Sydney

Title: Are humans aware of their timing errors: Temporal error monitoring

Authors: *B. AKDOGAN, F. BALCI;
Psychology, Koc Univ., Istanbul, Turkey

Abstract: One of the core features of human and animal timing ability is that time intervals can be judged with high average accuracy but with limited precision. For instance, when participants are asked to repeatedly reproduce a time interval based on their subjective experience, their reproduction times will form a nearly Gaussian distribution with a mean equivalent to the target interval and manifest substantial variance around the mean. This phenomenon indicates that some of our temporal judgments are deviations from the target times, which can be referred to as timing uncertainty. Although recent work suggests that the degree of timing uncertainty can be accessible to the decision-maker, whether and how humans can monitor trial-based errors in their timing behavior constitutes an understudied topic. Therefore, in four complementary experiments, we investigated whether individuals know if and to what extent they are early or late with respect to a target duration in an individual trial. Specifically, participants were tested in the temporal reproduction task and were then asked to judge the accuracy of their temporal estimates in the absence of explicit feedback. After each reproduction, they were prompted to rate their confidence in their temporal estimates and to indicate whether they under- or over-reproduced the target interval. The results revealed an inverse relationship between confidence ratings and the degree of temporal deviations, such that the participants reported lower levels of confidence with the increase in the divergence in their temporal estimates from target intervals. Furthermore, it was shown that participants successfully distinguished whether their

reproduction times were shorter or longer than the target intervals. These findings suggest that humans are able to monitor not only the magnitude but also the direction of the errors in their temporal judgments at a level indicating a clear *temporal error monitoring* ability.

Disclosures: **B. Akdogan:** None. **F. Balci:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.05/GGG17

Topic: H.02. Human Cognition and Behavior

Title: Hemispheric asymmetries in the temporal processing of spatial targets

Authors: *R. XU, L. WELCH;
Cognitive, Linguistic and Psychological Sci., Brown Univ., Providence, RI

Abstract: Multiple lines of evidence suggest an asymmetry in the time-course of perceptual processing between the two visual fields. For example, recent behavioral (Matthews et al, 2013), electroencephalography (Verleger et al, 2011) and rTMS (Verleger et al, 2010) research describe a processing advantage for targets in the left visual field (LVF). This advantage is thought to reflect differences in the initial allocation of attention, with compensation that occurs in later stages of processing to prevent the false perception that with simultaneous events, the right event lags the left. However, it is unclear whether observation of an LVF advantage can be partially attributed to early sensory features of an object that captures attention in an involuntary manner because previous studies employed target pairs that differed in color. To test whether there are inherent functional differences in visual processing between the two hemispheres, we removed the color difference between target pairs while preserving other experimental features. Eighteen young adults were asked to judge the simultaneity (SJ; did the two targets appear at the same time?) and temporal order (TOJ; was the first target an odd number?) of two targets in two rapid serial visual presentation (RSVP) displays. The two RSVPs were presented simultaneously, one in LVF and the other in RVF, and each contained one black numeric target embedded in a stream of black letter distractors. Consistent with previous findings (Matthews et al., 2013), our results demonstrate a clear temporal advantage for left targets in the TOJ, but not SJ, task. These results show that the LVF advantage found previously does not depend on target pairs differing in low-level saliency (color). A comparison of the perceived subjective equality (PSE) for the odd-numbered target presented to LVF and to RVF revealed the magnitude of the perceived-timing difference to be ~190ms, comparable to two cycles of attention's reported temporal resolution (Rogers-Ramachandran & Ramachandran, 1998). Since retinal stimulation during the two tasks

was identical, we cannot make any stimulus-driven interpretations for the TOJ v SJ task differences. This suggests that subjects perceived left targets significantly sooner than right targets during the temporal order task, but compensated for this difference in their simultaneity judgments. We argue that judgments of simultaneity and temporal order may rely on different neural mechanisms, and the visual field asymmetry observed for the TOJ cannot be attributed to having targets with different perceptual saliencies.

Disclosures: **R. Xu:** None. **L. Welch:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.06/GGG18

Topic: H.02. Human Cognition and Behavior

Support: JSPS 15H01690

Title: Hyperscanning study on synchronized singing between two people using fNIRS

Authors: ***N. OSAKA**[1], T. MINAMOTO[2], K. YAOI[1], M. AZUMA[3], M. OSAKA[3];
[1]Kyoto Univ., Kyoto, Japan; [2]NICT Cinet Osaka Univ., Suita, Japan; [3]Osaka Univ., Suita, Japan

Abstract: Our brain have been developed to communicate with others, however it seems unclear how our brain achieves interactive communication. Here, we report the neural synchronization for singing and humming between two people, simultaneously measuring two brain activities using an fNIRS-based hyperscanning . Using a functional near-infrared spectroscopy (fNIRS), brain activity of two peoples was measured while they performed a cooperated humming or singing with face-to-face and face-to-wall (preventing them from observing other's face by a wall). The results showed a significant increase in the neural synchronization in the left inferior frontal cortex (IFC) in both the singing and humming regardless of existence of the wall, in comparison to the single singing/humming. On the other hand, the right IFC showed an increase in the neural synchronization during humming but not singing, possibly due to higher dependence on musical processing. Those results suggest a usefulness of the fNIRS-based hyperscanning in natural social interaction.

Disclosures: **N. Osaka:** None. **T. Minamoto:** None. **K. Yaoi:** None. **M. Azuma:** None. **M. Osaka:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

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Topic: H.02. Human Cognition and Behavior

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People Programme (Marie Curie Actions) of EU FP7 2007-2013 under REA grant agreement no. 600209

Title: Rhythm processing deficits in nonfluent variant primary progressive aphasia relate to atrophy of the supplementary motor area

Authors: *J. SCHAEVERBEKE[1], M. GRUBE[2,3], R. BRUFFAERTS[1,4], V. NEYENS[1], E. DRIES[4], T. GRIFFITHS[2], R. VANDENBERGHE[4,1];
[1]KU Leuven, Leuven, Belgium; [2]Newcastle Univ., Newcastle, United Kingdom; [3]Tech. Univ. of Berlin, Berlin, Germany; [4]UZ Leuven, Leuven, Belgium

Abstract: Primary progressive aphasia (PPA) patients exhibit deficits in processing of rhythm and timing of sequences of non-linguistic acoustic stimuli, in particular the non-fluent variant (NFV) of PPA (Grube et al, 2016). The current study aimed to determine the neuroanatomical basis for these deficits. A consecutive case series of 17 PPA patients (65.7 ± 8.8 years) (four logopenic variant (LV), six NFV, seven semantic variant (SV)) and 22 cognitively intact controls (63.8 ± 6.5 years) participated. All participants underwent a T_1-weighted MRI, which was analyzed using voxel based morphometry (VBM, SPM8), and completed a psychoacoustic test battery including four rhythm tasks (single time-interval duration discrimination; isochrony deviation detection; strongly and weakly metrical pattern discrimination). The outcome measure for each of the tasks was the threshold obtained by adaptively adjusting the difference between reference and target stimuli. For each task, a voxel-wise linear regression was conducted between modulated cortical gray matter (GM) and task performance, with age and gender as nuisance variables. The significance threshold was set at voxel-level uncorrected $P<0.001$ and cluster-level family wise error (FWE)-corrected $P<0.05$, and Bonferroni-corrected for the number of regressions (n=4). In PPA patients, performance on strongly metrical pattern discrimination correlated with GM loss in the left supplementary motor area (SMA) and the medial wall of the superior frontal gyrus ($P_{FWE\text{-}corrected}= 0.04$, 829 voxels, $Z=4.73$; -18, 5, 66;

Z=4.28; -6, 20, 60). Patients who were more impaired on discrimination of strongly metrical patterns had less GM in this cluster (rho=-0.94). No other significant correlation was found in the PPA group, and no significant correlation was found in the controls. Atrophy of SMA may be causally involved in both the rhythm processing deficits and in the characteristic non-fluent speech apraxia, which is a core criterion of NFV PPA. This finding is consistent with the SMA playing a key role in one shared sensory-motor timing system that is relevant for speech production and rhythm perception.

Disclosures: J. Schaeverbeke: None. **M. Grube:** None. **R. Bruffaerts:** None. **V. Neyens:** None. **E. Dries:** None. **T. Griffiths:** None. **R. Vandenberghe:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01 NS089470

NIH Grant K08 NS078100

NARSAD Young Investigator Grant

Title: Optogenetic stimulation of frontal D1 neurons compensates for impaired temporal control of action in dopamine-depleted mice

Authors: *Y.-C. KIM[1], S. HAN[2], R. RUGGIERO[2], S. ALBERICO[2], K.-H. CHEN[2], N. S. NARAYANAN[1];
[1]Neurol., [2]Univ. of Iowa, Iowa City, IA

Abstract: Disrupted midbrain dopamine contributes to cognitive symptoms of PD. Past work has implicated frontal neurons expressing D1 dopamine receptors (D1DRs) in temporal processing; here we investigate if these neurons are sufficient to compensate for midbrain dopamine dysfunction. We study elementary cognitive processing using an interval timing task. This task requires subjects to estimate a brief interval of time over several seconds. Interval timing is ideal to study cognitive function depends on dopamine, involves executive functions such as working memory and attention to time, involves similar neuronal processing in humans and rodents, and is consistently impaired in human diseases involving dopamine. Animals with depleted dopamine in mesocortical circuits showed impaired interval timing. We report three main results. First, both PD patients and mice with disrupted midbrain dopamine have attenuated

delta activity (1-4 Hz) in the medial frontal cortex (MFC) during interval timing. Secondly, MFC neurons expressing D1DRs had distinct patterns of neuronal activity and optogenetically stimulating these neurons facilitated temporal processing by MFC neurons. Finally, stimulating MFC D1DR neurons specifically at delta frequencies (2 Hz) rescued deficits in temporal control of action caused by disrupting midbrain dopamine. Our results suggest that cortical networks can be targeted to improve dopamine-dependent cognitive processing.

Disclosures: **Y. Kim:** None. **S. Han:** None. **R. Ruggiero:** None. **S. Alberico:** None. **K. Chen:** None. **N.S. Narayanan:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.09/GGG21

Topic: H.02. Human Cognition and Behavior

Support: JSPS KAKENHI Grant Number 26119511

AIST AIRC YA27038

Title: A Synergistic model of the cerebellum and the basal ganglia for temporal information processing

Authors: ***O. KATAKURA**[1], T. YAMAZAKI[1,2];
[1]Grad. Sch. of Communication Engin. and Informatics, The Univ. of Electro-Communications, Tokyo, Japan; [2]Artificial Intelligence Res. Center, AIST, Tokyo, Japan

Abstract: Timing perception is an essential sense for our living. Despite of its importance, the neural mechanisms remain unclear. Many lines of studies have suggested that at least, the cerebellum and the basal ganglia play critical roles in temporal information processing. It is thought that the cerebellum processes less than 1 second for precise motor control, and the basal ganglia processes more than 1 second for more cognitive tasks. In particular, electrophysiological studies have shown that in time reproduction tasks, the cerebellum and the basal ganglia exhibit different temporal profiles of their neuronal activity patterns, suggesting that these two areas work together synergistically to represent the passage of time, which is proposed as a relay hypothesis (Tanaka et. al. 2013). In this study, we built a simple model of the cerebellum and the basal ganglia by extending internal clock models (Yamazaki and Tanaka 2005). We modeled the granular layer (GL, the network between the granule cells and the Golgi cells in the cerebellum) and the network between the external globus pallidus (GPe) and the sub-

thalamic nuclei (STN) as internal clocks. These neurons became active and inactive in turn with time, and the populations of active neurons represent time. On the other hand, the other components including the cortex (Ctx), the striatum (Str), the internal globus pallidus (GPi), the thalamus (Tha), the pontine nuclei (PN), the Purkinje cells (Pkj), and the cerebellar nuclei (CN), were simplified as single neurons. We modeled the direct, indirect, and hyper-direct pathways of the cortico-thalamo-cortical system and the cortico-cerebellar system. Each internal clock had plasticity on the synapses from GL to Pkj and those from STN to GPi, respectively. After repetitive training to learn the passage of time, activities of the CN and Tha showed peaks at the learned timing. The activity of CN showed narrow peak, that is, the activity increased just before the learned timing. In contrast, that of Tha shown wide peak, that is, the activity increased constantly from the onset to the learned timing. These results imply that the cerebellum could encode short and precise timing and the basal ganglia could encode long and broad timing, supporting a relay hypothesis on the representation of time by the cerebellum and the basal ganglia. The cortico-thalamo-cortical recurrent system and cortico-cerebellar system could cause interplay between the cerebellum and the basal ganglia.

Disclosures: O. Katakura: None. **T. Yamazaki:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.10/GGG22

Topic: H.02. Human Cognition and Behavior

Title: Interval timing in adult stutters - Role of beta connectivity in default mode-related functional segregation.

Authors: A. GHADERI[1], M. NAZARI[1], *W. H. MECK[2];
[1]Psychology, Univ. of Tabriz, Tabriz, Iran, Islamic Republic of; [2]Duke Univ. Dept. of Psychology and Neurosci., Durham, NC

Abstract: Stuttering is a speech disorder related to deficits in motor control across time and abnormal movement planning or preparation. It is associated with frequent interruptions and pauses in speech, particularly at the beginning of words and sentences, leading to unplanned sound prolongations. Evidence suggesting timing impairments in stuttering includes anomalies within the basal ganglia dopaminergic system and abnormal beta oscillations in the putamen, both of which share a close association with interval timing. Moreover, altered topological properties of brain connectivity networks have emerged as an important feature of neurological dysfunction and altered time perception. Consequently, the aims of this study were to establish

the nature of the interval timing deficit in adult stutters using an auditory, duration-discrimination task reliant on a maximum likelihood procedure. Sub-second (250-ms) and supra-second (2.5-s), 1kHz pure tones were used as baselines in a three-alternate forced choice task. Independent thresholds were generated three times using 30 trial blocks for each base duration. Following this, we recorded EEG activity in participants during a resting state or default mode. A graph theoretical analysis was applied to the EEG data in order to investigate the brain's topological properties in stutterers compared to controls. EEG coherence in different frequency bands was used as edges and various thresholds were applied and binary adjacent matrices were created. Finally, clustering coefficients and global efficiency scores were used to report functional brain segregation and integration in the stutterers compared with normal participants. These results show that individuals who stutter (n=18) exhibit impairments in their temporal discrimination thresholds for both sub-second and supra-second auditory durations compared to controls (n=18). Furthermore, stutterers exhibit an abnormal functional brain connectivity network in both the standard beta band (12.5-25 Hz) and the high beta band (25-30 Hz). Consistent with previous findings, this beta band abnormality occurred during resting state brain activity indicating a deficit in the default-mode network. Both clustering coefficient and global efficiency measures were impaired in stutterers. Overall, our results suggest that, in the beta band (12.5-30 Hz), individuals who stutter exhibit a decreased level of functional integration and increased modularity that directly contributes to the observed deficits in timing and time perception. These findings are discussed within the context of the striatal-beat frequency model of interval timing.

Disclosures: **A. Ghaderi:** None. **M. Nazari:** None. **W.H. Meck:** None.

Poster

085. Human Cognition: Temporal Processing I

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Program#/Poster#: 85.11/GGG23

Topic: H.02. Human Cognition and Behavior

Support: R01 NS089470

K08 NS078100

5 T32 GM007337

Title: Prefrontal-subthalamic delta/theta coherence shapes temporal processing during interval timing

Authors: *R. KELLEY, J. GREENLEE, N. NARAYANAN;
Univ. of Iowa, Iowa City, IA

Abstract: Prefrontal networks may control subthalamic neurons via the hyperdirect pathway-- a monosynaptic projection from frontal cortex to the subthalamic nucleus. This pathway has the potential to powerfully influence cognitive processing. We used an interval-timing task to study PD-related cognitive dysfunction in human STN-DBS patients and in mice. During human STN-DBS implantation, we simultaneously recorded from STN (LFP) and PFC (EEG) while subjects performed an interval-timing task. Our data show low-frequency power bursts in both STN and PFC that align to the onset of interval-timing. Granger connectivity analysis of neural recordings demonstrates sustained top-down signaling between PFC to STN consistent with hyperdirect pathway-mediated temporal processing. We also found that single STN neurons could be modulated during timing tasks. Finally, we tested the hypothesis that low-frequency STN-DBS could rescue impaired interval timing performance. Our preliminary data indicate that 4 Hz STN DBS can improve time estimation. Our results suggest that the STN is a key integration site for temporal processing. This may point to a rapidly-translatable treatment strategy for cognitive symptoms of PD that extends the clinical applications of STN-DBS.

Disclosures: R. Kelley: None. **J. Greenlee:** None. **N. Narayanan:** None.

Poster

085. Human Cognition: Temporal Processing I

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Program#/Poster#: 85.12/GGG24

Topic: H.02. Human Cognition and Behavior

Support: CIHR Grant MOP133450

Title: The influence of frequency on perceived temporal rate is larger in demanding listening situations

Authors: *B. HERRMANN, I. S. JOHNSRUDE;
Dept. of Psychology, The Univ. of Western Ontario, London, ON, Canada

Abstract: Acoustic features co-vary in natural environments (e.g., a sound's pitch changes with changes in the sound's temporal rate). Such structural covariations provide redundancy in fast-changing, acoustically complex soundscapes and might support hearing in difficult listening situations. The current study investigated how changes in frequency induce (illusory) temporal rate change percepts. The illusion is thought to arise from expectations matching the learned

structural covariations in natural acoustic environments. However, it is unknown whether reliance on learned structural covariations depends on situational task demands or whether structural covariations are stably utilized. Here we ask whether the magnitude of the perceptual illusion increases in unpredictable listening situations and when perception occurs under distraction.
Participants listened to amplitude-modulated sounds with modulation rates (~5 Hz) either decreasing or increasing over time, and identified the direction of the rate change. Sounds also either decreased or increased in carrier frequency (~1300 Hz) over time, but participants were instructed to ignore such changes. Experiment 1 confirmed that perception of the sound's temporal rate was biased by frequency such that sounds were perceived as speeding up when frequency increased and as slowing down when frequency decreased (temporal rate-change illusion). In experiments 2 & 3, frequency and temporal rate changes were small or large, and participants were cued either to expect an easy or difficult rate change (Exp2) or frequency change (Exp3), or were given an uninformative neutral cue. The magnitude of the illusion increased when participants were uninformed (neutral) about the difficulty of the upcoming sound. In experiments 4-6, participants listened to the amplitude-modulated sounds and performed a congruent distractor task at different difficulty levels. Memory load in the distractor task (Exp4 and Exp5) only weakly affected the illusion magnitude, whereas the illusion magnitude increased with increasing number of objects to be tracked in a multiple object tracking task (Exp6).
In sum, when the difficulty of a sound's temporal rate change is unpredictable or sound perception is demanding because of a congruent distractor task, the influences of frequency changes on a sound's perceived temporal rate change are increased, leading to a magnified illusion. The results suggest that in difficult listening situations, listeners might rely more strongly on learned structural covariations for sound perception than in less demanding situations.

Disclosures: B. Herrmann: None. **I.S. Johnsrude:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.13/GGG25

Topic: H.02. Human Cognition and Behavior

Title: Regulation of electrophysiological surprise responses by temporal ordering

Authors: *M. SCHWARTZE, S. A. KOTZ;
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Abstract: Adaptation to a dynamic environment requires adequate reactive and predictive adjustments of behavior, potentially reflecting a neurocognitive bias to minimize surprise by maximizing expectations about changes in the environment. The efficiency of predictive adjustments of behaviour depends in part on the ability to exploit temporal information in order to tune into the course of events in the environment. In the current study we used three fundamental temporal ordering principles (isochrony, proximity, periodicity) to investigate their impact on the time-course, size, and topographical distribution of two electrophysiological markers of neurocognitive behaviour. The P50 and P3b components of the event-related potential of the electroencephalogram (EEG) were obtained from 24 participants who listened to sequences of 320 pure tone pairs consisting of equidurational (300 ms) 512 standards (600 Hz) and 128 pseudorandomly interspersed deviants (660 Hz) while they counted the deviants embedded in each sequence. Whereas the P50 has been linked to sensory gating (i.e., the gating out of old relative to new information), the P3b has been directly associated with an element of surprise and the updating of a mental model of the environment in response to a change. Results indicate that the temporal ordering principles tested can be used separately and in combination to step-wise down-regulate the P50 component (amplitude suppression) and to up-regulate the P3b component (amplitude enhancement). This pattern suggests that temporal ordering principles offer a means to systematically modulate, and thereby potentially also to optimize, the quality of basic sensory and more complex forms of perceptual neurocognitive behavoir.

Disclosures: M. Schwartze: None. **S.A. Kotz:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.14/GGG26

Topic: H.02. Human Cognition and Behavior

Support: German Research Foundation SH 166/3-1 to ZS

Title: Temporal averaging: geometric or arithmetic mean.

Authors: *Y. REN, Z. SHI;
Ludwig-maximilians-Universität, Muenchen, Germany

Abstract: Efficient encoding of information is essential to sensory system, especially for processing dynamic and complex sensory inputs. Over the past decades, extensive work has shown that we are able to summarize statistical properties, such as averaging, of various features (e.g., positions, orientations, speeds and frequencies) for a given group of objects both in visual and auditory modalities. However, to date little is known what type of averaging statistics has been used in temporal perceptual coding. To be more specific, we aim to identify whether a linear or logarithmic encoding is used for representing the mean of temporal intervals. To investigate this issue, here direct estimations of temporal intervals from both auditory and visual modalities were measured by asking participants to compare the average duration of a sequence of intervals to a single probe interval. Three sets of intervals (A, B, and C) were used for testing, where the arithmetic and geometric mean of the sequences were as follows: the arithmetic mean of sequence A was equal to that of sequence B (0.8s), but greater than the value of sequence C(0.725s), while the geometric mean of sequence A was equal to that of sequence B (0.710s) but smaller than the value of sequence C(0.789s). The results showed overall estimations of 0.701s, 0737s and 0.683s for the sequence A, B and C respectively, which is consistent with logarithmic averaging. These findings suggest that temporal averaging uses geometric mean as the main statistical summary process, where logarithmic encoded intervals are likely directly averaged before decoding process.

Disclosures: Y. Ren: None. **Z. Shi:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.15/HHH1

Topic: H.02. Human Cognition and Behavior

Support: Natural Science Foundation of China (31200760)

National High Technology Research and Development Program of China (863 Program) (2012AA011602)

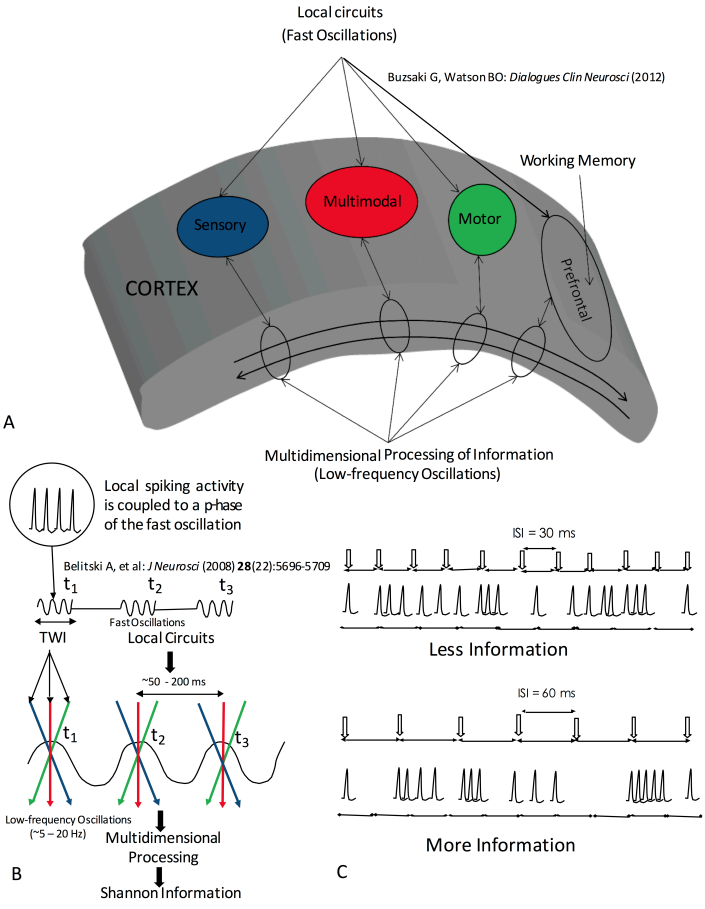
Fund for fostering talents in basic science (J1103602) to LC

Title: Brain oscillations in perception, timing and action

Authors: *D. S. GUPTA[1], L. CHEN[2,3];
[1]Biol., Camden County Col., Blackwood, NJ; [2]Key Lab. of Machine Perception (Ministry of

Education), [3]Dept. of Psychology and Beijing Key Lab. of Behavior and Mental Hlth., Peking Univ., Beijing, China

Abstract: Catching a thrown ball requires a tight coupling between perception and motor control. In a recent work (Gupta and Chen in: Current Opinion in Behavioral Sciences 2016, 8:161-166), we examined various studies, supporting the proposed multidimensional information processing across various perceptual and motor tasks. In this work, we examined how perception, timing, and action can be understood in terms of the coupling of gamma band oscillations, which represent the local activities of brain circuits, to a specific phase of long-range low-frequency oscillations. We proposed a temporal window of integration that emerges from cross-frequency coupling that serves to produce optimized action. In this poster, we will further look at more studies to understand how the information processing in multidimensional processing domains, resulting from the synchronization of local circuits in brain networks, may form an important basis of the interaction of the brain with the four-dimensional physical world.



Disclosures: D.S. Gupta: None. **L. Chen:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.16/DP08 (Dynamic Poster)

Topic: H.02. Human Cognition and Behavior

Support: EU Horizon 2020 FET Proactive grant 641100 TIMESTORM

Title: Resolving the dopamine paradox in interval timing: How a phasic dopamine release can reset the clock, whereas tonic dopamine fluctuations alter perceived time

Authors: *H. VAN RIJN[1], P. MOSTERT[2], P. A. M. MEDIANO[3], Z. FOUNTAS[3];
[1]Univ. of Groningen, Groningen, Netherlands; [2]Donders Inst. for Brain, Cognition and Behavior, Nijmegen, Netherlands; [3]Imperial Col., London, United Kingdom

Abstract: Typically, interval timing models assume that the subjective perception of time commences by the resetting of an internal clock, irrespective of whether this internal clock is hypothesized to be implemented as an pacemaker, as oscillating neurons, or as other structures that provide a temporally regular signal. Whereas most models are agnostic about the underlying signal that results in the resetting of the internal clock, the Striatal Beat Frequency model, the most prominent neurobiological model of the clock stage, assumes that a phasic release of dopamine resets the phases of clusters of oscillators. Interestingly, dopamine has been shown to have more subtle effects as well: when tonic dopamine levels are pharmacologically manipulated, the subjective experience of time is affected. Although both effects have been reported in multiple studies using multiple paradigms, no explanation has been given that can explain this paradoxical finding that a phasic boost resets the clock, but tonic fluctuations affect the internal clock in subtle but consistent ways (i.e., higher tonic dopamine levels resulting in a faster running internal clock). Here we will present a computational neuroscience model, implemented in BrainStudio (http://brain-studio.org) and based on biologically plausible neuron models, that explains how a phasic dopamine boost can reset an internal clock, but tonic dopamine levels affect the speed by which subjective time passes. By combining this more detailed implementation of the Striatal Beat Frequency model with an integrated process model of interval timing (Taatgen, Van Rijn, Anderson, 2007, Psychological Review, 114(3), 577-598; Van Rijn, Gu, Meck, 2014, Advances in Experimental Medicine and Biology, 829, 75-99), we can explain how the beginning of an interval is triggered, and what neurobiological mechanism explains cognitive and pharmacological distortions on subjective time.

Disclosures: **H. Van Rijn:** None. **P. Mostert:** None. **P.A.M. Mediano:** None. **Z. Fountas:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.17/HHH2

Topic: H.02. Human Cognition and Behavior

Title: A neural correlate of human time sense

Authors: C. K. DESAI[1], *M. I. LEON[2];
[1]Psychology, California State University, Bakersfield, Bakersfield, CA; [2]Psychology, Cal State Univ, Bakersfield, Bakersfield, CA

Abstract: We measured broadband EEG power from scalp electrodes positioned over the posterior parietal cortex of subjects performing a Go/No-Go temporal bisection task. During practice trials, subjects categorized one of two randomly presented tone stimuli as short or long. Each trial began by cueing the question, “Is it long?” or “Is it short?” At the termination of each tone, subjects decided “Yes” by reaching to depress a choice button, or “No” by remaining still. Experimental sessions included seven additional tones of intermediate durations, with all nine tones being randomly interleaved across trials. Subjects were tested with a short (0.57 sec - 1.74 sec) or a long (1.15 sec - 3.48 sec) set of durations across two different sessions. As the tone increased in duration, subjects demonstrated a greater probability of choosing long. In addition, the neural signal began to reflect the subject’s increasing likelihood of a long decision. The duration at which this first became evident coincided with the subject’s bisection point, where long and short decisions are equally likely. Our findings demonstrate a neural correlate of human time sense.

Disclosures: C.K. Desai: None. **M.I. Leon:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.18/HHH3

Topic: H.02. Human Cognition and Behavior

Support: MRC

EU FP7/2007-2013 Marie Curie 600209 (TU Berlin/IPODI)

Title: The links between perceptual timing and language skill in mid- vs. early adolescence: the St Thomas More School Project

Authors: *M. GRUBE[1,2], C. DAVISON[2], F. SMITH[2], S. KUMAR[2], T. D. GRIFFITHS[2];
[1]TU Berlin, Berlin, Germany; [2]Newcastle Univ., Newcastle-upon-Tyne, United Kingdom

Abstract: We have previously tested the relationship between auditory analysis and language skill in children in a series of experiments based on the systematic examination of multiple auditory domains (pitch, time and timbre) from simple features in single sounds or time intervals to complex sequences. The work demonstrated a relationship between pitch or temporal sequences and language skill in a large cohort of eleven-year-old school children [Grube et al: PMID 22951739]. The present study examined the specific relationship between time-sequence and language skill and how this changes over adolescence. Specifically, our previous data from eleven-year-old children demonstrated a relationship between the analysis of short isochronous rhythms and language skill whilst in young adults the analysis of longer more abstract, beat-based timing was important [Grube et al: PMID 24168197]. Here, we examined the relationship of both types of auditory skill and language skill in large cohorts of eleven- and fourteen-year-old children. 237 eleven year olds and 234 fourteen year olds each underwent 3 hours of testing in total (including both auditory and language). The groups represent complete year groups from a non-selective government-funded school. The four timing tasks comprised discrimination of single time intervals, deviation from isochronous rhythm, regularity detection (the ability to detect a 'roughly' regular beat) and metrical rhythm discrimination [Grube et al: PMID 20534501 and 22951739]. Language assessment included rhyme decision, spelling, word reading, non-word reading, rapid automated naming (digits and objects), and spoonerisms. Pairwise correlation analysis was carried out on data from ~ 190 eleven year olds and ~ 200 fourteen year olds. In the eleven years olds a limited but significant and consistent correlation between isochronous sequence analysis and language skills was demonstrated after accounting for non-verbal intelligence [average rho before: 0.23, and after: 0.19; $p < 0.05$, Bonferroni-corrected]. In the fourteen year olds a moderate to large and significant correlation was found between isochronous sequence analysis and language skill [average rho before: 0.33, and after: 0.22; $p < 0.05$, Bonferroni-corrected], and also between regularity detection and language skill [average rho before: 0.31, and after: 0.22; $p < 0.05$, Bonferroni-corrected]. The data confirm an important relationship between rhythmic and language skill consistent with the notion of a 'temporal scaffolding' mechanism common to generic auditory and speech domains. This specific nature of this relationship is not fixed but evolves over adolescence.

Disclosures: **M. Grube:** None. **C. Davison:** None. **F. Smith:** None. **S. Kumar:** None. **T.D. Griffiths:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.19/HHH4

Topic: H.02. Human Cognition and Behavior

Support: Wellcome Trust Grant WT099750MA

Title: Midbrain adaptation may set the stage for the perception of a musical beat

Authors: *V. G. RAJENDRAN[1], J. A. GARCIA-LAZARO[2], N. S. HARPER[1], N. A. LESICA[2], J. W. H. SCHNUPP[3,1];
[1]Univ. of Oxford, Oxford, United Kingdom; [2]Ear Inst., UCL, London, United Kingdom; [3]Biomed. Sci., City Univ. of Hong Kong, Kowloon Tong, Hong Kong

Abstract: It is a well-appreciated ability of humans (and possibly other species) to spontaneously perceive a quasi-periodic pulse in rhythmic sounds such as music. Recent work using electroencephalography (EEG) in humans has revealed a possible neural substrate of beat perception (Nozaradan et al., 2011; 2012). Through an approach termed "frequency-tagging," neural entrainment to the beat is identified as the selective enhancement of beat and meter-related frequencies in the amplitude spectrum of the EEG relative to the prominence of those frequencies in the envelope of the sound. This approach has led to a substantial body of work exploring sensory, motor, and cognitive aspects of beat perception, but sheds little light on where and how beat processing first emerges in the auditory pathway. We recorded from the inferior colliculus (IC) of anaesthetized gerbils in response to a subset of the rhythmic patterns used in these previous EEG studies. When we apply frequency-tagging analysis, the results are inconclusive: population local field potential (LFP) responses from gerbil IC would appear to entrain to beat and meter-related frequencies like in human EEG, but firing rates from single and multi-units at the same recording sites do not. However, time-domain analyses reveal that both LFP and spiking activity show larger responses to sounds occurring on the beat relative to otherwise identical sounds that are not on the beat. We split our 249 single and multi-units into clusters to reveal seven representative classes of neural responses that show characteristic differences in response latencies, strength of onset responses, and sustained activation. These clusters also differ in how suppressed responses become as a result of sounds in recent stimulus past. We quantified this sensory adaptation by fitting an exponential function to response strength as a function of time since the previous sound occurrence, and we find that the asymmetry between beat and non-beat responses is captured sufficiently by predictions based solely on these exponential fits. Together, this suggests that midbrain-level adaptation may play a role in biasing the neural representation of rhythmic patterns to favor the configuration that we as humans eventually perceive as a beat.

Disclosures: **V.G. Rajendran:** None. **J.A. Garcia-Lazaro:** None. **N.S. Harper:** None. **N.A. Lesica:** None. **J.W.H. Schnupp:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.20/HHH5

Topic: H.02. Human Cognition and Behavior

Title: The action congruency effect on the feelings of agency

Authors: *R. VASTANO[1,2], T. POZZO[1,3], M. BRASS[2];
[1]Robotics, Brain & Cognitive Sci., Fondazione Inst. Italiano Di Tecnologia, Genova, Italy; [2]Dept. of Exptl. Psychology, Ghent Univ., Ghent, Belgium; [3]Univ. de Bourgogne, INSERM U1093 Cognition, Action et Plasticité Sensorimotrice, Dijon, France

Abstract: Several studies suggest that the awareness and the feeling of control about one's own actions and ensuing effects are due to predictive mechanisms shaping the sense of agency. For instance, we experience a strong sense of agency when the predicted effects of an action match the perceived effects. In this vein, the sense of agency is considered retrospectively, after the action is completed and its consequences are known. However, recently it has been suggested that agency also has a prospective component. One aspect of this prospective component relates the fluency of action selection which prospectively informs our sense of agency.
In this study we investigate whether the awareness of an external stimulus, which affects the action selection and thus the intention-action-outcome flow, modulates the feelings of agency as indicated by the intentional binding effect. To this aim we used the imitation-inhibition task combined with a time estimation task. Participants lifted their index or middle finger in response to a number, while simultaneously observing either congruent (i.e. the same) or incongruent (i.e. the opposite) finger movements of a mirrored right-hand. Their lifting movements caused an auditory effect after a variable delay. At the end of each trial, participants estimated the time between their action and the ensuing effect.
The results show that the incongruent condition elicited a smaller temporal binding effect than the congruent condition. In order to investigate whether this effect was due to observing a congruent or incongruent finger movement, or to interference in general, we investigated intentional binding with a stroop-like task as well. In this task, no finger movements were shown and the participants performed finger lifting movements with a colored target. After their action the same auditory effect was delivered and they performed the time estimation task.
In accordance with a general interference hypothesis, we again found reduced temporal binding

in the incongruent condition. This effect presumably depends on the experience of conflict that disrupts the normal flow of information from intention, to action, to outcome elicited by the incongruent conditions. The influence of congruency on agency therefore does not depend on a specific type of stimuli.

Disclosures: **R. Vastano:** None. **T. Pozzo:** None. **M. Brass:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.21/HHH6

Topic: H.02. Human Cognition and Behavior

Support: ERC-YSt-263584

Title: Temporal expectation biases duration judgment

Authors: ***T. W. KONONOWICZ**, V. VAN WASSENHOVE;
CEA, NeuroSpin Center, INSERM, Univ. Paris-Sud, Gif S/ Yvette, France

Abstract: Timing processes in humans are typically classified into implicit and explicit timing: explicit timing requires explicit usage of the temporal dimension to estimate duration of a given time interval. On contraire, implicit timing entails the use of the temporal dimension to create temporal expectation with respect to the upcoming stimulus. The simplest form of temporal expectation is linked to the buildup of anticipation informed by the hazard rate function, that is the probability that an event will occur given that it has not yet occurred. As temporal expectation is known to affect sensory processing, we set out to test how temporal expectation affects duration perception in order to address the interplay between implicit and explicit timing processes which are rarely considered.
We asked participants to discriminate time intervals while recording brain signals using combined EEG and MEG. On each and every trial the standard duration (860ms) was presented, followed by the presentation of the comparison interval (770, 860, 950ms; 10%, 80%, 10% of all trials respectively). Crucially, the inter-stimulus intervals (ISI), that is interval between the SI offset and the CI onset, were uniformly distributed which allowed participants to build up a steady anticipation function with regard to the occurrence of the comparison interval, as evidenced by the proportion of ‘short’ and ‘long’ responses. To investigate how the expectation of the comparison interval affects duration perception, we split trials according to their short and long ISI (‘early’, ‘late’). Within these two groups we investigated differences in the oscillatory power and inter-trial coherence (ITC) between trials subjectively perceived as ‘short’ and ‘long’.

For the ‘late’ trials the processing of the comparison interval has been associated with the modulation of alpha and beta power, and the modulation of the ITC, predominantly in the theta band. Specifically, the trials perceived as long exhibited larger alpha and beta desynchronization and increased ITC, suggesting increased expectation level in the ‘longer’ trials. Importantly, no difference between the ‘short’ and ‘long’ trials was observed for the ‘early’ trials. Together, these results demonstrate that temporal expectation modulated duration judgments by desynchronization of oscillatory power and enhancement of ITC.

Disclosures: T.W. Kononowicz: None. **V. van Wassenhove:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.22/HHH7

Topic: H.02. Human Cognition and Behavior

Support: KAKENHI-25119003

KAKENHI-25119006

Title: Role of interhemispheric cortical connections in time perception: a case study with agenesis of the corpus callosum

Authors: *M. OKAJIMA[1], A. FUTAMURA[2], M. HONMA[2], M. KAWAMURA[2], Y. YOTSUMOTO[1];
[1]Dept. of Life Sci., The Univ. of Tokyo, Tokyo, Japan; [2]Dept. of Neurol., Showa Univ. Sch. of Med., Tokyo, Japan

Abstract: When we pay attention to the duration of a given event while ignoring other irrelevant events, our perception of the target event duration is susceptible to the other irrelevant distractor events. Our previous study demonstrated that the duration of a target stimulus is perceived as longer in the presence of a flickering distractor stimulus, and that such effect is larger when the position of the distractor is ipsilateral rather than contralateral to the target stimulus. These results indicate that the effect of an intrahemispheric distractor on the perception of the target stimulus duration is greater than that of an interhemispheric distractor.In this study, we examined the perception of the target stimulus duration and the effects of distractors in a split-brain patient. We hypothesized that because of the lack of interhemispheric cortico-cortical connections in this patient, the effects of an interhemispheric distractor would be absent or lesser than those of an intrahemispheric distractor. One patient (ambidextrous, female, 51 years old) with agenesis of

the corpus callosum and age-matched control subjects participated in the experiment. The subjects were instructed to reproduce the duration of the target stimulus while ignoring the simultaneously displayed distractor stimulus. Both stimuli were either stable or flickering at 10 Hz. The results showed that, in the patient, the reproduced duration of the target stimulus was longer when the position of the stable or flickering distractor was contralateral rather than ipsilateral to the target stimulus. In addition, the variance of the reproduced durations was larger when the flickering distractor was contralateral. Such trends were not observed in the control subjects.In the split-brain patient, there was a difference in the effects of interhemispheric and intrahemispheric distractors on the perception of the target stimulus duration. Contrary to our expectations, the interhemispheric distractor impaired the time reproduction task more than the intrahemispheric distractor, especially when the distractor was flickering. These results lead to the following conclusions. First, there is an interhemispheric communications of temporal information even in the absence of the corpus callosum, suggesting the involvement of subcortical areas in temporal information processing. Second, the corpus callosum is necessary to inhibit this interhemispheric interference of temporal information. Finally, the ability to focus on the duration of a certain event is based on the cortico-cortical connections that prevent other simultaneous events from interfering.

Disclosures: **M. Okajima:** None. **A. Futamura:** None. **M. Honma:** None. **M. Kawamura:** None. **Y. Yotsumoto:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.23/HHH8

Topic: H.02. Human Cognition and Behavior

Title: Time-perception training modulates fMRI activity in the cortical basal ganglia circuit

Authors: *I. C. SÁNCHEZ[1], H. MERCHANT[2];
[2]Behavioral and cognitive neurobiology, [1]Inst. of Neurobio., Queretaro, Mexico

Abstract: It is well known that intensive training in specific perceptual timing tasks leads to performance improvement and that this improvement can generalize to a different, untrained, timing task. Here, we tested whether training in a perceptual timing task could improve performance during motor timing. We recruited 19 subjects (women and men). Average age was 26.5 years. Participants performed two tasks: The first was a synchronization-continuation task (SCT) that consisted of the presentation of a gray square on a screen that appeared and disappeared at a rate of 850 ms. Participants pressed a button synchronizing their 9 responses to

the appearance of the square (Synch). After the visual cue disappeared subjects had to continue pressing the button at the same pace for other 10 taps (Cont). For the discrimination task (DT), participants had to discriminate between two time intervals of different durations, selecting the longest one. One was a standard interval with a duration of 850 ms and the other was a comparison interval ranging from 566 to 1330 ms. Following the first imaging session where subjects performed the SCT in the magnet, they underwent 7 consecutive days of training in the DT. Imaging was repeated in the 7th day, identically to the first session. For the DT we calculated the discrimination threshold for each subject and day, and fitted a power function to the data. For the SCT, inter-interval variability was calculated and compared between the pre-training and the post-training sessions. We identified the brain regions active during the Synch and Cont. We evaluated differences between pre and post-training sessions and between the phases (Synch and Cont). Analysis of the behavioral data of the Cont showed two distinct groups of participants: A group of Learners (11, L) and a group of Non-Learners (7). Only the subjects that belonged to the L had a significant decrease in its inter-interval variability after training. This clearly reflects that the 850 ms internal representation was improved and this enable the subjects to perform better during the SCT. We found increased BOLD signal during the Cont of the SCT in the L after training in regions: cerebellum Crus I and occipital lobe. We performed a contrast between the Synch and the Cont after training. During the Synch we found activation of: Brodmann Area 44 and 45, anterior intra-parietal sulcus, thalamus, caudate, hippocampus dentate gyrus, occipital lobe (V1, V2, V3 and V4) and cerebellum (Crus I and II). In contrast, we found larger activation during the Cont in the following regions: Supplementary motor area, premotor cortex, right cingulate gyrus, primary somatosensory cortex, primary auditory cortex and insula.

Disclosures: I.C. Sánchez: None. **H. Merchant:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.24/HHH9

Topic: H.02. Human Cognition and Behavior

Support: Kakenhi 25119002

Kakenhi 25240022

Kakenhi 16H02890

Title: Reversal of tactile temporal order judgment correlates with the phase of posterior alpha rhythm

Authors: *T. TAKAHASHI[1,2], S. KITAZAWA[1,2];
[1]Osaka Univ., Osaka, Japan; [2]Ctr. for Information and Neural Networks (CiNet), Natl. Inst. of Information and Communications Technol., Osaka, Japan

Abstract: The subjective temporal order of tactile stimuli, delivered one to each hand with an interval of 100-300 ms, is often inverted when the arms are crossed but not when they are uncrossed. On the basis of data from behavioral and neuroimaging studies (Yamamoto & Kitazawa, 2001,Takahashi et al., 2013), we proposed a motion projection hypothesis in which the brain reconstructs the temporal order by integrating an apparent motion signal with the hand locations in space (Kitazawa et al., 2008). Because the posterior alpha rhythm reportedly modulates apparent motion (e.g. Valera et al., 1981), our hypothesis predicts that the illusory reversal is also modulated by the alpha rhythm.
To test the hypothesis, we recorded cortical magnetoencephalographic (MEG) activity by using a 160-ch magnetometer from 16 healthy right-handed volunteers, while the participants carried out a tactile temporal order judgment task under the arms crossed condition with a fixed stimulation interval of 100 ms (left-then-right or right-then-left). Because the alpha band signals reportedly consist of a few distinct components, we applied independent component analysis to the MEG data and eventually revealed five independent components that contributed the alpha rhythm: 1) a peri-PO component that has major current sources near the parieto-occipital (PO) sulcus and the precuneus, 2) a calcarine component, 3) a tau component with current dipoles in the bilateral primary auditory cortices, 4), and 5) the right and left mu components with dipoles in the right and left Rolandic areas.
We compared the distribution of the phase of each alpha-band component between trials with correct judgment and those with inverted judgment, and found that the illusory reversal correlated with the phase of the alpha rhythm in the peri-PO component, but not with the others. The rate of inverted judgment increased to 0.51 at a “preferred” phase but decreased to 0.37 at the opposite phase. The peri-PO region including the precuneus has been shown to provide an allocentric coordinate that is implicated for visual stability (Uchimura et al., 2015). Thus the results support the prediction from the motion projection hypothesis, and highlight the importance of the peri-PO alpha component in organizing temporo-spatial perception.

Disclosures: **T. Takahashi:** None. **S. Kitazawa:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.25/HHH10

Topic: H.02. Human Cognition and Behavior

Title: Neural entrainment during beat perception and its relation to psychophysical performance

Authors: *M. J. HENRY, J. A. GRAHN;
The Univ. of Western Ontario, London, ON, Canada

Abstract: The ability to pick up on regularities in environmental stimuli is apparent in infancy and supports language learning, movement coordination, and parsing auditory scenes into "objects". Here, we were interested in the seemingly unique sensitivity humans show to temporal regularities in rhythm: they spontaneously feel a "beat" in rhythmic sequences. In particular, we examined how synchronization of neural oscillations with auditory rhythms might give rise to beat perception, and in turn how entrained neural oscillations might affect psychophysical performance. In the current electroencephalography (EEG) study, participants detected near-threshold targets (changes in spectral bandwidth) embedded in long (~19-s) simple or complex auditory rhythms. Simple rhythms were composed of intervals related by integer ratios (1:2:3:4), and had a regular grouping that resulted in standard and target events always being present at "on-beat" locations given a duple meter. Simple rhythms thus induced a relatively strong sense of a beat. Complex rhythms were also composed of intervals related by integer ratios, but were grouped irregularly and thus did not induce a strong beat percept. We compared EEG spectral power at beat-related frequencies (1.25, 2.5, and 5 Hz, where 5-Hz was the base inter-tone interval) for simple and complex rhythms. We observed significantly stronger spectral power at 1.25 Hz for simple compared to complex rhythms in particular for individuals that were "good beat perceivers" as determined by a behavioral measure of beat-tapping variability. This result indicates stronger subharmonic entrainment for rhythms that gave rise to a strong sense of beat. We did not observe power differences between rhythm types at 2.5 or 5 Hz. We also examined power-envelope fluctuations in the beta (13-30 Hz) frequency band, which have been previously linked to temporal prediction of events comprising isochronous sequences. Beta power fluctuations at 5 Hz (the base inter-tone interval) were stronger for simple than for complex rhythms, suggesting that temporal prediction of upcoming events is sharpened in the presence of a strong beat percept. We found that targets were better detected when beta power increased in anticipation of a target, but that this effect was similar for simple and for complex rhythms. Thus, although beta fluctuations were stronger in the presence of a beat, high beta power was universally beneficial for psychophysical performance. The results provide the first link between electrophysiological correlates of beat perception and the psychophysical consequences of beat perception.

Disclosures: **M.J. Henry:** None. **J.A. Grahn:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.26/HHH11

Topic: H.02. Human Cognition and Behavior

Support: Vanier Canada Graduate Scholarship, NSERC

Canadian Institutes of Health Research

Title: Neural oscillatory entrainment to auditory rhythmic stimuli represents pitch as well as time

Authors: ***A. CHANG**, K. CLAYWORTH, D. J. BOSNYAK, L. J. TRAINOR;
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Abstract: Neural oscillatory activities in auditory cortex entrain to external temporal regularity. Previous studies have shown that the power of induced beta oscillations (15 - 25 Hz) in auditory cortex entrain to the rate of a presented isochronous tone sequence (e.g. Fujioka et al., 2012), with reductions in beta power following sound event onsets and with the slope of the rebound predicting the onset of the next sound event. Also, the power of beta oscillations prior to the onset of a tone reflects the accuracy of temporal perception (Arnal et al., 2015). However, given that both time and pitch are important dimensions for auditory information, we hypothesize that beta power entrainment co-represents both dimensions prior to an event's occurrence. Our first study aimed to investigate whether beta power entrainment reflects predictions for pitch as well as temporal information. We employed rhythmic auditory oddball sequences containing occasional pitch deviants. The EEG results showed that beta power entrainment is affected by the predictability of pitch changes: (1) beta power entrainment depth prior to the onset of a deviance was reduced when it was unpredicted, and (2) beta power entrainment after a pitch deviant was disrupted by the degree to which it was unpredictable. These results suggest that in addition to predicting when an event will occur, beta power entrainment also reflects predictions of what pitch will occur. The second study aimed to investigate whether the degree of beta power entrainment to an auditory rhythm might also facilitate the perception of pitch information. We employed rhythmic and non-rhythmic auditory oddball sequences and recorded EEG. Participants were required to discriminate whether each deviant pitch was higher or lower than the context pitches. Psychophysical analyses showed higher sensitivity of pitch discrimination (steeper slope of the psychometric function) and shorter response times with the rhythmic

compared to non-rhythmic sequence, suggesting that temporal regularity facilitates pitch perception. The analyses of neural responses are ongoing. Preliminary results indicate that beta entrainment prior to deviance determines the sensitivity of pitch discrimination. Together, our series of studies show that predictions for both time and pitch are represented in dynamic changes in the power of beta oscillations.

Disclosures: **A. Chang:** None. **K. Clayworth:** None. **D.J. Bosnyak:** None. **L.J. Trainor:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.27/HHH12

Topic: H.02. Human Cognition and Behavior

Support: KAKENHI (16K12969)

KAKENHI (26119521)

KAKENHI (25242058)

SCOPE (152309011)

Title: Neurofunctional coupling in tactile simultaneity judgment

Authors: T. KIMURA[1], T. KOCHIYAMA[2], T. KURODA[3], M. IWATA[1], H. KADOTA[1], *M. MIYAZAKI[3];
[1]Res. Inst., Kochi Univ. of Technol., Kami, Japan; [2]Brain Activity Imaging Ctr., ATR, Seika-cho, Japan; [3]Dept. of Computer Science, Fac. of Informatics, Shizuoka Univ., Hamamatsu, Japan

Abstract: In this study, we demonstrate the neurofunctional coupling that underlies tactile simultaneity judgment (SJ). Prior to the current study, our research group investigated differences in the neural correlates of tactile SJ and temporal order judgment (TOJ) using fMRI (Miyazaki et al. 2012, SfN 910.03; 2016, Sci Rep 6: 23323). We found that while the TOJ > SJ contrast exhibited stronger brain activation in the left-dominant motor-control network, the SJ > TOJ contrast exhibited stronger activation only in the posterior insula. We hypothesized that the posterior insula plays a role in discriminating tactile simultaneity. Meanwhile, the SJ > TOJ contrast should subtract brain activation involved in the common temporal processing components for SJ and TOJ. In the current study, we conducted fMRI to identify the neural correlates for the whole temporal processes for tactile SJ, using a time-irrespective control task.

During fMRI, participants ($N = 24$) received a pair of tactile stimuli across both index fingers in each trial. The stimulus onset asynchrony was -50 ms (left earlier), 0 ms, or +50 ms (right earlier). The number of driven pins in each tactile stimulator was 2 or 6 [difference in the number of pins: -4 (left greater), 0, or +4 (right greater)]. In two sessions (32 trials/session), the participants judged whether the stimulus onsets were simultaneous between both hands (SJ). In the other two sessions, they judged whether the number of stimulus pins were same (NJ). The SJ > NJ contrast revealed significant activation in the right inferior parietal lobule (IPL)/supramarginal gyrus (SMG) extending to the superior temporal gyrus (STG), and in the right inferior and middle frontal gyri ($Z > 2.58$, $P < 0.05$ FWE corrected at the cluster level). The posterior insula was thus not observed in the SJ > NJ contrast, since the insula was activated in both SJ and NJ. However, psychophysiological interaction analysis exhibited functional coupling between the right IPL/SMG and the left posterior insula with surrounding areas (e.g., STG, inferior frontal gyrus). The results suggest that the posterior insula is involved not only in discriminating tactile simultaneity but also in discriminating more general tactile coincidence. Hayashi et al. (2015, PLoS Biol 13(9); e1002262) indicated that the perceived visual duration is represented in the right IPL. We speculate that the left posterior insula discriminates coincidence of onset timing between tactile stimuli according to tactile temporal intervals represented in the right IPL.

Disclosures: **T. Kimura:** None. **T. Kochiyama:** None. **T. Kuroda:** None. **M. Iwata:** None. **H. Kadota:** None. **M. Miyazaki:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.28/HHH13

Topic: H.02. Human Cognition and Behavior

Support: EU Horizon 2020 FET Proactive grant 641100 TIMESTORM

Title: Temporal and numerical magnitude processing: two dimensions, one processing system?

Authors: ***N. SCHLICHTING**, R. DE JONG, H. VAN RIJN;
Dept. of Psychology, Univ. of Groningen, Groningen, Netherlands

Abstract: Magnitudes or quantities of different dimensions (e.g., time, number, speed or space) have been shown to interact when perceived simultaneously. These findings have been interpreted as evidence for a common magnitude system, in which magnitude information from different dimensions are encoded concurrently. Certain ERP-components (e.g., the CNV) and

brain areas (e.g., the SMA) have been linked to the perception of temporal magnitudes specifically. However, it is of yet unclear whether these EEG components are really unique to time perception or reflect the perception of magnitudes in general. In the current experiment, we recorded EEG while participants had to make judgments about duration (*time* dimension) or numerosity (*number* dimension) in a classical comparison task. Stimuli consisted of a series of blue dots appearing and disappearing dynamically on a black screen. Each stimulus was characterized by its duration (i.e., the interval between appearance of the first dot and disappearance of the last dot) and the total number of dots it consisted of. Thus, each stimulus could vary simultaneously and independently in both *time* and *number*. Participants had to judge whether the second stimulus (S2) presented in a given trial was shorter/longer (*time* dimension) or consisted of more/less dots (*number* dimension) than the first stimulus (S1), whereby either S1 or S2 was the standard stimulus with a duration of 1.8 s and consisting of 30 dots. Six different comparison intervals (1.23, 1.49, 1.63, 1.98, 2.18 and 2.64 s) and number magnitudes (20, 25, 27, 33, 36 and 44 dots) were used. Participants were informed in advance whether a judgment on *time* or *number* had to made. Behavioral results show that response accuracy, measured by the Weber Ratio and the Point of Subjective Equality, did not vary across tasks. Thus, the *time*- and *number*-task were successfully equated for difficulty. We will present EEG data analyzed in the time- (ERPs) and (time-) frequency-domain to investigate possible differences or similarities in the neural mechanisms underlying the processing of magnitudes of different dimensions.

Disclosures: **N. Schlichting:** None. **R. de Jong:** None. **H. van Rijn:** None.

Poster

085. Human Cognition: Temporal Processing I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 85.29/HHH14

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant NS092079

Title: Preserved rhythm-based temporal predictions in cerebellar degeneration

Authors: ***A. BRESKA**, R. B. IVRY;
Psychology, Univ. of California Berkeley, Berkeley, CA

Abstract: The ability to time, whether estimating the duration of events or predicting the moment of upcoming events' onsets, is essential for successful interaction with the world. Animal models, imaging, and neuropsychological research have demonstrated a key role for the cerebellum in tasks requiring precise timing, but have also suggested constraints on the

functional domain of cerebellar timing. For example, patients with cerebellar degeneration are impaired in explicit perceptual timing (duration judgments) in the context of discrete events but not rhythms, as well as in motor timing in the context of discrete movements but not circle drawing. These dissociations suggest the hypothesis that, whether timing is perceptual or motor, the cerebellum is not necessary for timing continuous cyclic dynamics, but is essential for timing discrete events.To test this hypothesis, the current study examined the impact of cerebellar degeneration on temporal predictions, which constitute a form of perceptual timing, in rhythmic and non-rhythmic contexts. Each trial consisted of a stream of visual stimuli: a preparatory sequence, a predefined warning signal, and a target that required a speeded response. In the 'Rhythm' condition, the preparatory sequence was rhythmic, with the warning signal and target continuing the rhythm such that they predictably occurred on the beat. In the 'Random' condition, the intervals of the preparatory sequence were jittered in time around the fixed interval of the rhythmic stream, providing no information regarding the time of the target. In the 'Interval' condition, the preparatory stream consisted of two stimuli such that the interval between them was identical to the interval between the WS and the target. This enabled forming temporal prediction based on memorizing this discrete interval, in the absence of a rhythmic context. To prevent predictive motor responding, the target did not appear on in 25% of the trials in each condition (catch trials). Patients with cerebellar degeneration showed no deficit in rhythm-based temporal predictions, with faster responses in the Rhythmic condition compared to the Random condition; the degree of facilitation was similar to that of age-matched controls. The patients also had faster responses in the Interval condition compared to the Random condition, but this effect did not reach statistical significance and was smaller than the benefit demonstrated by the controls. These results support the generalizability of the hypothesis that the cerebellum is not necessary for timing in continuous cyclic contexts, and also the dissociation between rhythm- and memory-based temporal predictions.

Disclosures: **A. Breska:** None. **R.B. Ivry:** None.

Poster

086. Human Cognition: Aging I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 86.01/HHH15

Topic: H.02. Human Cognition and Behavior

Support: Irish Research Council

Title: Can coordinated movement training enhance scores on tests of cognitive function in older adults?

Authors: *I. A. CREMEN[1], R. G. CARSON[2];
[1]Trinity Col. Inst. of Neurosci., Trinity Col. Dublin, Dublin 2, Ireland; [2]Trinity Col. Inst. of Neurosci., Trinity Col. Dublin, Dublin, Ireland

Abstract: Over the past decade, a vast research literature has emerged dealing with attempts to harness brain plasticity in older adults, with a view to ameliorating cognitive function. Since cognitive training has shown restricted utility in this regard, attention has turned increasingly to interventions that use adjunct procedures such as motor training or physical activity. As evidence builds that these have some efficacy, it becomes necessary to disentangle the characteristics of these interventions that are responsible for gains in cognitive function. In the present study, the mechanisms through which motor training interventions may lead to enhancement of cognitive function were investigated using a behavioural motor training intervention. Forty healthy community dwelling older adults (aged 65 to 90), participated in a randomised, double-blinded intervention. Participants were randomly allocated to a motor training group or an active control group. The intervention consisted of five thirty-minute sessions on consecutive days. The motor training programme was designed to accentuate multisensory integration, spatial planning and the precise coordination of muscle activity. Motor training required incremental improvements in the performance of a complex target acquisition task executed using ballistic movements of the upper limb. An active control group carried out training requiring no movement, but was otherwise equivalent and demanded comparable levels of attention. Cognitive function (motor reaction time and cognitive processing speed) was measured using the Choice Reaction Time Task (CRT) pre- and post-intervention. Both groups showed comparable results in the baseline CRT measures, however only the motor training group improved on measures of cognitive processing speed post-test, as measured by the CRT ($P>0.0156$, $r=0.416$). The active control group failed to exhibit improvements in cognitive processing speed. This result lends support to the notion that interventions emphasising a motor coordination element can lead to improvements on tests of cognitive function.

Disclosures: I.A. Cremen: None. **R.G. Carson:** None.

Poster

086. Human Cognition: Aging I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 86.02/HHH16

Topic: H.02. Human Cognition and Behavior

Support: R01 AG041915

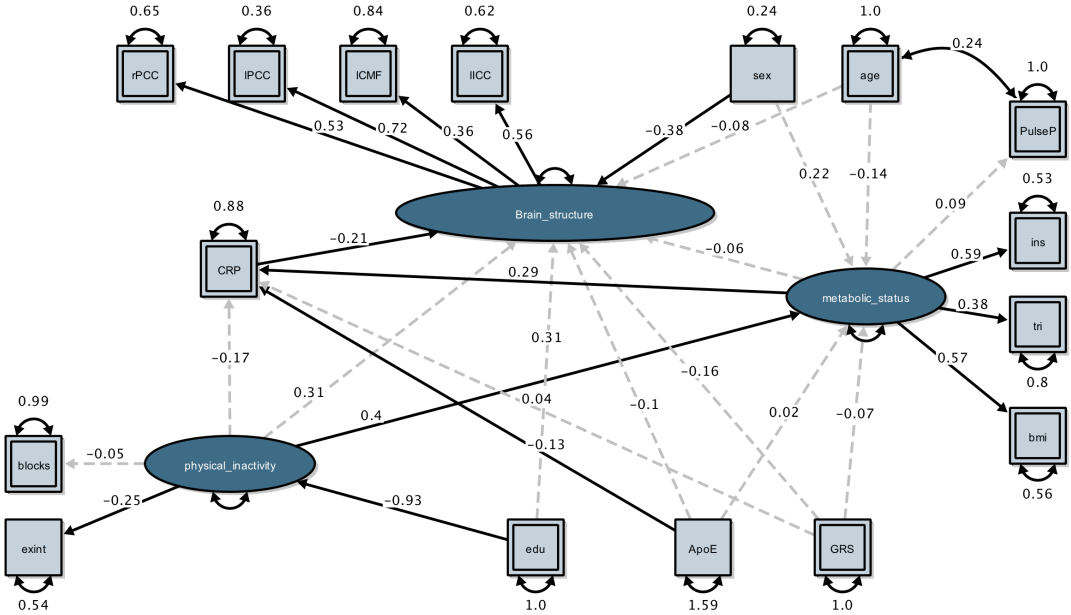
R01 AG040060

P50 AG005142

Title: Aging brain structure is related systemic inflammation & metabolic risk factors

Authors: *F. W. CORLIER[1], G. HAFZALLA[1], L. H. KULLER[2], O. L. LOPEZ[3], J. T. BECKER[4], P. M. THOMPSON[1], M. N. BRASKIE[1];
[1]Imaging genetics center, laboratory of neuroimaging, USC, Marina del Rey, CA; [2]Dept. of Epidemiology, [3]Dept. of Neurol., [4]Dept. of Phychyatry, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Systemic low-level inflammation is associated with brain atrophy and neurocognitive decline in Alzheimer's disease (AD) and normal aging. It is vital to know which risk factors trigger inflammation in old age how to prevent irreversible brain damage. Recent genome-wide association studies (GWAS) discovered AD risk polymorphisms in genes related to inflammation and immune function. Here, we investigated whether systemic inflammation mediates the established relationship between genetic and environmental AD risk factors and cortical thickness in AD-sensitive brain regions. We included 209 healthy older adults of European descent with available MR scans at year 9 of the longitudinal Cardiovascular Health Study (CHS).We estimated cortical thickness (cTh) 10 bilateral AD-sensitive brain regions using FreeSurfer. We used multiple linear regression to identify relationships between cTh in each region of interest and year-2 serum C-reactive protein (CRP), a measure of systemic inflammation, controlling for age, sex, education, and *APOE* genotype. Regions whose cTh was significantly associated with CRP included bilateral posterior cingulate, and left middlefrontal and isthmus of the cingulate cortices. We further investigated the significant associations between peripheral inflammation and those significantly related brain regions using a structural equation model (Figure 1) that included *APOE*4, a composite genetic risk score (GRS) of other AD-related genetic variants associated with immune function, physical activity measures and cardiovascular risk. We found that greater inflammation was associated with thinner cortex, (β= -0.21). Greater physical inactivity intensity was associated with higher cardiovascular risk (β=0.40) but not directly to more inflammation, Greater cardiovascular risk was associated with more inflammation (β=0.29). Our results suggest a link between systemic markers of chronic inflammation and cortical thickness. This relationship may partially mediate the effect of cardiovascular risk on brain integrity.



Path diagram representation of the structural model. Quality of fit: RMSEA= 0.007, SRMR=0.049, CFI = 0.997, TLI 0.995. Elipse shaped variables represent latent variables, square shaped variables represent manifest variables. Variables with a double edged case are continuous variables and were scaled prior to analysis.Double arrows represent correlations, and simple headed ones are for regressions. Plain arrows indicate significant loadings, dashed ones indicate relationships not statisticaly significant (Zscore <1.96) but that were takent into account in the overall fit indices.

Brain_structure represents the latent process of cortical thinning apparent through the values of cortical thickness in right (**rPCC**) and left (**lPCC**) posterior cingulate cortices, left isthmus of the cingulate cortex (**lICC**), and left caudal middle frontal (**lCMF**), all four significantly correlated to CRP blood levels in a preliminary multiple regression that controlled for age, sex, education and APOE genotype and adjusted for false discovery rate accros 16 regions evaluated. **Metabolic_status** measures the cardiovascular risk factors apearent through heart pulse pressure (**PulseP**), blood fasting insulin (**ins**) and triglycerides (**tri**), and body mass index (**bmi**). **Physical_inactivity** is inversely related to the number of blocks walked (**blocks**) and intensity (**exint**) of physical exercise performed in the weeks preceding the visit. **edu**, education (in years), **ApoE**, Apolipoprotein E associated risk score (Alzheimer's odds ratio associated to each genotype for individuals of European descent), **GRS**, genetic risk score obtained by cumulating the specific AD-odds related to single nucleotide polymorphysims in *CR1* (rs6701713), *MS4A* (rs670139 and rs610932), *CD33* (rs3865444), *ABCA7* (rs3764650), *EPHA1* (rs11767557), *IL1a* (rs180587), *IL1b* (rs1143634), *CLU* (rs11136000) genes, for individuals of European descent (Alzgene meta-analysis of caucasian studies).

Disclosures: F.W. Corlier: None. **G. Hafzalla:** None. **L.H. Kuller:** None. **O.L. Lopez:** None. **J.T. Becker:** None. **P.M. Thompson:** None. **M.N. Braskie:** None.

Poster

086. Human Cognition: Aging I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 86.03/HHH17

Topic: H.02. Human Cognition and Behavior

Support: NIA RO1 AG034613

Title: Forced-choice and old/new test formats reveal a stable age-related impairment of performance on the Mnemonic Similarity Task

Authors: *D. J. HUFFMAN[1], C. E. L. STARK[2];
[1]Neurobio. and Behavior, Univ. of California Irvine, Irvine, CA; [2]Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA

Abstract: Previous studies from our lab have indicated that healthy older adults are impaired in their ability to mnemonically discriminate between previously viewed stimuli and similar lure stimuli (e.g., Stark et al., 2013, 2015). These studies used old/similar/new and old/new test formats, including a version with confidence ratings. Previous studies have shown that the forced-choice test format (e.g., "Did you see object A or object A' during the encoding phase?") can sometimes yield different results than the old/new test format (e.g., "Did you see this object during the encoding phase?"). Additionally, the forced-choice test format relies on different assumptions (e.g., decision criteria) than the old/new test format; hence, converging evidence from these approaches would bolster the conclusion that healthy aging is accompanied by impaired performance on the Mnemonic Similarity Task. Consistent with our hypothesis, healthy older adults exhibited impaired performance on a forced-choice test format that required discriminating between a target and a similar lure (i.e., A vs A') but not on a format that required discriminating between a target and an unrelated foil (i.e., A vs X). We also tested the hypothesis that age-related impairments on the Mnemonic Similarity Task could be modeled within a global matching framework. Consistent with our hypothesis, we found that decreasing the probability of successful feature encoding in the models resulted in a similar pattern of results to the empirical data. Collectively, our behavioral results extend to the forced-choice test format the finding that healthy aging is accompanied by an impaired ability to discriminate between targets and similar lures, and our modeling results suggest that a diminished probability of encoding stimulus features is a candidate mechanism for memory changes in healthy aging. We will also discuss the ability of global matching models to account for findings in other studies that have used mnemonic similarity tasks.

Disclosures: D.J. Huffman: None. **C.E.L. Stark:** None.

Poster

086. Human Cognition: Aging I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 86.04/HHH18

Topic: H.02. Human Cognition and Behavior

Title: Management of prospective memory deficits in mild cognitive impairment

Authors: T. HUM-HYDER, *S. A. RASKIN;
Trinity Col., Hartford, CT

Abstract: Mild Cognitive Impairment (MCI) is an intermediate level of cognitive decline that is "greater than expected for an individual's age and education level" (Gauthier et al. 2008). MCI is believed to predict a trajectory towards a later diagnosis of Alzheimer's Disease (AD), which is corroborated by autopsied MCI patients who had neurofibrillary pathology in the entorhinal cortex, hippocampus, and amygdala (Markesbery 2010). Prospective memory is the ability to remember to do something. Using attention processing training, prospective memory training, and Virtual Week, we looked at the potential for attenuation of symptomology for mild cognitive impairment individuals.

Disclosures: T. Hum-Hyder: None. **S.A. Raskin:** None.

Poster

086. Human Cognition: Aging I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 86.05/HHH19

Topic: H.02. Human Cognition and Behavior

Title: Familiar context effects on pattern separation in aging

Authors: A. LAWRENCE[1], *L. RYAN[2];
[2]Evelyn F. McKnight Brain Inst., [1]Univ. of Arizona, Tucson, AZ

Abstract: Research suggests that recognition memory performance declines with age (Yassa et al., 2011). However, recent work in our laboratory indicates that although older adults perform more poorly at object recognition, their recognition is boosted to the same degree as younger adults when the object is presented in the same context at study and test. Older adults may be using relatively spared scene recognition processing to boost recognition of objects presented in a scene. However, other studies suggest that older adults are simply biased by familiar background scene information to falsely recognize a similar object. We tested these two alternative hypotheses using a continuous recognition pattern separation paradigm. Young adults (n=32, mean age=19) and older adults (n=30, mean age= 71) were given three continuous recognition tasks consisting of objects, scenes, and objects in scenes. Participants indicated whether each image in the series was either the same as, similar to, or different from an image they had seen previously. Both younger and older adults performed more poorly recognizing similar scenes and objects in a scene than recognizing similar object on a white background ($t = -2.584$, $p < .01$). Interestingly, both younger and older adults demonstrated significantly greater bias to falsely recognize objects presented in scenes than objects presented on a white background ($t=2.785$, $p<.01$). This suggests that contextual information may be biasing individuals to falsely recognize similar lure objects. These findings also suggest that the degree

to which an individual is biased to falsely recognitize a similar object is not related to age, as has been previously suggested (Gutchess et al., 2007), but rather to the presence of a familiar background context.

Disclosures: A. Lawrence: None. **L. Ryan:** None.

Poster

086. Human Cognition: Aging I

Location: Halls B-H

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Program#/Poster#: 86.06/HHH20

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Support: Graduate School of Systemic Neurosciences (GSN), LMU, funded by the German DFG Excellence Initiative)

DFG FI 1424/2-1

Alzheimer Research Initiative (AFI)

Zentrum Seniorenstudium München

Title: The influence of alertness training on visual processing speed in healthy older adults

Authors: *M. PENNING[1,2], P. REDEL[3], H. J. MÜLLER[3], T. SALMINEN[3,4], T. STROBACH[4,5], S. MOELBERT[3,6], T. SCHUBERT[3,4], K. FINKE[3];
[1]Dept. Psychology & Pedagogy, Gen. and Exptl. Psychology, Ludwig-Maximilians-Universität München, Muenchen, Germany; [2]Grad. Sch. of Systemic Neurosciences, Muenchen, Germany; [3]Dept. Psychology & Pedagogy, Gen. and Exptl. Psychology, Ludwig-Maximilians-Universitaet Muenchen, Muenchen, Germany; [4]Dept. of Psychology, Humboldt-Universitaet zu Berlin, Berlin, Germany; [5]Med. Sch. Hamburg, Department of Psychology, Germany; [6]Dept. of Psychosomatic Med., Univ. of Tuebingen, Tuebingen, Germany

Abstract: Visual processing speed markedly declines during aging, which is assumed to have significant consequences for independent daily life. Hence, the idea of improving this critical attentional function in older adults seems auspicious. However, in any intervention it is crucial to exactly determine the underlying mechanisms of possible gains, as diverse attentional and motor functions could be involved. Potential benefits should be based on an improvement in the targeted function, i. e. visual processing speed. We investigated the effects of a cognitive training of fast motor responses (the CogniPlus intrinsic alertness training designed specifically for the rehabilitation of neuropsychological patients) on visual parameters assessed ‘purely’ without any

motor confounds on the basis of the Theory of Visual Attention (TVA, Bundesen, 1990). We were especially interested in TVA parameter processing speed *C* in our training group (*n* = 25). To control for unspecific effects, we also tested two demographically matched control groups, an active control group (*n* = 25) performing an unspecific working memory task and a passive (*n* = 25) control group without any training. We found a significant group × training interaction, in which only for the alertness training group, the processing speed *C* was improved, while there was no change in either control group. Hence, the training of fast motor responses enhanced 'pure' visual processing speed in healthy elderly adults. This crucial and specific transfer to the targeted parameter allows for exact conclusions about the underlying neuro-cognitive changes and has important implications for the targeted rehabilitation of cognitive functions.
We will also look at the correlation between processing speed gain and intrinsic functional connectivity.

Disclosures: **M. Penning:** None. **P. Redel:** None. **H.J. Müller:** None. **T. Salminen:** None. **T. Strobach:** None. **S. Moelbert:** None. **T. Schubert:** None. **K. Finke:** None.

Poster

086. Human Cognition: Aging I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 86.07/HHH21

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant F31AG049564

NIH Grant R01AG034570

Title: Medial temporal lobe structure, function, and protein aggregation affect memory encoding in aging

Authors: *S. M. MARKS[1], S. N. LOCKHART[1], W. J. JAGUST[1,2];
[1]Helen Wills Neurosci. Inst., Berkeley, CA; [2]Life Sci. Div., Lawrence Berkeley Natl. Lab., Berkeley, CA

Abstract: Successful memory processes require large-scale networks, many of which are affected by changes associated with aging. Early Alzheimer's pathology preferentially targets these networks and has been associated with memory impairment. This study sought to investigate the impact of pathology on the hippocampus and its associated network during episodic memory. Forty-three cognitively normal older adults and 20 young controls participated in a functional MRI paradigm designed to emphasize pattern separation, a function attributed to

the hippocampus. A subset of older adults underwent Pittsburgh Compound B and AV-1451 PET scans to measure beta-amyloid (Aβ) and tau neurofibrillary tangles, as well as structural and resting-state MRI. Cortical Aβ values were used to identify Aβ-positive individuals. Tau values corresponding to Braak 1/2 and 3/4 staging were quantified using Freesurfer parcellations. Functional connectivity between retrosplenial cortex and medial temporal lobes (MTL) was assessed using seed-based correlation of resting-state time series. Older adults performed worse on the memory task ($t_{(41)} = -6.40$, $p < 0.001$). Voxelwise analyses revealed greater activation in older adults relative to young controls during encoding of subsequent hits and false alarms (lures incorrectly identified as old). Clusters associated with subsequent hits were found in right anterior hippocampus and spanned all subfields ($p < 0.001$, $k > 250$). Clusters associated with subsequent false alarms were found in bilateral anterior hippocampus, left hippocampal tail and right perirhinal/parahippocampal cortex ($p < 0.05$, $k > 500$). There were no MTL areas where young controls activated more than older adults. Aβ status and tau were unrelated to the degree of activation within significant voxelwise clusters; however, there were complex relationships between brain activation, tau deposition, and connectivity. Specifically, parahippocampal thickness may influence memory deficits, such that thinner cortex is associated with increased false alarm activation ($r^2 = 0.20$, $p = 0.007$), decreased retrosplenial-parahippocampal connectivity ($r^2 = 0.28$, $p = 0.003$), and increased parahippocampal tau ($r^2 = 0.31$, $p = 0.02$). These results suggest that widespread activation differences underlie age-related changes in pattern separation. This activation appears related to atrophy of the parahippocampal cortex, which is in turn related to changes in connectivity and tau accumulation.

Disclosures: **S.M. Marks:** None. **S.N. Lockhart:** None. **W.J. Jagust:** None.

Poster

086. Human Cognition: Aging I

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Program#/Poster#: 86.08/HHH22

Topic: H.02. Human Cognition and Behavior

Support: NIA Grant AG045571

NIA Grant AG013854

Title: Vulnerability and integrity of von economo neurons in human anterior cingulate cortex across the cognitive lifespan

Authors: ***T. GEFEN**, S. PAPASTEFAN, F. RAHMANI, E. ROGALSKI, S. WEINTRAUB, E. H. BIGIO, M.-M. MESULAM, C. GEULA;

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Abstract: Age is the strongest risk factor for Alzheimer's Disease (AD), likely due to typical "wear-and-tear" that occurs in permanently post-mitotic neurons across the human lifespan. The Alzheimer dementia stage is preceded by a prodromal amnestic mild cognitive impairment stage (aMCI) where both AD pathology and cognitive impairment are less pronounced. What remain unknown are the intrinsic vulnerabilities of specific neuronal subpopulations that potentially contribute to neurodegeneration and cognitive decline across the lifespan.
von Economo neurons (VENs) are highly specialized spindle-shaped cells unique to the anterior cingulate cortex (ACC) and frontoinsular cortex in primate hominids, and are implicated in higher-order cognition. VENs were found to be reduced in certain neurodegenerative diseases such as behavioral variant Frontotemporal Dementia, whereas cognitive "SuperAgers" (age 80+ individuals with memory scores equal-to-or-above individuals ~25 years younger) were found to have an abundance of VENs in the ACC. The current study sought to determine whether VENs are preferentially reduced in individuals with amnestic dementia due to AD versus other healthy cognitive aging outcomes.
Postmortem brain specimens stained with Nissl were analyzed using modified stereological methods (StereoInvestigator, MBF) from the following cohorts (N=5, per group): SuperAgers, cognitively-normal elderly (age 65+), cognitively-normal young (age 20-60), elderly individuals diagnosed with aMCI (age 65+) and elderly individuals diagnosed with amnestic dementia (age 65+) with severe postmortem AD pathology. VEN density and total neuronal counts were obtained in the ACC based on cellular morphology.
Stereological results revealed lowest VEN counts in the AD group, followed by higher counts in aMCI, older, and younger groups, respectively. VEN counts and total neuronal counts in the AD group were significantly lower compared to old and young groups ($p<0.01$), but not the aMCI group. Consistent with prior findings, SuperAgers continued to show the highest VEN counts compared to all other groups ($p<0.05$). Total neuronal counts in the ACC were highest in young controls.
Similar to other neurons in human ACC, VENs are indeed vulnerable to loss in AD, but appear to be preserved in individuals with well-preserved cognition.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: "This study was supported by a grant of the Korean Mental Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea(Grant Number: HM15C0995)."

Title: A study on the utilizing status and demand for smartphone and mobile healthcare application in elderly adults

Authors: *J.-S. AHN[1,2], S.-Y. KIM[2], J. HEO[2], W.-J. CHOI[2,3], J. PARK[2,4];
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Abstract: Objective: This study aims to examine the status and the needs of smartphone usage, patterns of use, interest in utilizing mobile applications and preferred healthcare types with the purpose of providing mobile mental healthcare services that are efficient in improving the older adults' health conditions and quality of life. Methods: Data were collected through self-designed questionnaires with seniors and in-depth interviews with experts and social workers at a local center for dementia. The questionnaire consisted of Sociodemographic information, mobile devices utilizing characteristics, alcohol, smoking, the Korean version revised center for epidemiologic studies depression scale(CESD-R), a self-reported depression and self-reported sleep disturbance. the data of survey on 37 seniors and in-depth interviews with 7 experts were analysed. Results: 1) Most experts recommended the use of smartphones because using a smartphone exert a positive impact on the lives of the elderly. Many elderly adults were also interested in application for health care and health related education. If elderly adults want to receive health information via smartphones, applications that provide disease information or manage medication are recommended . In addition, most of the experts said that the elderly customized, detailed and sustainable smartphone education is necessary for the efficient use of smartphone. 2) As a preliminary analysis result of questionnaire survey on the utilizing status and demand for smartphone and mobile healthcare application of the elderly adult (65+) visited Seodaemun-gu Center for Dementia, there are differences in education and income between with and without smartphones. Functions commonly used in smartphones were voice calls, internet search and Social network services(in particular, Kakao talk). The preferred health care system via smartphone were information of medical disease and mental health. The majority opinion is that utilization is low due to the use of smartphones and functions are difficult overall. One of the main advantages of smartphones is that can easily get knowledge and communicate in real time. and disadvantages of smartphones were too complicated to use and too expensive to maintain.Conclusions: These results of the study will show the direction for more in-depth studies on mental health care application based on mobile devices for the elderly. In the future, the development of mental health care services related application that reflect these demands would be preferable.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: H2020-MSCA-IF-2015 Grant 708842

Title: Objective electrophysiological evidence for reduced efficiency of face identity processing in aging

Authors: *S. LITHFOUS, J. LIU-SHUANG, B. ROSSION;
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Abstract: Failures to identify other people through their face constitutes one of the most frequent complaints of elderly people and has been widely reported in previous studies. However, the results showed some discrepancy, suggesting that this complaint is difficult to measure behaviorally, due to decreased efficiency and slowing down in general cognitive and motor processes with age. Here we isolated face identity processing with a highly sensitive and objective approach in EEG, Fast Periodic Visual Stimulation (FPVS), in an oddball paradigm (Liu-Shuang et al., 2014). Fifteen young (mean age: 22.5 ± 1.25 years; 10 women) and 15 healthy elderly subjects (mean age: 61.5 ± 2.38 years; 10 women) participated in the study. Identical faces were presented through sinusoidal contrast modulation at a base frequency of 6 Hz (i.e. 6 images/s) during 64 s sequences, with different faces appearing every 5^{th} face (i.e. 6/5 = 1.2 Hz). Participants simply had to detect color changes of the fixation cross, with no difference in accuracy and RTs between groups. Sequences contained either upright or inverted faces and were presented randomly, with 4 sequences per orientation (i.e. 8 sequences in total). While the response at 6 Hz reflects general visual processing, previous studies in young adults showed that face individualization elicits a specific response at 1.2 Hz and harmonics (2.4 Hz, etc.). The general visual processing response was localized over medial occipital electrodes and did not differ between groups. However, the face individualization response over right occipito-temporal electrodes was reduced in elderly subjects compared to the young subjects. Moreover, the difference in amplitude of face individualization responses between upright and inverted faces was reduced in the elderly compared to young subjects. Using a sensitive and objective approach in EEG, these results demonstrate a decline of face individualization in aging independently of general cognitive factors, and suggest that holistic processing – i.e., the ability to perceive all features of a face at a single glance in a unified representation - may explain this decline.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant 1R01AG039103

Title: The relationships between age, fMRI correlates of familiarity and recognition memory

Authors: *M. A. DE CHASTELAINE, J. T. MATTSON, T. H. WANG, B. E. DONLEY, M. D. RUGG;
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Abstract: Studies investigating the effects of age on episodic memory performance generally report larger age-related deficits in recollection- versus familiarity-based recognition. Nevertheless, the neural correlates of familiarity are often reported to differ with age. Here, we used an associative recognition task to investigate the relationships between age, the neural correlates of familiarity, and recognition memory performance. Young, middle-aged and older participants (n = 136) were scanned during an associative recognition test following a study phase in which word pairs were visually presented in the context of an elaborative encoding task. Test items comprised studied, rearranged (items studied on different trials) and new pairs. fMRI familiarity effects were operationalized as greater activity for studied test pairs incorrectly identified as 'rearranged' than for correctly rejected new pairs. The reverse contrast was employed to identify 'novelty' effects. Simple recognition memory (proportion of studied items identified as studied or rearranged minus new item false alarm rates) was lower for the older relative to the younger group, while recognition for the middle-aged group did not differ from either the young or older groups. Whole brain analysis identified 7 small clusters (bilaterally in the supramarginal gyrus, caudate nucleus and precuneus, and the left superior frontal gyrus) where familiarity or novelty effects were larger for the younger group relative to the two older groups. These age-related differences were overshadowed however by the identification of an extensive number of canonical familiarity- sensitive regions that were age-invariant. Some of these canonical regions, such as the intra-parietal sulcus and caudate nucleus, also showed age-invariant positive correlations with recognition performance. Novelty effects in bilateral hippocampus covaried reliably across participants with recognition memory, and also with an index of recollection. The latter findings indicate that fMRI hippocampal novelty effects reflect a generic memory signal.

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Poster

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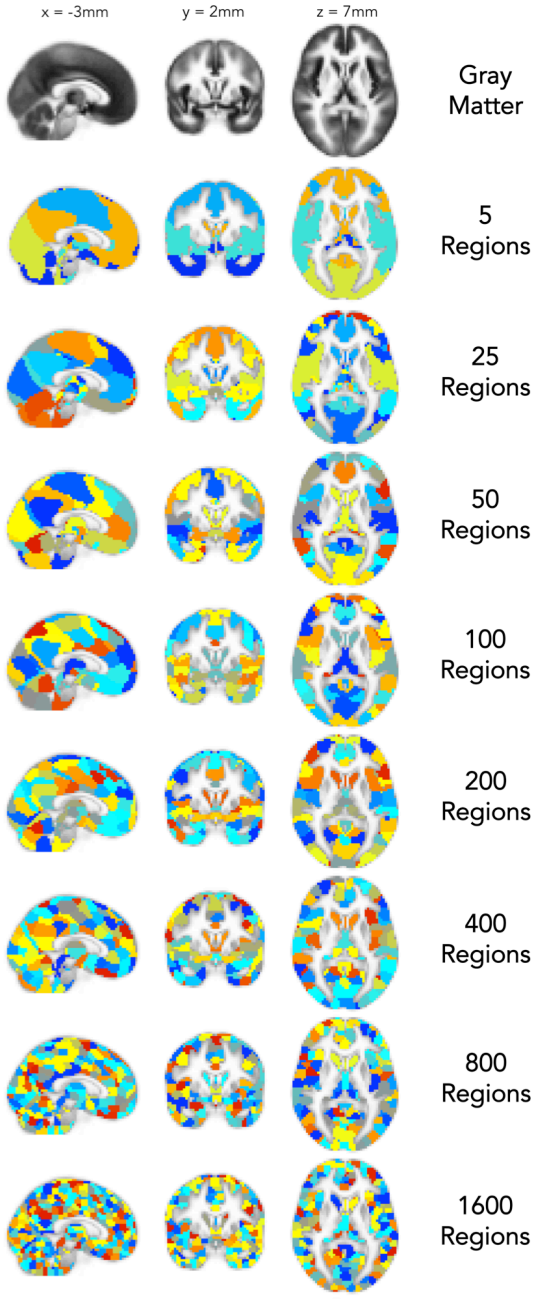
UAB Comprehensive Center for Healthy Aging

Title: Training the aging brain: a multi-level network analysis

Authors: *J. M. HICKS[1], R. NENERT[1], L. A. ROSS[2], K. M. VISSCHER[1];
[1]Univ. of Alabama At Birmingham, Birmingham, AL; [2]The Pennsylvania State Univ., University Park, PA

Abstract: One strategy shown to be effective in improving cognitive performance of older adults is known as Speed of Processing (SOP) training. SOP training shows reliable effects that lead to improvements in performance on tasks similar to the training paradigm as well as important life activities. While SOP training yields reproducible behavioral effects, the neural mechanisms underlying these effects are largely unknown. We aim to determine how SOP training affects the resting-state brain networks of older adults. Yet because the brain is a vastly complex structure with many interconnected units, understanding the organization of these connections and how they change through training is a challenging endeavor. A further challenge is how to accurately define these interconnected units of the brain. Current analytical methods require the experimenter to make assumptions about how the brain is subdivided into parts within a network, but clearly, these assumptions can influence the outcomes of analyses. Thus, we use resting-state functional connectivity data along with a k-means clustering algorithm to create parcellations of cortical and subcortical gray matter across multiple spatial scales. The resulting parcellations demonstrate validity as they identify previously defined brain regions, group regions that are known to be connected, and exhibit a high degree of bilateral symmetry. We found a significant correlation between the degree of behavioral improvement with training and a change in global

network characteristics, suggesting SOP training may improve cognitive performance by altering network structure. Further, the relationship to behavior exists primarily at a limited set of spatial scales, suggesting that behaviorally relevant network changes occur at a favored range of spatial scales. This work shows that the spatial scale of parcellations influences the sensitivity of analyses of network structure and that improvements in speed of processing may be a result of subtle changes in network structure.



Disclosures: **J.M. Hicks:** None. **R. Nenert:** None. **L.A. Ross:** None. **K.M. Visscher:** None.

Poster

086. Human Cognition: Aging I

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01 AG015019

PHS grant T32 AG000175

Title: Cortisol and limbic system volumetrics across the adult lifespan

Authors: *S. D. MOFFAT[1], G. ENNIS[1], E.-M. QUINTIN[2], U. SAELZLER[1], C. HERTZOG[1]; [1]Psychology, Georgia Inst. of Technol., Atlanta, GA; [2]McGill Univ., Montreal, QC, Canada

Abstract: The hippocampus, anterior cingulate cortex, and amygdala, regions within the limbic system, are rich in glucocorticoid receptors and play an important role in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis. Research suggests that chronic elevations of cortisol may have a deleterious influence upon the structure of some limbic regions, especially during older adulthood when neuroplasticity is reduced. For example, increased exposure to cortisol has been related to smaller hippocampal volume in older adults. However, few studies have examined the relationship of cortisol to extra-hippocampal limbic structures across the adult lifespan. We investigated whether the relationship between cortisol and specific regions of interest (ROI) within the limbic system would be moderated by age. Specifically, we assessed diurnal cortisol output (area under the curve or AUC) and diurnal cortisol slope and related these measures to hippocampal and amygdalar volume and caudal and rostral anterior cingulate cortical thickness. Participants (M_{age} = 53.1 years, SD = 18.04, range = 23 - 83, N = 51) collected 7 salivary cortisol samples on 10 consecutive days: upon waking, 30 minutes later, and then approximately every three hours until 9 PM. Magnetic resonance imaging (MRI) was performed 2.4 years later on a 3T Siemens scanner with an MPRAGE pulse sequence. MRI data were processed with Freesurfer. Hippocampal and amygdalar volumes were adjusted for intracranial volume. There were significant interactions between age and cortisol on left and right caudal anterior cingulate thickness and left and right amygdalar volumes. In older but not younger adults, a flatter diurnal cortisol slope was related to a thinner right caudal anterior cingulate cortex and higher diurnal cortisol output was related to smaller left and right amygdalar volumes. These results will be discussed in the context of allostatic load and its influence upon neural structure in older adulthood.

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Poster

086. Human Cognition: Aging I

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant RO1 AG019714

NIH Grant T32 GM084907

Title: Investigating the interaction of expectation and entropy in speech comprehension by young and older adults using eye-tracking

Authors: N. D. AYASSE, *A. WINGFIELD;
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Abstract: Although older adults are known to be able to use context effectively to understand spoken language, not all situations are as straightforward as they are in the laboratory; at times, the context may fit multiple semantic competitors, and choosing the correct one can be crucial for correct comprehension. Given the inhibitory control deficit and working memory reduction common in aging, it is critical to understand how older adults can achieve comprehension. An experiment is reported to explore the interaction of constraining contextual cues (expectancy) and the degree of competition (response entropy) using sentences with either high or low expectancy for a sentence-final (target) word and with either high or low response entropy. These target words were then paired with either a high or low contextual competitor in a variation on a visual world eye-tracking paradigm. Participants heard recorded sentences while being presented visually with two potential target words on a screen. Participants were instructed to move a cursor over the target word as quickly as possible as the sentence unfolded. As this task is being performed, their eye movements to the referents on the screen were recorded. Results support the expectation that lower expectancy and higher entropy slow comprehension, and that expectancy and entropy interact such that participants are differentially slower to comprehend a sentence with low expectancy and high entropy. Results will be discussed in terms of adult aging and individual difference effects. Work supported by NIH Grants RO1 AG 019714 and T32 GM 084907

Disclosures: N.D. Ayasse: None. **A. Wingfield:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: BMBF EMOTISK FKZ 16SV7243

Title: Inferring the intentions of others across the lifespan

Authors: ***A. REITER**[1], A. DIACONESCU[3], B. EPPINGER[2], S.-C. LI[2];
[1]Lifespan Developmental Neurosci., [2]TU Dresden, Dresden, Germany; [3]ETH Zürich, Zürich, Switzerland

Abstract: Inferring others' mental states is a core function of social cognition (Frith & Frith, 2005, *Curr Biol*). What are the other's intentions - can I trust him or her? The appropriate interpretation of others' behavior as well as adapting our own behavior flexibly to it is key for human interactions. Emerging findings indicate that these mechanisms change over the course of the lifespan (e.g., Bailey et al., 2015, *Cogn Emot*). However, to date, the underlying computational and neurophysiological mechanisms are poorly understood. To this end, we applied a social learning paradigm (cf. Behrens et al., 2008, *Nature*; Diaconescu et al., 2014, *PLOS CB*) in younger (20-30 years) and older (60-80 years) adults . Using hierarchical Bayesian learning models, we investigate social learning in an uncertain social environment, i.e., when the intentions of a young vs. older adviser change dynamically over the course of a probabilistic reinforcement learning task. Comparing the predictions of different logistic models in accounting for the participants' choices, we find that both age groups integrate social and statistical information to guide decision-making; furthermore, social information is particularly weighted in trials where the statistical information is ambiguous ($\Delta BIC \geq 52$). Both age groups use social information less when advice is unreliable ($t=3.72$, $p<.001$). That is, they adapt their behavior flexibly to changing social conditions. However, difference scores for social and statistical information were found to differ on a trend level between age groups ($t=1.81$, $p=.07$), indicating that the elderly may be less sensitive to statistical information, whereas they value social advice similarly as younger adults. By means of computational modeling in an age comparative design, we demonstrate that a Bayesian hierarchical generative model can describe the observed behavior of both age groups Based on this computational model, we will further explore the effects of aging on dealing with uncertainties in social information. The applied paradigm in combination with computational modeling of inter-individual differences in social learning appears as a promising step towards mechanistic accounts of lifespan differences in socio-emotional processes.

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Poster

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Topic: H.02. Human Cognition and Behavior

Title: Reduced white matter integrity and aberrant increases of cerebral blood flow in the episodic memory network in cognitively normal older adults with β-amyloid pathology

Authors: *H. OH[1], A. E. LEVITANUS[2];
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Abstract: While approximately 30% of cognitively normal older adults present with beta-amyloid (Aβ) deposition, a pathological hallmark of Alzheimer's disease, the understanding of early neural markers that are associated with Aβ pathology is largely limited. Previous studies using functional magnetic resonance imaging have shown Aβ-related hyperactivation in brain regions highly implicated in episodic memory. To better understand the impact of Aβ deposition on the absolute level of functional and structural correlates in the episodic memory network, we examined whether and how Aβ deposition relates to cerebral blood flow (CBF) and white matter structure integrity (WMI) among cognitively normal older adults. We assessed cerebral blood flow and white matter integrity that were measured by arterial spin labeling and diffusion tensor imaging (DTI), respectively, in cognitively normal older adults who participated in the Alzheimer's Disease Neuroimaging Initiative (ADNI). A level of Aβ deposition was quantified by 18F-Florbetapir positron emission tomography (PET). Forty and 47 cognitively normal older adults (mean age = 72.8, 25 females) were included for CBF and DTI results, respectively, and classified into either Aβ+ or Aβ− according to the criteria set by the ADNI processing pipeline. Effects of dichotomized and continuous Aβ measures on CBF and WMI in a priori regions of interest were assessed using analyses of covariance and multiple regression models. For behavioral measures, baseline scores and slopes representing longitudinal changes in the 11-item ADAS-cog and RAVLT immediate recall and percent forgetting were assessed and related to biomarker measures. For CBF, higher Aβ deposition was associated with lower CBF in entorhinal cortex but increased CBF in posterior cingulate cortex. A higher level of CBF in posterior cingulate cortex was further associated with greater longitudinal decline in RAVLT among Aβ+ older adults. For WMI, higher Aβ deposition was associated with significant reduction in integrity in most association fibers including superior longitudinal fasciculus, inferior fronto-occipital fasciculus, cingulum, and body of corpus callosum. The present results suggest that, despite cognitively normal functioning, Aβ-related differences in cerebral blood flow and white matter tract integrity are present in the episodic memory network in cognitively normal older adults. These functional and structural differences associated with Aβ pathology

would be important to be accounted for in cognitive aging research, while serving as an early biomarker for pathological brain aging associated with Alzheimer's disease.

Disclosures: H. Oh: None. **A.E. Levitanus:** None.

Poster

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Support: Marga and Walter Boll Foundation

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Title: Reduced acetylcholinesterase activity is associated with impaired deactivation of the precuneus and the temporoparietal junction during episodic memory encoding in prodromal Alzheimer's disease

Authors: *J. KUKOLJA[1], N. RICHTER[1], Ö. A. ONUR[1], L. KRACHT[3], M. TITTGEMEYER[3], B. NEUMAIER[4], M. SCHMIDT[2], M. DIETLEIN[2], G. R. FINK[1];
[2]Dept. of Nuclear Med., [1]Univ. Hosp. Cologne, Cologne, Germany; [3]Max-Planck-Institute for Metabolism Res., Cologne, Germany; [4]Inst. for Nuclear Chemistry, Inst. for Neurosci. and Med., Res. Ctr. Jülich, Jülich, Germany

Abstract: *Background*: The cardinal symptom of Alzheimer's disease (AD), progressive loss of episodic memory impairment, is attributed to extensive neuronal degeneration, especially of cholinergic populations. Cholinergic transmission enhances encoding of new information into long-term memory. However, this enhancement requires simultaneous suppression of off-line consolidation of previously encoded content. We hypothesized that both processes are impaired in patients with mild cognitive impairment (MCI) due to AD. We tested whether a local cholinergic deficit underlies a disequilibrium of activations and deactivations during memory encoding.
Methods: Cerebral acetylcholinesterase (AChE) activity, a surrogate marker for the integrity of cholinergic networks, can be measured *in vivo* using positron emission tomography (PET) with the tracer [^{11}C]N-methyl-4-piperidyl acetate (MP4A). In the present study we used MP4A-PET and fMRI to relate neuronal activity to AChE activity. fMRI was measured during encoding of visual items in 20 healthy elderly subjects and 20 patients with amnestic MCI. The subsequent

memory effect was evaluated by contrasting items which were subsequently remembered against those which were forgotten.
Results: Across groups, a typical deactivation of the precuneus, posterior cingulate, angular and supramarginal gyri was observed during encoding of subsequently remembered items. In the right supramarginal and angular gyri this deactivation was significantly greater in the control group than in patients. Within this area, AChE activity was significantly decreased in patients than in controls. Across groups, a significant positive subsequent memory effect was observed in the anterior hippocampus. In the right fusiform gyrus controls showed greater activation than patients.
Conclusion: In an approach combining cholinergic PET and fMRI, we show that a local cholinergic deficit was associated with an impaired suppression of regions which are typically deactivated during long term memory encoding. We conclude that the cholinergic system is crucially involved in redistributing neural network resources in active tasks, but that this process is disturbed in MCI due to AD. The failure to suppress regions which are usually active during rest and are thought to be involved in off-line processes like memory consolidation may contribute to deficits in encoding of new information. These results indicate that cholinergic therapy may be beneficial in patients showing a relevant cholinergic deficit even at earlier disease stages than manifest AD.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant AG029523

NIH Grant AG047972

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Title: Changes in functional connectivity across the lifespan reflect declines in cognitive efficiency

Authors: *M. P. TURNER, N. A. HUBBARD, L. M. HIMES, M. A. MOTES, B. RYPMA; Sch. of Behavioral and Brain Sci., Univ. of Texas at Dallas, Richardson, TX

Abstract: Background: Functional connectivity (FC) is a measure of the extent to which brain regions undergo activity synchronously in time and form a task-relevant network. Models of cognitive efficiency suggest that optimal task performance results from simultaneously maximizing the speed of cognitive processes and minimizing expenditure of cognitive resources. Previous studies have shown that structural connectivity in the brain changes with advancing age. In this study, we used functional magnetic resonance imaging during performance of a processing speed task to determine how FC differs across multiple healthy groups of different ages (younger, middle-ages, and older), as well as groups with compromised white-matter (Multiple Sclerosis; MS). Method: Five groups of participants, 11 healthy younger adults, 11 healthy middle-aged adults, 19 healthy older adults, 15 middle-aged MS patients, and 12 older MS patients (total N = 70) were scanned during performance of an fMRI-adapted version of the Digit Symbol Substitution task (DSST). To measure FC, dorsolateral prefrontal cortex (DLPFC), known to be heavily involved in processing speed tasks, was chosen as a seed region. FC was assessed for each participant and group via comparison of voxels in the brain with those in this DLPFC seed region. Results: Each participant's FC was plotted as a function of DSST reaction time. A quadratic polynomial regression was significant ($p < 0.0001$), and demonstrated an inverted-U shaped relationship between degree of FC and behavioral performance. Conclusions: The changing relationship between FC and performance across the lifespan can be explained in the context of cognitive efficiency models. When processing paths are more direct, neural activity is reduced and processing speed is faster. When the integrity of direct processing links between nodes is reduced, more indirect links must be traversed, so neural activity is increased and processing speed is slower. Younger adults who perform faster utilize more direct (i.e., efficient) connections, than those who perform slower. These individuals use less direct connections, increasing both reaction time and DLPFC activity. In older adults, however, connections between cortical regions are known to degrade. Faster performing older adults may be able to make use of intact indirect connections, while those with slower performance have fewer intact connections, limiting performance and DLPFC activity.

Disclosures: M.P. Turner: None. **N.A. Hubbard:** None. **L.M. Himes:** None. **M.A. Motes:** None. **B. Rypma:** None.

Poster

086. Human Cognition: Aging I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 86.19/HHH33

Topic: H.02. Human Cognition and Behavior

Support: AG19731

Title: Neuromodulatory effects on memory networks in aging are topographically selective and modulated by structural network density

Authors: *S. W. DAVIS[1], D. MURPHY[2], B. LUBER[3], S. H. LISANBY[4], R. CABEZA[2]; [2]Ctr. for Cognitive Neurosci., [1]Duke Univ., Durham, NC; [3]Exptl. Therapeut. & Pathophysiology, [4]Div. of Translational Res., NIH, Bethesda, MD

Abstract: Functional neuroimaging studies have shown that, compared to younger adults, older adults show a more distributed activation pattern, perhaps as a consequence or a benefit of changes in structural morphology. It has been suggested that the distributed activation in older adults reflects compensation for reduced neural resources, but this hypothesis is difficult to test using only imaging data, which is only correlational. To address this issue, we manipulated the causal relationship between neural resources and distributed activity in older adults using transcranial magnetic stimulation (TMS). We defined local and distributed connectivity using graph techniques: local connectivity measured within modules, and distributed connectivity measured between modules. Given that functional connectivity depends on structural connectivity, we expected that success-related connectivity would be constrained by white matter structure. Thus, we predicted that (1) while 5Hz TMS would boost local activity and increase within-module connectivity, 1Hz TMS would depress local activity and increase more long-distance between-module connectivity, and that (2) that this effect would be constrained by white-matter connectivity, such that the 5Hz-related increase in connectivity would be related to 1st-order connections, while 1Hz-related increases in between-module connections were related to indirect structural connectivity between more distant regions. Both predictions were confirmed, providing support for the compensatory interpretation of distributed activity in older adults and demonstrating the power of TMS in clarifying the interpretations of activation patterns observed in aging populations. These findings provide an integrated, causal explanation for changes in networks interactions associated with successful cognition in older adults.

Disclosures: S.W. Davis: None. **D. Murphy:** None. **B. Luber:** None. **S.H. Lisanby:** None. **R. Cabeza:** None.

Poster

086. Human Cognition: Aging I

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01AG047972

National Multiple Sclerosis Society RG4453A1/2

Think Ahead Group 2015 Research Award

Title: Age-related white matter micro- and macro-structural changes associated with functional connectivity decline in resting state network

Authors: L. HIMES[1], N. A. HUBBARD[1], M. TURNER[1], *B. P. RYPMA[1,2];
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Abstract: Healthy aging is associated with neural changes in both structure and function yet the relationships between these changes are not fully understood. One manifestation of age-related structural changes is a decrease in white matter integrity measured by fractional anisotropy (FA). Healthy aging is also associated with an increase in white matter lesions. One manifestation of age-related functional changes is a decrease in resting state functional connectivity. We investigated whether white matter integrity affected resting state networks across a wide-range age sample. We hypothesized that individuals with lower FA (older adults) would have less functionally connected resting-state networks compared to individuals with higher FA (younger adults). We also investigated to what extent white matter lesions are related to FA.
White matter integrity (FA) was estimated by diffusion tensor imaging (DTI). White matter lesion burden (LB) was estimated using T2-FLAIR magnetic resonance imaging. Twenty-two ($N = 22$) participants ages 21-66 ($M = 42.68$ [$SD = 12.26$]; 17 female) were selected. Resting state connectivity was measured using global brain connectivity (GBC), a measure of functional connectivity of each voxel within the resting network.
GBC decreased with age in nodes within multiple resting networks (all regions $p < .05$, $k > 11$), and LB significantly predicted GBC decreases in these regions (all regions $p < .05$, $k > 11$). FA was a significant predictor of age-related GBC decrease (all ps $< .05$). Further, LB increased with age ($r = .48$, $p < .05$) and was significantly predicted by FA ($r = .46$, $p < .05$).
Age-related FA decreases are associated with disconnection of multiple networks at rest. These findings may reflect a decrease in the overall cohesiveness of resting networks in aging due to white matter micro- and macrostructure damage. Functional decline of resting state networks and micro- and macro-structural decline might differentially affect cognitive changes in healthy aging.

Disclosures: **L. Himes:** None. **N.A. Hubbard:** None. **M. Turner:** None. **B.P. Rypma:** None.

Poster

086. Human Cognition: Aging I

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Topic: H.02. Human Cognition and Behavior

Support: CIHR Operating Grant MOP115011

CIHR Doctoral Award CGM137440

Title: Changes in functional organization of the human brain with age

Authors: *S. HRYBOUSKI[1], F. OLSEN[2], J. MCGONIGLE[3], R. CARTER[2], P. SERES[2], N. MALYKHIN[2];
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Abstract: Introduction: The predominant theory of brain aging suggests that association cortices are most affected by the aging process, while primary visual, somatosensory, motor and auditory systems of the brain are not. Independent Component Analalysis (ICA) can be used to detect age-related changes in brain network dynamics. In the current study we tested the hypothesis that functional networks, which are localized to frontal, temporal, and parietal association cortices, show reduction in functional connectivity with age, while primary sensory and motor networks remain preserved in healthy cognitive aging. **Methods:** A total of 140 healthy volunteers (18-88 years old) were recruited for this study. Participants were excluded if they had unstable medical illness, history of psychiatric or neurological disorders and the use of medications that might affect brain structure or function. Older individuals with Mild Cognitive Impairment (MCI) and dementia were also exlcuded from the study. T2*-weighted images were acquired on a 1.5T Siemens Sonata system. SPM12, FSL 5.0, and ANTS software packages were used to preprocess the functional data. Network analyses were performed using GIFT (v4.0) software. The number of components for the spatial GroupICA analysis was assessed using ICASSO bootsrapping procedure (500 iterations) and Group-Information-Guided ICA was used to estimate resting-state networks in each volunteer. We evaluated age-related changes functional connectivity by (1) examining independent component (IC) spatial maps to study intra-network properties; (2) comparing network signal using multitaper approach; (3) investigating age-related changes in between-network connectivity. **Results:** Based on previous literature we identified visual, sensorimotor, default-mode, auditory, ventral attention, and frontal-parietal networks. Results showed general reduction in intra-network connectivity with age not limited to association cortices only. Spectrum analysis showed that fMRI signal from older individuals shows reduced low-frequency (0.01-0.10 Hz) power. Similar to reduction in intra-network communication, analysis of between-network connectivity revealed broad reductions in cross-

network communication with age. **Conclusions:** Our findings demonstrate broad changes in within- and between-network functional connectivity in healthy aging, which are not restricted to the association cortices.

Disclosures: S. Hrybouski: None. **F. Olsen:** None. **J. McGonigle:** None. **R. Carter:** None. **P. Seres:** None. **N. Malykhin:** None.

Poster

086. Human Cognition: Aging I

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

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Topic: H.02. Human Cognition and Behavior

Support: University of Michigan Geriatric Center

Title: GABA levels in occipital cortex decline with age and correlate with fluid processing ability

Authors: *T. A. POLK[1], J. M. CARP[2], B. R. FOERSTER[3], L. OSSHER[5], M. PETROU[3], M. SIMMONITE[4], D. H. WEISSMAN[4];
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Abstract: Work in non-human primates has shown that the selectivity of neural receptive fields in visual cortex declines with age (*Nat. Neurosci.* **3:**382) and that administering gamma-aminobutyric acid (GABA), the brain's primary inhibitor neurotransmitter, reverses such age-related declines in neural selectivity (*Science* **300**:812). Furthermore, reduced neural selectivity is associated with reduced fluid processing ability in older humans (*Jnl Neurosci* **30**:9253), suggesting that age-related declines in GABA might contribute to age-related declines in cognition. Here, using magnetic resonance spectroscopy at 3T, we aim to explore whether GABA levels in visual cortex decline with age in human beings and whether individual differences in GABA are related to fluid processing ability. We used a MEGA-PRESS sequence with frequency spectral editing to distinguish GABA from other metabolites (*NMR Biomed* **11**:266). Spectra were obtained from a 3 x 3 x 2.5 cm voxel placed in the occipital cortex of 17 young adults (mean age 20.7 years) and 18 older adults (mean age 76.2 years). Participants also performed 11 fluid processing tasks outside the scanner. Occipital GABA levels were significantly reduced in the older subjects compared to the younger subjects. Furthermore, higher GABA levels correlated with better performance in 10 out of the 11 behavioral tasks. We also

performed a principal component analysis on the behavioral data and regressed the first principal component against GABA levels. We found that higher GABA levels were significantly associated with better performance across tasks as assessed by this summary measure. These results indicate that age-related declines in GABA may contribute to age-related declines in fluid processing ability.

Disclosures: **T.A. Polk:** None. **J.M. Carp:** None. **B.R. Foerster:** None. **L. Ossher:** None. **M. Petrou:** None. **M. Simmonite:** None. **D.H. Weissman:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: FIS/IMSS/PROT/G15/1458

Title: Differences in execution efficiency between young and elderly healthy adults during an incidental/ intentional learning/memory visuospatial task

Authors: M. JUNCO-MUÑÓZ[1], O. MEJÍA-RODRÍGUEZ[2], J. CERVANTES-ALFARO[4], *M. OLVERA-CORTES[3];
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Abstract: The development of explicit access to the implicit learned information called incidental learning, is an important learning mechanism and along with visuospatial information highly vulnerable to aging. The effect of aging on an incidental (INC)/intentional (INT)-learning/memory tests for visuospatial location information was assessed in twenty four right handed participants grouped by age in a young adult (n=14, 25-45 years old, 5 female) and an elderly adult group (n=10, 65-85 years old, 7 female). All participants were screened with the RAVEN, BDI and BAI inventories to be included. The study was approved by the Research and Ethics Committee of the IMSS. A virtual maze which included 7 common objects at specific placements was presented on a PC to each participant; then, these 7 objects and 7 new objects were presented to them and the subjects responded, if the object was in the maze and where (INC1-remember). Following, the objects in the maze were intentionally studied by the participants (INT-test). The 7 objects presented in the maze, the 7 objects presented in the INC1

test and 7 new objects were presented to the participants (INC2). They had to remember if the object was in the maze and indicate where, also, if the object was seen in the INC1 test or was new (INC2). The total number of remembered correct objects (RCO-INC1&INC2/RCO-INT) and correctly remembered positions (RCP-INC1&INC2/RCP-INT) (positions marked with the exact location of the objects' figures in the maze) were measured. Recognition errors were classified as false-negative or false positive, position errors (index error) and inversions (incorrect object in a correct position). The data were compared using the Mann-Whitney U test and correlations (Pearson) between behavioural variables and age, years of study and CI were made. Statistical significance was considered at $p < 0.05$. The young adult group had a higher number of total RCO and RCP in INC1&INC2 and INT test than the elder adult group. The elder adult group had more false-negative errors in the INC tests and both more false-positive and negative errors in the INT test. A negative correlation between years of study and RCO/RCP in the INT was observed, but not correlation existed in the INC test. A negative correlation between the number of correctly remembered positions and age as well as a positive correlation between the error and age were observed for the INT test. Elderly adults showed mayor number of position errors in INC1, whereas in the INT showed only object errors with increased false positive errors. The differential accuracy under INC/INT learning conditions suggest a difference in the underlying retrieval processes used to perform the tasks.

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Poster

086. Human Cognition: Aging I

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 86.24/HHH38

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant K01AG031301

Title: Prefrontal gray matter volume mediates age effects on self-initiated encoding strategies

Authors: R. A. HUSA[1], B. A. GORDON[2], *B. A. KIRCHHOFF[1];
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Abstract: Aging is associated with decreased self-initiated use of effective elaborative encoding strategies. Currently, relatively little is known regarding what factors drive age differences in self-initiated use of encoding strategies. Prior research has shown that prefrontal cortex plays an

important role in supporting complex self-initiated strategic processes. The goal of the present research is to investigate whether declines in prefrontal regional gray matter volume with age make a significant contribution to age differences in self-initiated use of elaborative encoding strategies. The relationships among age, prefrontal regional gray matter volumes, and self-initiated use of encoding strategies were in examined in 39 healthy younger adults (age range 18-35) and 38 healthy older adults (age range 65-80). Freesurfer was used to measure gray matter volume in 12 prefrontal regions of interest. Self-initiated encoding strategy use was assessed by asking participants to give retrospective item-by-item strategy reports after performing a verbal intentional encoding task. Participants reported the frequency with which they used rote repetition, visual imagery, sentence generation, and/or personal relevance strategies as well as no strategy to encode individual words. A composite elaborative encoding strategy score was calculated for each participant by determining the frequency with which he/she used any elaborative encoding strategy (visual imagery, sentence generation, and/or personal relevance) to encode the studied words. Preliminary results from this study revealed that age was negatively correlated with the composite measure of self-initiated use of elaborative encoding strategies and self-initiated use of the personal relevance strategy. Importantly, left caudal middle frontal gray matter volume mediated the effect of age on self-initiated use of elaborative encoding strategies. In addition, right caudal middle & bilateral lateral orbital frontal gray matter volumes mediated the effects of age on self-initiated use of the personal relevance encoding strategy. These results suggest that age differences in prefrontal regional gray matter volume are a significant contributor to age differences in self-initiated use of effective elaborative encoding strategies.

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Poster

086. Human Cognition: Aging I

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Topic: H.02. Human Cognition and Behavior

Support: Research Grant Council (Hong Kong HKU-17613815)

Chow Tai Fook Charity Foundation

Henderson Warmth Foundation

Title: Moderation effect of serum cortisol on neurocognitive association in non-demented elderly subjects

Authors: *A. C. LAW[1,2], W. K. W. LAU[3], M.-K. LEUNG[4], T. M. C. LEE[4];
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Abstract: Dysregulation of the hypothalamus pituitary adrenal (HPA) axis accompanied by elevated basal cortisol levels are often associated with poorer cognitive functioning during aging. The current study aimed to investigate the mechanistic relationships between serum cortisol levels, regional brain volumes, and cognitive processing speed in a group of non-demented elderly people. Forty-one male and female healthy elderly participants were included. Blood samplings were done in the late morning to minimize the diurnal effect of cortisol. Serum cortisol levels were measured using commercially available enzyme-linked immunosorbent assay (ELISA). Regional gray / white matter volumes (GMV/WMV) were obtained by whole-brain anatomical scanning. Cognitive processing speeds were assessed by digit-symbol and symbol search test, from the Chinese version of the Wechsler Adult Intelligence Scale - third edition (WAIS-III). Mediation and moderation analyses were performed to study the mechanistic relationship among serum cortisol levels, regional brain volumes, and processing speeds. Whole-brain regression analysis revealed that serum cortisol levels positively predicted the WMV of the right thalamus, the GMV of the left thalamus and right cerebellar tonsil, and negatively predicted the WMV and GMV of the left middle temporal gyrus (MTG). Furthermore, significant moderation effects of serum cortisol were observed on the relationship between GMV of the left MTG and processing speed, as well as GMV of the left thalamus and processing speed. This study provided the first piece of evidence supporting the moderating effects of serum cortisol levels on the relationship between regional brain volumes and processing speeds in the healthy elderly subjects. This observation enriches our understanding of the role of cortisol in brain morphology and cognitive functioning in older individuals.

Disclosures: **A.C. Law:** None. **W.K.W. Lau:** None. **M. Leung:** None. **T.M.C. Lee:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: RO1 AG019714 from NIA

T32 AG000204 from NIH

Title: Influence of age, hearing acuity and speech prosody on processing effort revealed through pupil dilation.

Authors: *N. M. AMICHETTI[1], E. ATAGI, 02454[2], Z. BROWN[2], A. WINGFIELD[2];
[1]Neurosci., Brandeis Univ., Waltham, MA; [2]Brandeis Univ., waltham, MA

Abstract: Speech prosody refers to the pitch contour, word stress, pauses and final vowel lengthening of words immediately prior to clause boundaries. It is important for sentence comprehension as it signals to the listener the location of syntactic boundaries. As the prosodic pattern of speech normally coincides with the syntactic boundaries, this provides an advantage as it reduces processing effort. To examine the effects of prosody on sentence comprehension and effort, we utilized sentences in which the prosodic pattern was placed in conflict with the syntactic structure -thereby disrupting the processing advantage normally provided by prosody. To investigate the effects of age and reduced hearing acuity, normal-hearing young adults and older adults with good-hearing and older adults with mild-to-moderate hearing loss listened to sentences with either a neutral prosodic pattern, a prosodic pattern that coincided with the syntactically defined clause boundary or with a prosodic pattern that was conflicting and were asked to recall post-listening. Pupil dilation was used as a measure of processing effort, with larger dilations reflecting larger amounts of effort, and was continuously recorded throughout the experiment. For all groups, recall performance varied by condition in a graded fashion with better performance in the congruent condition and incongruent condition producing the most errors. This pattern was observed within each group in the pupillometry traces, with the incongruent sentences eliciting the greatest amount of pupil dilation, followed by sentences heard in the neutral condition and congruent condition. Further, the size of the dilations varied across groups, with the older adults showing larger pupil excursions than the young adults, and especially so for the older adults with a hearing impairment. Of interest is when older adults show larger pupil dilations even when the resolution of syntax and prosody is successful, implying that while older adults may reach comparable levels of performance, they do so at a higher cognitive cost.

Disclosures: **N.M. Amichetti:** None. **E. Atagi:** None. **Z. Brown:** None. **A. Wingfield:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant T32 AG000175

NIH Grant UL1TR000454

Title: The relationship of glucose metabolism to cognition in non-diabetic older adults

Authors: *G. E. ENNIS[1], U. SAELZLER[1], G. UMPIERREZ[2], S. D. MOFFAT[1];
[1]Psychology, Georgia Inst. of Technol., Atlanta, GA; [2]Sch. of Med., Emory Univ., Atlanta, GA

Abstract: The prevalence of prediabetes, a condition of glucose intolerance between normal glucose control and diabetes, has increased significantly in older adults aged 65 to 74 years, from 41.3% in 1999 – 2005 to 47.9% in 2006 – 2010 (Casperson, Thomas, Beckles, & Bullard, 2015). This is concerning because prediabetes may worsen cognition and neural structure during an age period when cognition typically declines. The relationship of the three types of prediabetes – impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and combined IFG/IGT - to cognitive function in older adults is not clear. In a group of non-diabetic older adults (M_{age} = 71.1 years, *SD* = 5.2), we compared cognitive functioning in IFG, IGT, and IFG/IGT groups to a normal glucose tolerance (NGT) group. We also examined the relationship of fasting glucose and 2-hr post load glucose to cognitive impairment identified in the IFG, IGT, and/or IFG/IGT groups. Participants were assigned to NGT or prediabetes groups based on results from a 2-hour oral glucose tolerance test. Participants were tested using a battery of cognitive tests that assessed processing speed, working memory, executive functioning, and episodic memory. Our pilot sample included 5 with NGT, 5 with IFG, 5 with IGT, and 6 with IFG/IGT. There were no significant age or education differences between the groups. We found that the IGT and IFG/IGT groups performed significantly worse on episodic and working memory tasks relative to the NGT group. Fasting glucose was not significantly related to cognitive functioning; however, higher 2-hr post load glucose was significantly related to worse episodic and working memory performance. These findings suggest that glucose intolerance, even prior to the development of diabetes, may result in cognitive dysfunction in older adults. Results will be discussed in the context of potential physiological mechanisms, such as insulin resistance, that may be responsible for cognitive decline in older adults with prediabetes.

Disclosures: **G.E. Ennis:** None. **U. Saelzler:** None. **G. Umpierrez:** None. **S.D. Moffat:** None.

Poster

086. Human Cognition: Aging I

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 86.28/III2

Topic: H.02. Human Cognition and Behavior

Title: Behavioural and neural deficits in error detection related to higher age

Authors: *E. NIESSEN[1], G. R. FINK[2], H. HOFFMANN[3], P. H. WEISS[1], J. STAHL[3];
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Abstract: With increasing age, cognitive control processes steadily decline. Prior research suggests that healthy older adults have a generally intact performance monitoring system, but show specific deficits in error awareness, i.e., the ability to detect committed errors. We examined the neural processing of errors across the lifespan (73 participants; age range 20-72 years) by analysing the error (-related) negativity (Ne/ERN) and the error positivity (Pe) using an adapted version of the Go/Nogo task. At a stable overall error rate, higher age was associated with a greater proportion of undetected errors. While the Ne/ERN could be associated with the processing of errors in general, the Pe amplitude was modulated by detected errors only. Furthermore, the Pe amplitude for detected errors was significantly smaller in older adults, in contrast to the Ne/ERN amplitude which was similar across all ages. Structural path models suggest that through those age-related changes in Pe amplitude, an indirect effect on the sensitivity index d' was observed. Data show behavioural and neural deficits in error detection associated with aging.

Disclosures: E. Niessen: None. **G.R. Fink:** None. **H. Hoffmann:** None. **P.H. Weiss:** None. **J. Stahl:** None.

Poster

087. Human Long-Term Memory: Spatial and Episodic

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 87.01/III3

Topic: H.02. Human Cognition and Behavior

Support: R03NS093052

Title: Patients with hippocampal damage demonstrate impairments in spatiotemporal binding and precision but not spatial strategy

Authors: *B. KOLARIK[1], T. L. BAER[1], K. SHAHLAIE[2], G. GURKOFF[2], A. P. YONELINAS[1], A. D. EKSTROM[1];
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Abstract: Separate lines of research suggest roles for the human hippocampus in both spatial navigation and episodic memory yet reconciling these different perspectives has proven

challenging. Specifically, past studies suggest that hippocampal damage causes dense amnesia but also impairs the ability to employ an allocentric (landmark) based strategy to find hidden targets. We have previously demonstrated, in a single patient with bilateral damage to the medial temporal lobes (MTL), relatively intact coarse (allocentric) spatial memory for locations in a virtual water maze, but impairments in the precision of the spatial representation relative to controls (Kolarik et al, 2016). These findings are consistent with a novel model of hippocampal function termed the precision and binding model (PBM) (Yonelinas, 2013), which postulates roles for the hippocampus in complex, high-resolution binding as part of a larger role in both spatial navigation and episodic memory. In the current study, we extend these findings by including additional features in our experimental design such as a longer delay between acquisition and probe trials, and employing a larger cohort (N=5) of MTL patients. Participants navigated a virtual room on a desktop computer, learning the locations of 3 separate hidden targets over multiple training trials. After a filled delay of 10 minutes, participants were instructed to navigate to each of the three locations, three times each. By requiring participants to remember not only the locations, but also the order in which they were learned, we are able to test the hypothesis that MTL damage impairs spatiotemporal binding. Replicating our previous effects, we found that patients demonstrated only limited allocentric memory deficits, with a specific impairment instead in the precision of their trajectories relative to controls. Importantly, we also noted a tendency for patients, compared to controls, to go to the wrong target, indicating an error in remembering which target to visit when. To quantify this, we calculated the direction and magnitude of swap errors (e.g., correct target = 1 but patient navigates to target 2 = +1 swap error). Patients made more swap errors than controls after the delay, which differ from a perseverative error because patients did not simply visit the most recent target but rather visited the targets in the wrong order. Critically, however, there was no evidence of patients wandering randomly in the environment. Together, our findings suggest a role for the hippocampus in spatial precision and spatiotemporal binding, consistent with the Precision and Binding Model.
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Poster

087. Human Long-Term Memory: Spatial and Episodic

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01NS076856

NIH Grant R21NS087527

Title: Novel episodic memory paradigm reveals common and dissociable brain areas involved in processing spatial and temporal information

Authors: *J. S. LIEBERMAN, A. M. SCHEDLBAUER, A. D. EKSTROM;
Ctr. for Neurosci., Univ. of California Davis, Davis, CA

Abstract: A critical component of episodic memory is the spatial and temporal information associated with a given event. While prior studies of episodic "source" memory have identified neural correlates of context encoding and retrieval as a whole, these paradigms do not attempt to dissociate between spatial and temporal elements of source memory. To address this issue, participants encoded a series of objects in one of two contexts (either a spatial location or a temporal event), and then were tested on their memory of which contexts the objects were encoded in. Participants underwent whole-brain 3T fMRI scanning during both the encoding and retrieval parts of the experiment. Functional scans were acquired at 2x2x2.2 mm voxel resolution. During encoding, each trial consisted of a 6-s period in which the participant was briefly visually presented with an object and cued to imagine it in either a spatial or temporal context, followed by another 6-s period in which they rated how vividly they were able to imagine the item in context on a 1 to 4 scale (1 = "unable to imagine" and 4 = "imagined very vividly"). Retrieval trials consisted of an item recognition followed by a source recognition question, each of which lasted 6 seconds. Participants were first shown an object and asked to indicate if they had seen the object earlier in the experiment. They were then asked to decide whether they had placed the object in the spatial or temporal context, or if it was a new object. We used univariate analyses in SPM to examine differences and commonalities in which brain regions were recruited during correct retrieval of the two contexts. Preliminary results in 8 participants showed that correct retrieval recruited the hippocampus and other areas of the temporal lobe, regardless of whether the item had been encoded in the spatial or temporal context. However, spatial retrieval was associated with significantly greater activation than temporal retrieval in several locations in posterior parts of the brain, such as the occipital pole, cuneus, lingual gyrus, intracalcarine cortex, and lateral occipital cortex. Temporal retrieval showed significant activation in a wider range of locations within the frontal, parietal, and occipital lobes such as the lateral and orbital frontal cortex, superior parietal lobule, and occipital fusiform cortex, as well as subcortical regions such as the thalamus, caudate nucleus, and putamen. Our findings replicate past results from the lab suggesting dissociable components of spatial and temporal retrieval but also suggest commonalities with past source memory retrieval paradigms, providing a novel means for understanding the neural basis of episodic memory.

Disclosures: **J.S. Lieberman:** None. **A.M. Schedlbauer:** None. **A.D. Ekstrom:** None.

Poster

087. Human Long-Term Memory: Spatial and Episodic

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 87.03/III5

Topic: H.02. Human Cognition and Behavior

Support: R01NS076856

R21NS087527

Title: A graph theory approach to spatiotemporal contextual encoding and retrieval in human episodic memory

Authors: *A. SCHEDLBAUER[1], J. S. LIEBERMAN[2], A. D. EKSTROM[3];
[1]Univ. of California Davis, Davis, CA; [3]Psychology, [2]Univ. of California, Davis, Davis, CA

Abstract: Episodic memories capture events from our daily lives and often involve rich contextual details, particularly the spatial and temporal components of the experience. Encoding and retrieval of these features is thought to rely on regions within the medial temporal lobe (MTL), with current theories proposing additional important contributions from neocortical areas, such as prefrontal and parietal cortices. However, current work has yet to 1) describe the interactions between these disparate regions *and* 2) compare the overlapping and distinct global networks involved in both encoding and retrieval of contextual information. To address these issues, participants underwent functional magnetic resonance imaging (fMRI, N=8) while completing an episodic memory task in which they were instructed to mentally place a specific object in a self-generated spatial or temporal context. After each item presentation, subjects rated their success in visualizing the object in the appropriate context using a confidence judgment (1 low - 4 high). Using a multivariate rather than a univariate approach, we conducted a functional connectivity analysis using beta-time series correlations to determine the interactions between 46 regions throughout the brain. Resulting encoding and retrieval network topology was examined and quantified using graph theoretical techniques. Examining encoding during high versus low confidence and retrieval during successful versus unsuccessful trials revealed dense, large-scale increases in connectivity that typified network topology (uncorrected for differences in trial numbers). Furthermore, when comparing the changes in connectivity of specific areas (i.e. change in z-score of node degree and betweenness centrality) during spatial and temporal encoding and retrieval, regions within the MTL (hippocampus, parahippocampal gyrus) maintained relatively stable connectivity patterns. In contrast, neocortical regions (e.g. superior frontal gyrus, precuneus, lingual gyrus) significantly altered their connectivity to other regions during encoding versus retrieval. The differential connectivity patterns for spatial and temporal encoding and retrieval suggested that network signatures distinguish between different aspects of contextual memory processing. This preliminary work emphasizes the importance of modeling

task-related network dynamics to understanding the neural basis of episodic memory encoding and retrieval.

Disclosures: **A. Schedlbauer:** None. **J.S. Lieberman:** None. **A.D. Ekstrom:** None.

Poster

087. Human Long-Term Memory: Spatial and Episodic

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Topic: H.02. Human Cognition and Behavior

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R21NS087527

R03NS093052

Title: Navigation in virtual reality with vestibular and proprioceptive input diminishes orientation-dependent spatial representations

Authors: ***M. J. STARRETT**[1], J. D. STOKES[1], O. KREYLOS[2], A. D. EKSTROM[1];
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Abstract: Representations of spatial information from the environment are critical for accomplishing cognitive operations in everyday life. A ubiquitous finding in studies of human spatial navigation shows that while participants are able to accurately recall information about relative locations of objects in an environment, participants perform better when recalling information aligned to preferred orientations, such as the surrounding spatial geometry of a room. One limitation of these studies, however, is they are typically conducted in room-sized environments or employ real-world environments that are difficult to control experimentally. We developed a virtual reality (VR) interface that incorporates visual input and self-based cues to assess orientation dependence of spatial representations in a large-scale, virtual environment. Participants wore an Oculus head-mounted display and were secured in an omnidirectional treadmill. After being familiarized with eight target stores, participants were placed in a virtual city with dimensions of approximately 310x220 meters. Beginning from a unique starting location, participants navigated to each of the eight stores in a randomized order. After reaching the final store, the environment was removed from view and participants completed 28 trials of the judgement of relative directions (JRD) pointing task ("Imagine standing in store A, facing store B; point to store C") using a controller to orient an arrow toward the target direction. Critically, each trial involved participants imagining orientations that were either aligned

(orthogonal) or misaligned with the streets of the city. Participants completed six blocks of navigation and JRD. Results showed that pointing error on the JRD task did not differ for aligned and misaligned trials initially, but that this difference emerged after repeated exposure to the environment, suggesting that orientation-dependent representations did not emerge until participants had considerable familiarity with the environment. To further probe the effects of familiarity with the surrounding spatial geometry on the orientation-dependence of spatial representations, we included a condition in which participants were briefly exposed to a map of the city prior to beginning the experiment. Preliminary findings suggest that participant spatial representations were initially orientation dependent. Together, these findings suggest that orientation-dependent representations may be an artifact of testing in small-scale rooms and/or familiarity with the environment and thus do not emerge until participants have learned the surrounding spatial geometry.

Disclosures: **M.J. Starrett:** None. **J.D. Stokes:** None. **O. Kreylos:** None. **A.D. Ekstrom:** None.

Poster

087. Human Long-Term Memory: Spatial and Episodic

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Topic: H.02. Human Cognition and Behavior

Support: NIH/NINDS grant NS087527

Title: A novel, network-based approach to memory modulation

Authors: ***K. KIM**[1], A. SCHEDLBAUER[2], M. ROLLO[1], A. D. EKSTROM[2], N. TANDON[1]; [1]Univ. of Texas Houston, Houston, TX; [2]Univ. of California Davis, Davis, CA

Abstract: Direct brain stimulation via intracranial electrodes implanted for electrocorticography (ECoG) permits modulation of endogenous electrical signals with significantly greater spatial and temporal specificity than non-invasive approaches. It also provides access to deep brain structures, such as the hippocampus, that are difficult, if not impossible, to target non-invasively. Stimulation studies of memory structures, however, have produced mixed results, with some reporting improvement, some impairment, and others, no consistent changes. We hypothesize that to effectively affect cognitive function using brain stimulation, it is essential to modulate the connected set of nodes comprising a network - rather than local activity - that is involved in the function of interest. Memory retrieval recruits the medial temporal lobes (MTL) as well as parts of parietal and prefrontal cortex. In particular, coherent (i.e., phase synchronized) low-frequency

oscillations between these brain regions are critical for successful memory retrieval (Watrous et al., 2013). Here we used frequency-specific, multi-target electrical stimulation to modulate such low-frequency coherence between the memory network nodes. Using ECoG data collected while the patients performed a memory retrieval task, we first mapped frequency-specific, coherent, oscillatory activity (pairwise phase coherence, Vinck et al., 2010) between different brain regions associated with successful memory retrieval. Then we identified two nodes that a) were significantly connected in the network during successful retrieval, and b) exhibited significant coupling difference for temporal vs. spatial retrieval. In a subsequent session, electrical stimulation was applied to these target nodes while patients performed a spatiotemporal retrieval task. Theta-band frequency (4Hz) stimulation was delivered on a trial-by-trial basis for two seconds preceding the retrieval cue onset, with a fixed phase-lag (e.g., 0°, 180°) between the two target regions. Stimulation with a phase-lag (180°) impaired temporal retrieval while not affecting spatial retrieval. Our preliminary findings thus suggest that stimulating the core nodes at the appropriate phase-lag may effectively modulate the network function, and while in this case it impaired memory processes, this sets the ground work for further studies.

Disclosures: K. Kim: None. **A. Schedlbauer:** None. **M. Rollo:** None. **A.D. Ekstrom:** None. **N. Tandon:** None.

Poster

087. Human Long-Term Memory: Spatial and Episodic

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UC-Davis Faculty Senate grant

Chancellor's Fellowship

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T Chen Fong Research Excellence Scholarship

Title: Mental simulation of routes during navigation involves adaptive temporal compression

Authors: *A. E. ARNOLD[1], G. IARIA[3], A. D. EKSTROM[2];
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Abstract: Mental simulation is a hallmark feature of human cognition, allowing features from memory to be flexibly used during prospection. While past studies demonstrate the preservation of real-world features such as size and distance during mental simulation, their temporal dynamics remain unknown. Here, we compare mental simulations to navigation of routes in a large-scale spatial environment to test the hypothesis that such simulations are temporally compressed in time, a hypothesis based on findings from place cell recordings in the rodent hippocampus during pre-play/replay events. Additionally, we hypothesized that the temporal compression rate would be adaptive, changing based on the speed at which environmental features used for the simulations were originally experienced. Over three separate experimental conditions that varied movement speed (*N* for condition 1: 28, condition 2: 26, condition 3: 26), our results show that simulations occurred at 2.39x the speed it took to navigate a route, increasing in compression (3.57x) for slower movement speeds. Additionally, participant self-reports of vividness (medium movement condition: $\beta = -0.19$, $p = 0.004$) and spatial coherence (fast movement condition, $\beta = -0.21$, $p = 0.003$) of the simulations were significant predictors of simulation duration, providing an important link between subjective experiences of simulated events and how spatial representations are combined during prospection. These findings suggest that simulation of spatial events involve adaptive temporal mechanisms, mediated partly by the fidelity of memories used to generate the simulation.

Disclosures: **A.E. Arnold:** None. **G. Iaria:** None. **A.D. Ekstrom:** None.

Poster

087. Human Long-Term Memory: Spatial and Episodic

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Program#/Poster#: 87.07/III9

Topic: H.02. Human Cognition and Behavior

Support: R01NS076856

R21NS087527

R03NS093052

Title: Low-frequency oscillations in the human hippocampus during real-world and virtual navigation

Authors: *M. COPARA[1], A. D. EKSTROM[2], J. GOTMAN[3], V. D. BOHBOT[4];
[2]Psychology, [1]UC Davis, Davis, CA; [3]Montreal Neurolog. Inst., McGill Univ., Montreal, QC, Canada; [4]Dept. of Psychiatry, Douglas Inst. - McGill Univ., Verdun, QC, Canada

Abstract: Low frequency oscillations (LFO) are a hallmark of spatial navigation in the rodent hippocampus. While past studies have also identified LFOs in the human hippocampus during virtual navigation (VR), these oscillations are of lower prevalence and peak at a lower frequency than those in the rodent. To date, however, technical barriers have prevented recordings of LFOs in the human hippocampus during free ambulation and comparing with recordings in VR. We surmounted this barrier by recording intracranial hippocampal EEG from ambulating patients with intractable epilepsy while they performed both real-world and virtual navigation tasks. Specifically, patients freely ambulated in a real-world environment by searching for five hidden locations in a room; they also navigated a virtual maze on a desktop computer using a joystick. Raw traces during searching for hidden locations revealed clear evidence of low-frequency rhythmicity, with power spectral density (PSD) plots showing peaks around 7-9Hz on some electrode contacts and 1-4 Hz on other contacts, both showing significantly greater power than stop periods in the same session. Similarly, we observed increased LFOs during recall of the previously learned targets and during real-world walking, in which patients ambulated in an unconstrained manner with no specific task demands. Our findings suggest that real-world movement elicits LFOs and that these are reduced during stopping periods. During VR navigation, we again found significant levels of LFOs, although raw traces and PSDs suggested a lower peak frequency than during real-world navigation. Comparing the distribution of significant contacts during virtual navigation with real-world searching demonstrated a significant cross-over interaction, with a greater number of contacts showing increases in the delta band (1-4 Hz) during virtual than real-world searching compared with the theta/alpha band (8-12 Hz). Together, our findings help resolve an important debate regarding LFO in the human hippocampus. First, we show prominent LFOs during real-world searching, walking, and recalling locations, which were present across the low frequency spectrum (1-12 Hz). These effects were consistently higher during movement than stopping periods, implicating movement itself as a generating mechanism for these oscillations. Second, we found that virtual movement induced oscillations peaking within a lower frequency band than real-world searching. Together, these findings confirm previous theoretical proposals suggesting that virtual movement in humans may shift the frequency-wise prevalence to a lower range than real-world movement.

Disclosures: **M. Copara:** None. **A.D. Ekstrom:** None. **J. Gotman:** None. **V.D. Bohbot:** None.

Poster

087. Human Long-Term Memory: Spatial and Episodic

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Topic: H.02. Human Cognition and Behavior

Support: NIH MH104606

Title: Evidence for oscillatory coding akin to phase precession in the human hippocampus

Authors: *S. E. QASIM[1], A. J. WATROUS[1], I. FRIED[2], J. JACOBS[1];
[1]Biomed. Engin., Columbia, New York, NY; [2]Neurosurg., UCLA, Los Angeles, CA

Abstract: Theta phase precession is a well established example of temporal coding in the rodent hippocampus, whereby the oscillatory phase when a neuron spikes decreases across successive cycles of the theta rhythm. Given that hippocampal oscillations are thought to underlie behavior in other species and tasks, we sought to test for a similar temporal code between neuronal spiking and oscillations in humans. We analyzed single-neuron and local-field-potential (LFP) data recorded from surgical patients performing a virtual navigation task. During spiking episodes a significant number of cells in amygdala and hippocampus (11% and 15%, respectively) exhibited significant circular-linear correlation between “low theta” (1-4 Hz) LFP phase and spike latency. These patterns were more prominent during periods of movement rather than stillness in our virtual reality task. Though a small number of cells exhibited this relation with the phase of “high theta” (4-10 Hz), this pattern was not robust statistically in any particular region.
Our data show, for the first time we know of in humans, the existence of a relation between timing in a spiking episode and the phase of neuronal oscillations. This pattern appears to be similar to the phase precession observed in rodents. Because this effect was focused in the low theta band, it recapitulates previous findings indicating a special role for oscillations in this range in human navigation.

Disclosures: **S.E. Qasim:** None. **A.J. Watrous:** None. **I. Fried:** None. **J. Jacobs:** None.

Poster

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Topic: H.01. Animal Cognition and Behavior

Support: ONR N00014-13-1-0672

Title: Recurring spatiotemporal patterns in cortical high gamma and their behavioral correlates: evidence for human memory replay

Authors: *X. JIANG[1], I. SHAMIE[2], S. CASH[4], T. THESEN[5], E. HALGREN[3]; [1]Neurosciences, Univ. of California San Diego, La Jolla, CA; [3]Radiology, Neurosciences, and Psychiatry, [2]UCSD, La Jolla, CA; [4]Neurosurgery, Epilepsy Div., Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA; [5]Comprehensive Epilepsy Ctr., New York Univ. Sch. of Med., New York, NY

Abstract: Animal studies support the hypothesis that in slow-wave sleep, replay of waking neocortical activity under hippocampal guidance leads to memory consolidation. However, no direct electrophysiological evidence exists in humans. We analyzed 4-day long human intracranial recordings to reveal consistent spatio-temporal patterns of cortical High Gamma present during waking. In all subjects, more of the waking patterns (most of which were novel) had more matches with activity in the sleeps following the reference waking period than with activity in preceding sleeps. This predominance of matches to after-waking sleep activity was also observed for patterns that occurred in waking time periods that involve performing tasks with multisensory stimuli (e.g. watching movies), as well as for patterns that overlap with human face-containing movie frames for subjects that watched the same movie. In addition, High Gamma activity in matched patterns was coupled to elevated neocortical spindle power, slow oscillation power, and broad-band hippocampal activation. We therefore conclude that replay of large-scale cortical activation patterns during sleep may embody a predicted neural mechanism of human memory consolidation.

Disclosures: **X. Jiang:** None. **I. Shamie:** None. **S. Cash:** None. **T. Thesen:** None. **E. Halgren:** None.

Poster

087. Human Long-Term Memory: Spatial and Episodic

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant R01-NS074450

Title: Multivariate pattern analysis of human intracranial electrocorticography predicts speed during virtual navigation

Authors: *A. A. ROBBINS[1], P. HORAK[1], A. C. CONNOLLY[1], B. JOBST[1,2];
[1]neurology, Geisel Sch. of Med., Lebanon, NH; [2]Neurol., Dartmouth-Hitchcock Med. Ctr., Lebanon, NH

Abstract: Spatial navigation is often used in experimental neuroscience to investigate the mechanisms of learning and memory. These tasks allow for insight into the dynamics of the brain processed that occur in order to complete navigational tasks. In this study we recorded intracranial EEG (iEEG) from depth and cortical electrodes that were implanted in patients with intractible epilepsy. The virtual navigation task was built using the "Source Engine" (Valve™), and was designed to mimic rodent pellet chasing. In this task subjects collect 64 coins which randomly appear one at a time in a grid-like fashion within the environment. Power within six different frequency bands (delta, theta, alpha, beta, low gamma and high gamma) was used to train a linear discriminant analysis (lda) classifier to predict the speed of movement within the environment. This classification method was able to accurately predict movement speed (stopped, slow or fast) in the six subjects that were tested ($p < 0.01$). Power from electrodes within the frontal, temporal and hippocampal regions were the most prominent regions with predictive accuracy. Within those regions, the theta band was more often predictive than the beta, low gamma and high gamma bands ($p=0.0047$); but not the delta or alpha bands. These results are consistent with previous studies showing that slow frequency oscillations change with speed in virtual environments. The inclusion of frontal and temporal lobe electrodes in our analysis suggests that regions other than the hippocampus are responsive to changes in speed and may be important for sensory integration of memory processes.

Disclosures: **A.A. Robbins:** None. **P. Horak:** None. **A.C. Connolly:** None. **B. Jobst:** None.

Poster

087. Human Long-Term Memory: Spatial and Episodic

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Samsung PhD studentship

Title: An isotropic 3D spatial representation of a multi-level building in the human brain

Authors: *M. KIM, E. A. MAGUIRE;
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Abstract: The world is three-dimensional (3D) but most previous neuroscientific studies have investigated spatial memory and navigation on a horizontal two-dimensional plane, leaving much unknown about how 3D spatial information is represented in the brain. Gravity adds complexity to 3D navigation by imposing energy constraints on movement along the vertical dimension, and it has been proposed that the brain might use a planar encoding strategy, with consequently reduced sensitivity to the vertical dimension. However, a previous fMRI study we conducted revealed that vertical and horizontal place information was equally well encoded in the hippocampus (Kim et al., submitted). This previous experiment was based within a 3D lattice-like virtual environment, whereas 3D space is often compartmentalized by walls and floors. Thus, vertical and horizontal spatial information might be asymmetrically represented within multi-level buildings. We addressed this issue by investigating behavioural and fMRI responses while participants moved within a virtual reality multi-level building (Figure 1A). If 3D space is isotropically processed, the neural representation of each place should be equally distinguishable, predicting a similar degree of repetition suppression for both vertical and horizontal axes. By contrast, if vertical information is under-represented, the neural representation of two locations that only differ by floor (e.g. A1 and C1 in Figure 1A) would be more similar, and hence show a greater repetition suppression effect. Preliminary results suggest that the human hippocampus has a symmetric representation of space in this building (Figure 1B). In addition, we found that retrosplenial cortex, another structure implicated in navigation and spatial processing, contained global context information (e.g. the specific room within the building) with equal sensitivity to

vertical and horizontal axes (Figure 1B). These results suggest that the 3D world can be isotropically represented in the human brain even when space is compartmentalized.

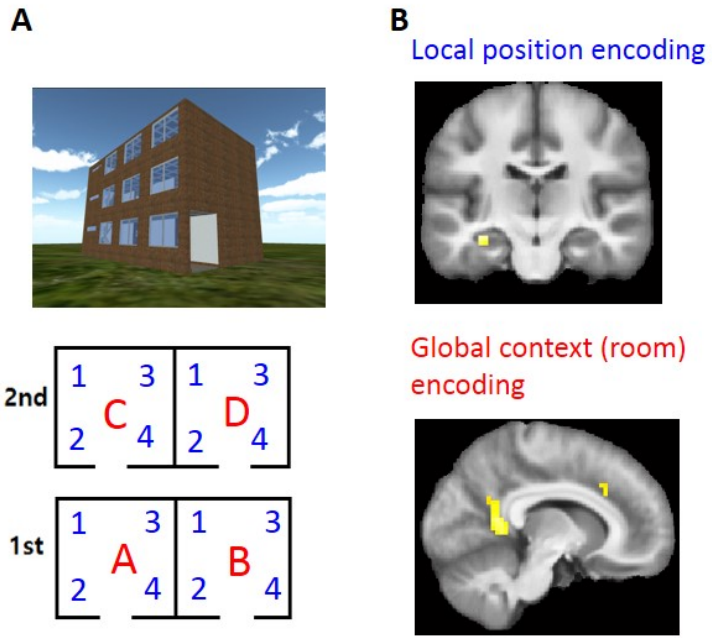

Figure 1. **A** Top panel, a view of the outside of the virtual building. Bottom panel, a schematic of the 16 locations within the building, which had two main floors. **B**, Top panel, anterior hippocampus contained significant local position information as revealed by repetition suppression effects. Bottom panel, retrosplenial cortex encoded room information.

Disclosures: **M. Kim:** None. **E.A. Maguire:** None.

Poster

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Support: Medical Research Service of the Department of Veterans Affairs

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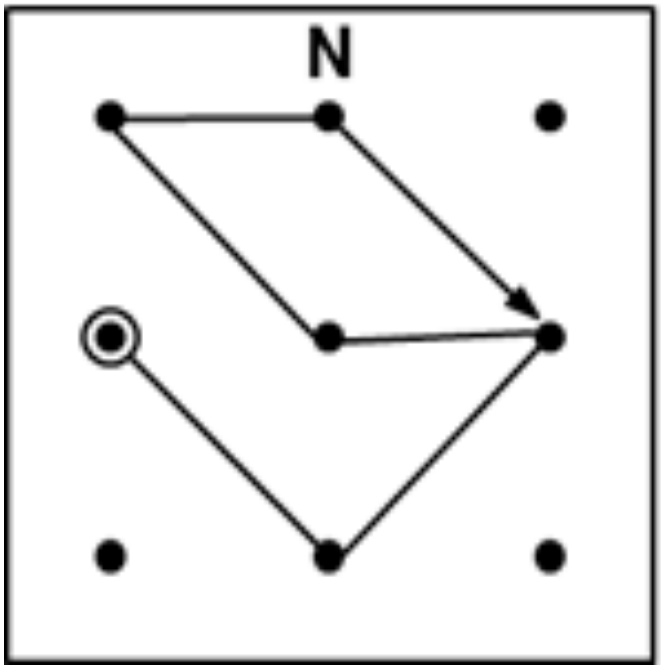
Title: The role of the human hippocampus in map reading and route following

Authors: *Z. J. URGOLITES[1,4], S. KIM[5], R. O. HOPKINS[6,7], L. R. SQUIRE[4,1,2,3];
[1]Dept. of Psychiatry, [2]Dept. of Neurosciences, [3]Dept. of Psychology, UCSD, La Jolla, CA; [4]Veterans Affairs San Diego Healthcare Syst., San Diego, CA; [5]Dept. of Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA; [6]Dept. of Psychology and Neurosci. Ctr., Brigham Young Univ., Provo, UT; [7]Dept. of Medicine, Pulmonary and Critical Care Div., Intermountain Med. Ctr., Murray, UT

Abstract: Two views have been central to discussions about the function of the hippocampus. One view grew out of work with humans and emphasizes the importance of the hippocampus for memory. By this view, the hippocampus is important for the formation of declarative (long-term) memory, not for immediate memory or for its maintenance by what is ordinarily termed working memory. The second view grew out of work with rodents and emphasizes the importance of the hippocampus for spatial navigation. The suggestion is that the hippocampus is needed to perform certain on-line spatial computations. In one sense these views are compatible because spatial memory is a type of memory. However, a puzzle arises when one turns to tasks of spatial navigation that are manageable within working memory. Do these tasks depend on the hippocampus? In the present study, participants navigated an array of dots on the floor by following paths indicated on maps (Figure 1). The paths involved 1 to 9 turns. Participants held the map in their hands in a fixed position throughout each trial. Thus, the role of long-term memory in the task was minimal. Nevertheless, participants needed continually to translate the spatial coordinates of the map into geographical coordinates, because the orientation of the hand-held maps was frequently rotated away from true north as participants made turns. Patients with lesions limited to the hippocampus (N=5) performed similarly to controls (N=13) across all path lengths ($p > 0.10$). Patients and controls also had similar and constant error rates at each turn regardless of whether a turn occurred early or late in the path ($p > 0.10$). In contrast, patients were markedly impaired at remembering factual details about the experiment ($p < 0.01$). These findings show that the hippocampus is essential for forming long-term declarative memory and that it does not support the spatial computations needed for map reading and route following when the task is manageable within working memory.

Figure 1. A sample map from the experiment. This map consists of 5 turns.



Disclosures: **Z.J. Urgolites:** None. **S. Kim:** None. **R.O. Hopkins:** None. **L.R. Squire:** None.

Poster

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Program#/Poster#: 87.13/III15

Topic: H.02. Human Cognition and Behavior

Title: Functional connectivity in the human medial temporal lobe during memory-guided spatial navigation

Authors: ***S.-F. WANG**[1], V. CARR[3], S. E. FAVILA[4], J. BAILENSON[2], A. D. WAGNER[1]; [1]Dept. of Psychology, [2]Dept. of Communication, Stanford Univ., Stanford, CA; [3]Dept. of Psychology, San Jose State Univ., San Jose, CA; [4]Dept. of Psychology, New York Univ., New York, NY

Abstract: The medial temporal lobe (MTL) plays a critical role in both spatial navigation and episodic memory. Previous studies suggest that, within the MTL, parahippocampal cortex (PHC) is important for representing spatial environments while perhinal cortex (PRC) is important for representing objects within an environment. Data in rodents indicate that neurons in hippocampal

subfields dentate gyrus (DG), CA3, and CA1 differentially respond to the global environment, locations within environments, and valence of objects. A central question is how do these MTL cortical regions and hippocampal subfields interact to support memory-guided spatial navigation. To answer this question, we combined high-resolution fMRI with a virtual reality paradigm in which subjects relied on memory to navigate through two different macroscopic environments to traverse to reward-associated locations. We applied functional connectivity (FC) analyses to determine how activity covaries across MTL cortical and hippocampal subregions while subjects navigated to learned target locations within each environment. Analysis of activity throughout the entire task (i.e., during both navigation and non-navigation periods) revealed that activity in DG/CA3 and CA1 more strongly covaried with that in PHC (DG/CA3 and PHC: $r = 0.23$, $p<0.01$; CA1 and PHC: $r = 0.27$, $p<0.01$) than with that in PRC and entorhinal cortex (ERC) (DG/CA3 and PRC: $r = 0.13$; DG/CA3 and ERC: $r = 0.12$; right-CA1 and right-PRC: $r= 0.12$; CA1 and ERC: $r = 0.14$). Within MTL cortex, PRC and ERC activity covaried ($r = 0.37$). When the same analyses were repeated for the navigation period only, correlations between hippocampal subfields and PHC were even stronger. These results suggest that, during memory-guided spatial navigation, PHC and hippocampal subfields increase their functional interactions, forming a functional network that enables successful navigation to goal-relevant locations. Future analysis will examine how FC within the MTL varies with macroscopic environments and locations and how FC between the MTL and the reward system vary with the value of the memory-guided navigational target.

Disclosures: **S. Wang:** None. **V. Carr:** None. **S.E. Favila:** None. **J. Bailenson:** None. **A.D. Wagner:** None.

Poster

087. Human Long-Term Memory: Spatial and Episodic

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Program#/Poster#: 87.14/III16

Topic: H.02. Human Cognition and Behavior

Title: The hippocampus operates in a continuous manner during spatial memory

Authors: *B. M. JEYE, J. M. KARANIAN, S. D. SLOTNICK;
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Abstract: There is a long-standing debate as to whether recollection is a threshold/all-or-none process or a continuous/graded process. In the current spatial location memory fMRI study, we examined activation profiles in the hippocampus to determine the nature of processing in this region. During encoding, participants viewed abstract shapes in the left or right visual field.

During retrieval, the same shapes were presented at fixation and participants classified each shape as previously in the “left” or “right” visual field followed by an “unsure”-“sure”-“very sure” confidence rating. The contrast of accurate versus inaccurate memory for items previously presented in the left visual field (left-hits > left-misses) produced two activations in the hippocampus ($p < 0.001$, corrected for multiple comparisons to $p < 0.05$). The analogous contrast for items previously presented in the right visual field did not produce any significant activations in the hippocampus. The corresponding activation profiles were generated by plotting the magnitude of activity as a function of memory strength for left items (left-miss-“very sure”, left-miss-“sure”, left-miss-“unsure”, left-hit-“unsure”, left-hit-“sure”, left-hit-“very sure”) and right items (right-hit-“very sure”, right-hit-“sure”, right-hit-“unsure”, right-miss-“unsure”, right-miss-“sure”, right-miss-“very sure”). The threshold model dictates that there is a threshold in the activation profile above which only high memory strength items show activation (e.g., left-hit-“very sure” activity > 0 and right-miss-“very sure” activity = 0), while the continuous model dictates that the activation profiles will be completely overlapping (i.e., all activations should be > 0). In support of the continuous model of recollection, the activation profiles were completely overlapping, as all of the activation magnitudes were significantly greater than zero. Receiver operating characteristics (ROCs, hit rates versus false alarm rates) were generated and fit with the two-high threshold model and the unequal variance continuous model, but both models adequately fit each ROC. Although an equal variance continuous model did not adequately fit a hippocampal spatial memory ROC in a previous study, the present differential activity for items in the left and right visual fields and adequate fit for the unequal variance model indicate that the equal variance model does not reflect hippocampal function. The present results demonstrate that the hippocampus operates in a continuous manner during spatial location memory and highlight the utility of analyzing activation profiles to determine the nature of neural processing.

Disclosures: **B.M. Jeye:** None. **J.M. Karanian:** None. **S.D. Slotnick:** None.

Poster

087. Human Long-Term Memory: Spatial and Episodic

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 87.15/III17

Topic: H.02. Human Cognition and Behavior

Support: Wabash College

Title: Use of hippocampally-dependent navigation strategies is associated with low self-reported stress and high trait mindfulness

Authors: D. BOWES[1], A. RAINS[1], K. KONISHI[2], L. DAHMANI[2], V. D. BOHBOT[2], *N. C. SCHMITZER-TORBERT[1];
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Abstract: Mindfulness training (mindfulness-based stress reduction, MBSR) has been found to be effective in reducing stress and improving cognitive function, and to produce changes in regional grey matter density in brain areas including the hippocampus. Most studies of MBSR's effects on cognitive function have focused on improvements in attention and working memory, but some studies have found improvements in declarative memory following MBSR training. Other work has shown that even in participants who have not completed mindfulness training, regional grey matter density in the hippocampus is positively associated with measures of dispositional (trait) mindfulness. Here, we investigated if dispositional mindfulness was associated with the use of spatial navigation strategies in a set of virtual navigation tasks. Participants (n = 111) were recruited through Amazon's Mechanical Turk service after completing the Five Facet Mindfulness Scale (FFM). Participants completed up to four automated versions of existing navigation tasks: the 4 on 8 virtual maze (Iaria, Petrides, Dagher, Pike, & Bohbot, 2003), the Concurrent Spatial Discrimination Task (CSDLT, Etchamendy, Konishi, Pike, Marighetto, & Bohbot, 2012), a wayfinding task set in a virtual town and a virtual water maze. Results from the behavioral tasks fit our expectations based on previous research (performance on the wayfinding and water maze task was strongly related, and flexible performance on the CSDLT was associated with better performance on the wayfinding and water maze task). Only the 4 on 8 task was associated with trait mindfulness, with participants who reported using hippocampus-dependent place spatial strategies also reporting higher scores on the Describe FFM subscale but not other FFM subscales. In a second study, participants completed the Calgary Symptoms of Stress Inventory (C-SOSI), and participants (n = 96) with high (C-SOSI > 50) and low (C-SOSI < 50) stress scores were recruited to complete the FFM and the 4 on 8 virtual maze. C-SOSI scores were negatively correlated with several FFM subscales, and participants reporting high levels of stress were less likely to use hippocampus-dependent spatial strategies (though, a small number of participants reporting very high scores, C-SOSI > 100, were more likely to use spatial strategies). These data provide some support for the claim that hippocampus-dependent spatial strategies may be associated with both dispositional mindfulness and self-reported stress. Programs which aim to reduce stress by increasing mindfulness (as in MBSR programs) may thus be associated with an increase in the use of hippocampally-dependent navigation strategies.

Disclosures: D. Bowes: None. **A. Rains:** None. **K. Konishi:** None. **L. Dahmani:** None. **V.D. Bohbot:** None. **N.C. Schmitzer-Torbert:** None.

Poster

087. Human Long-Term Memory: Spatial and Episodic

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Program#/Poster#: 87.16/III18

Topic: H.02. Human Cognition and Behavior

Support: CIHR MOP11149

Title: The functional role of hippocampal subregions: a high-resolution fMRI study of memory

Authors: *M. C. MACGILLIVRAY[1], S. HRYBOUSKI[2], Y. HUANG[3], C. R. MADAN[5], P. SERES[3], R. CARTER[3], N. V. MALYKHIN[4];
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Abstract: Introduction: While it is well accepted the hippocampus plays a role in memory, the role of its subregions is less well understood. The hippocampus can be divided into subregions along its longitudinal axis. Human and animal literature has suggested the posterior hippocampus is involved in spatial navigation, while the role of the anterior hippocampus is may be involved in the stress response. The main goal of the present study was to investigate the functional role of hippocampal subregions using high-resolution fMRI.
Methods: Twenty-three healthy volunteers were recruited for this study. Participants were excluded if they had unstable medical illness, history of psychiatric or neurological disorders and the use of medications that might affect brain structure. Written informed consent was obtained and the research was approved by the University of Alberta Health Research Ethics Board. Participants viewed a 4x4 grid containing symbols in various locations and were cued to focus on certain aspects of the grid (symbol, location or both conditions). A breathing belt and heart rate (BioPac inc) monitor were used to collect physiological measurements to correct for breathing and heart rate.
Functional volumes were acquired using a 2D T2*-Weighted EPI sequence (Voxel Size:1.5x1.5x1.5 mm^3) on a 4.7T Varian Inova system. High resolution 2D T2-weighted FSE scan were acquired coronally for hippocampal tracing. 3D T1-weighted MPRAGE images were acquired for creation of CSF and WM nuisance regressors.
Results: Behavioural results show that participants performed best during the symbols condition (95%) while performance on the more difficult location and both conditions was (78%).
The strongest activation during encoding was observed in the both condition across the entire hippocampus, and in the body and tail. In addition, more activation occurred in the hippocampal tail vs. hippocampal head across all conditions.
Conclusions:

Analyses suggest differential processing of memory task conditions by hippocampal subregions. The hippocampus appears to be the most involved in encoding of the both condition vs. symbol and location conditions. The hippocampal tail is more active than the head across conditions.

Disclosures: **M.C. Macgillivray:** None. **S. Hrybouski:** None. **Y. Huang:** None. **C.R. Madan:** None. **P. Seres:** None. **R. Carter:** None. **N.V. Malykhin:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: R01 MH106512

R01 AG049002

Title: Hippocampal-cortical fMRI network distinctions between two types of item-context memory

Authors: ***S.-S. KIM**[1], M. HERMILLER[2], J. VOSS[2];
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Abstract: Accumulating evidence suggests functional distinctions between two cortical networks of the hippocampal system: the anterior-temporal (AT) and posterior-medial (PM) networks. The AT network has been associated with item-oriented and semantic memory whereas the PM network has been associated with context-oriented and episodic memory. However, these are broad memory constructs and few direct comparisons have been made to test functional selectivity of these networks for different types of semantic or contextual memory. We designed a novel human-subject fMRI task to test memory for item-scene associations versus item-location associations using very similar formats across memoranda type. These are widely considered as two examples of contextual memory that should be relatively selective for the PM network. Functional connectivity analyses indicated stronger interconnectivity of PM network regions than AT network regions for both memory types. However, neural correlates of item-scene memory differed from those of item-location memory in that: (1) evoked activity was significantly greater in prefrontal cortex for item-scene compared to item-location memory, (2) the opposite activity pattern (item-location > item-scene) was identified for parahippocampal regions, and (3) functional connectivity patterns included more connectivity among AT network regions (particularly frontal cortex) for item-scene than item-location associations. These

findings generally support the association between hippocampal-PM networks and contextual memory, but indicate that different types of contextual memory vary significantly in terms of network recruitment. Item-scene association memory was more heavily associated with additional recruitment of regions within the AT network, suggesting less PM-specificity for this type of contextual memory.

Disclosures: **S. Kim:** None. **M. Hermiller:** None. **J. Voss:** None.

Poster

087. Human Long-Term Memory: Spatial and Episodic

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Topic: H.02. Human Cognition and Behavior

Support: R01 Grant MH106512

Title: Identifying optimal transcranial magnetic stimulation sequences for enhancement of hippocampal-cortical network connectivity and memory

Authors: ***M. HERMILLER**[1], S. VANHAERENTS[1], T. RAIJ[3,2], D. BRIDGE[1], J. VOSS[1]; [1]Med. Social Sciences, Neurology, and Psychiatry, [2]Physical Med. and Rehabil., Northwestern Univ., Chicago, IL; [3]Rehabil. Inst. of Chicago, Chicago, IL

Abstract: Hippocampal-cortical networks identified by fMRI have established roles in memory encoding and retrieval. Our previous findings indicate that multi-day repetitive transcranial magnetic stimulation (rTMS) of the lateral parietal cortex can enhance associative memory and increase fMRI connectivity among multiple hippocampal-cortical network regions. However, these studies used 20-Hz rTMS trains, whereas patterned TMS such as theta burst stimulation (TBS) might be more optimal for modulation. In this study, we compared the effects of single-session intermittent TBS (iTBS), continuous TBS (cTBS), and 20-Hz rTMS on fMRI connectivity and on both item and source memory. On each of four separate experimental days, subjects (N=11) studied 96 unique words that were presented in one of eight colors. Colors were divided into two broad categories (e.g., blue or red) and each category was further divided into four shades (e.g., navy or cyan). In this way, memory for each color category and precise shade could be tested. Words were later tested in an old/new recognition test followed by forced-choice associative recognition of the source color. MRI-navigated TMS was delivered to lateral parietal cortex during the retention interval, using either rTMS, iTBS, cTBS, or sham on each of the four days, in counterbalanced order across subjects. Preliminary analyses indicate better source accuracy for the cTBS condition relative to all other stimulation conditions (sham p=0.06; iTBS

p=0.06; rTMS p=0.04). Changes in fMRI resting-state connectivity during the retention interval due to stimulation will also be discussed. These findings suggest that short-term effects of TMS on memory performance vary across stimulation frequencies and patterns. This research has implications for the optimization of noninvasive stimulation for enhancement of hippocampal-cortical memory networks.

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Poster

087. Human Long-Term Memory: Spatial and Episodic

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Program#/Poster#: 87.19/III21

Topic: H.02. Human Cognition and Behavior

Support: R21 MH108863

T32 MH67564

Title: The role of dorsolateral prefrontal cortex in viewing behaviors guided by episodic memory

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Abstract: Episodic memory is traditionally observed in humans through explicit/declarative responses. Eye-movement tracking, when recorded in parallel with traditional methods, can also be used to observe the expression of episodic memory, as with differences in viewing of familiar versus novel memoranda. This experiment aims to determine the relationship between episodic memory, explicit responses, and viewing behaviors and to test the causal role of the dorsolateral prefrontal cortex in these cognitive and behavioral phenomena. Subjects first studied scene-face pairs, then, during subsequent cued recall testing, were given a studied scene as a cue and after which they selected the associated face from a display of three previously studied faces, only one of which was originally paired with the scene. Previous research with this task [Hannula and Ranganath, Neuron 63, 592-599, 2009] shows that increased viewing of correctly selected faces, relative to incorrectly selected faces, occurs 500-750 ms after onset of the three faces. Furthermore, accurate responses corresponded to activity in the left lateral prefrontal cortex following cue presentation, whereas increased viewing of associated faces corresponded to activity in the medial temporal lobes during this period. We found that these increases in viewing

of correct faces occurred when subjects reported face recollection when given the scene cue, but not when recollection was absent ($P<0.01$). This suggests that disproportionate viewing of correctly matching faces represents an influence of successful explicit memory retrieval on viewing behavior, which is counter to previous interpretations of disproportionate viewing as indicators of implicit memory processing. Preliminary analyses suggest that continuous theta burst stimulation of the left dorsolateral prefrontal cortex using TMS causes attenuation of the 500-750-ms disproportionate viewing effect, but no change in explicit response accuracy. These results suggest that episodic memory retrieval sufficient to support accurate explicit memory responses influences viewing behavior, and that dorsolateral prefrontal regions are more selectively involved in this influence of memory retrieval on visual exploration than they are in explicit/declarative memory expression.

Disclosures: D.R. O'Young: None. **D.J. Bridge:** None. **J.L. Voss:** None.

Poster

087. Human Long-Term Memory: Spatial and Episodic

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Program#/Poster#: 87.20/III22

Topic: H.02. Human Cognition and Behavior

Title: Intranasal insulin modulates networks involved in an episodic memory task: an fMRI study

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Abstract: Intranasal insulin (INI) administration has been shown to improve memory in some patient and healthy adult populations. Importantly, the intranasal route of delivery for insulin has demonstrated elevated CNS insulin levels in humans without altering peripheral plasma insulin or glucose levels. Despite the demonstrated effects of INI on memory, no study to date has used functional magnetic resonance imaging (fMRI) to examine the neural mechanisms underlying insulin-related memory task performance in healthy adults. We hypothesized that INI administration would modulate activity levels in parahippocampal and hippocampal regions of the brain in response to a memory task and that INI administration would improve overall memory task performance. Lastly, we hypothesized that there would be no difference in peripheral glucose and insulin levels following INI administration compared to saline. Part 1 of the study involved 8 participants undergoing blood draws after receiving separate intranasal administrations of both insulin and saline. Samples were collected and analyzed to determine

peripheral levels of glucose and insulin following administration over a period of 90 minutes. Part 2 of the study involved 40 participants undergoing a randomized, double-blinded intranasal administration of either insulin (n=20) or saline (n=20) followed by a memory task. The encoding phase of the memory task involved participants viewing 180 images (60 indoor scenes, 60 outdoor scenes, 60 scrambled images) during an fMRI scan. The recall phase of the task was conducted one hour later, as participants viewed a new set of images that included the indoor and outdoor scenes seen previously in addition to 60 new similar scenes and were asked to determine if each image had been seen before. Part 1 of the study found no significant differences between INI and saline administration in plasma insulin ($p = 0.752$) or glucose ($p = 0.679$) levels after baseline correction. Part 2 identified INI dependent activations in the right rectus gyrus and frontal lobe regions in addition to memory task performance modulation with significantly elevated activations of bilateral fusiform and parahippocampal gyri for remembered images compared to forgotten images (all FWE corrected, $p < 0.05$). Importantly, insulin enhanced activity in regions which were correlated with remembering, specifically in the right parahippocampus, right mid cingulum, and bilateral rectus gyri. These findings suggest that INI may support memory encoding by increasing network activations in the parahippocampus via inputs from the prefrontal and cingulate cortex.

Disclosures: **D.C. Cockrell:** None. **S.E. Adams:** None. **R.J. Moran:** None.

Poster

087. Human Long-Term Memory: Spatial and Episodic

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Topic: H.02. Human Cognition and Behavior

Support: SFB 776A7

Medical Research Council, UK

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Title: Ultra-high resolution imaging of the medial temporal lobe at holistic episodic recollection

Authors: *X. GRANDE[1,2], J. A. BISBY[3,4], D. BERRON[1,2], E. DÜZEL[1,2,3], N. BURGESS[3,4]; [1]Inst. f. Kogn. Neurologie u. Demenzforschung, Magdeburg, Germany; [2]German Ctr. for Neurodegenerative Dis., Magdeburg, Germany; [3]Inst. of Cognitive Neurosci., [4]Inst. of Neurol., Univ. Col. London, London, United Kingdom

Abstract: A crucial characteristic of episodic memory is holistic recollection. Functionally this is reflected by comprehensive cortical reinstatement of the elements that form a memorized event. A recent study showed that the hippocampus is involved in the reinstatement of event elements, including those incidental to a task (Horner, Bisby, Bush, Lin, & Burgess et al., 2015). We obtained submillimetre (0.8 isotropic) resolution data at 7Tesla using the same multi-element event paradigm to investigate the specific involvement and the interplay of hippocampal subfields in the process of holistic episodic recollection. Theoretical models and animal studies propose that hippocampal subfield CA3 completes partial cues and subfield CA1 reinstates the completed pattern via backprojections in cortical regions. Therefore, we expect hippocampal subfields CA3 and CA1 to be involved in the holistic recollection of events upon partial cues. We hypothesize that the connectivity between subfield CA3 and CA1 reflects the amount of cortical reinstatement of incidental event elements. The study contributes to our understanding of the interaction between the hippocampal circuit and cortical regions to accomplish holistic episodic recollection.

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Poster

087. Human Long-Term Memory: Spatial and Episodic

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Topic: H.02. Human Cognition and Behavior

Support: DFG Grant WE 4269/3-1

Title: Hippocampal subfield volumes contribute to episodic memory development

Authors: ***A. KERESZTES**[1], A. R. BENDER[1], N. C. BODAMMER[1], M. WERKLE-BERGNER[1], Y. L. SHING[2,1];
[1]Ctr. for Lifespan Psychology, Max Planck Inst. for Human Develop., Berlin, Germany; [2]Div. of Psychology, Univ. of Stirling, Stirling, United Kingdom

Abstract: Successful memory formation and retrieval crucially depends on the ability to distinguish between overlapping memory representations. The CA3 and dentate gyrus (DG) subfields of the hippocampus (HC) have been implicated in the orthogonalization of mnemonic contents by implementing pattern separation on neural inputs to the HC (Yassa & Stark, 2011). It is still unclear how pattern separation fits into cognitive process models of human memory and how the structural maturation of the HC subfields is related to episodic memory development.

Challenges in addressing these problems are manifold, and include difficulties in disentangling multiple HC-related episodic memory processes and limitations of standard magnetic resonance imaging (MRI) methods for reliable delineation of HC subfields. To address these challenges, we used high-resolution structural MRI, acquired on a 3T scanner in a sample of children (N = 69, aged 6–14 years) and young adults (N = 33, aged 18-26 years) to investigate the relationship between structural maturation of HC subfields and pattern separation and episodic memory processes. Participants performed an incidental learning task designed to assess pattern separation behaviorally (Bakker et al., 2008; Stark et al., 2013). Participants also completed additional tests measuring recognition memory, recollection and familiarity, associative and item memory, as well as context memory. Entorhinal cortex, subiculum, CA1/2, and CA3/DG were manually segmented by experienced, reliable raters. We observed age-related increases in subiculum, CA1/2, and CA3/DG volumes, in behavioral measures of pattern separation, as well as in measures of episodic memory processes. Results revealed age-invariant associations between HC subfields and episodic memory processes: specifically, pattern separation was most strongly associated with DG/CA3 volume, whereas general recognition performance was most strongly related to CA1/2 volume. The present results corroborate earlier findings linking HC subfields to pattern separation, and extend this relationship into child development. More importantly, these results characterize the parallel trends of HC subfield development and pattern separation maturation, and establish their age-invariant contributions to episodic memory processes. In sum, by taking a developmental perspective, the present study provides an important step in bridging the gap between computationally inspired models of HC networks and episodic memory theories.

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Poster

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Support: EU FP7 WYSIWYD ICT-612139

EU Human Brain Rroject

Title: Episodic and autobiographical memory in humans and robots

Authors: *T. J. PRESCOTT[1], D. CAMILLERI[1], A. DAMIANOU[1], U. MARTINEZ[2], N. LAWRENCE[1];
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Abstract: Human episodic and autobiographical memory can be considered as an attractor network operating in a latent variable space whose dimensions encode salient characteristics of the physical and social world in a highly compressed fashion. Instantaneous memories then correspond to points in this latent variable space and episodic memories to trajectories through this space. Seeding such a mechanism with appropriate cues will allow retrieval of a past episode, but the same system can also serve to fill-in and enrich the representation of the current situation, providing the potential for more informed action. As part of the development of a broader brain-based architecture for social cognition in a humanoid robot we are exploring the hypothesis that suitably a configured systems-level model of human memory can support effective Synthetic Autobiographical Memory (SAM) for robots. The operation of the perceptual systems that provide input to episodic memory can be analogised to deep learning processes that identify psychologically meaningful latent variable descriptions. Deep Gaussian Process (DGP) neural network models have many attractive properties including the ability to discover highly compressed latent variable spaces, to form attractors that encode temporal sequences, and to act as generative models. The core element of our robot SAM system is therefore constituted by a set of DGP models that represent memories of multiple heterogeneous sensory modalities through a compressed latent feature space and a set of anchor points. The model "knows" how to combine these two elements to reconstruct past memory, or to generate fantasy memories (imagination). Chunking and pattern separation are also naturally manifested within this formulation. By linking sensory primitives of multi-modal memories to verbal descriptions of episodes stored elsewhere in the system the SAM model also provides a way to ground linguistic accounts in remembered experience. Our current implementation of robot SAM for the iCub humanoid robot is able to demonstrate effective memory formation and retrieval of human faces, actions, objects, and sequences of agents acting on objects. The system is also able to imagine possible sequences, such as a person's future action. Future work aims to extend this research to more brain-like models of episodic memory, incorporating insights from fMRI and neurobehavioural data concerning the interaction of the hippocampal system with primary and secondary sensory cortical areas, and thus provide an embodied test of theories and computational models of human episodic memory function.

Disclosures: **T.J. Prescott:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Director of Consequential Robotics. **D. Camilleri:** None. **A. Damianou:** None. **U. Martinez:** None. **N. Lawrence:** None.

Poster

088. Attentional Networks

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Topic: H.02. Human Cognition and Behavior

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Title: Modelling trans-thalamic coherence of cortical gamma-band activity in MEG

Authors: ***F. ROUX**[1], P. J. UHLHAAS[2], J. GROSS[2];
[1]Sch. of Psychology, Univ. of Birmingham, Birmingham, United Kingdom; [2]Inst. of Neurosci. and Psychology, Univ. of Glasgow, Glasgow, United Kingdom

Abstract: Emerging data from electrophysiological recordings in thalamo-cortical (TC) circuits indicate that thalamic alpha rhythms could coordinate cortico-cortical communication by establishing coherent gamma-band activity in distinct cortical areas during cognitive processing (Saalmann et al., 2012). This thalamic coordination of cortico-cortical communication could arise from the abundant non-reciprocal projections that route inputs from one cortical area to another cortical area by passing through higher-order thalamic nuclei such as the pulvinar (Theyel et al., 2009; Schmid et al., 2012). Given that alpha oscillations are a prominent feature of magnetoencephalographic (MEG) recordings, the analysis of whole brain activity with MEG could provide important insights into the role of thalamic alpha rhythms in establishing coherent gamma-band activity in distinct cortical areas. However, due to the rapid decay of magnetic fields, the localization of thalamic sources with MEG has remained challenging as thalamic generators may be shadowed by sources located much closer to the sensors. Here, we assess how differences in field strength produced by thalamic and cortical sources influence the reconstruction of TC interactions by simulating a set of coherent dipoles in the thalamus and cortex. Specifically, simulated thalamic and cortical regions were coupled through cross-frequency interactions at alpha (10Hz) and gamma (70Hz) frequencies and their amplitude levels were varied parametrically. Our simulations show that the coupling of cortical gamma-band power to the phase of thalamic alpha activity can be reconstructed from MEG-data if the amplitude of thalamic dipoles is larger than the amplitude of cortical dipoles. The reconstruction of TC interactions, by contrast, is deteriorated by weaker thalamic sources. The present findings suggest that TC interactions including TC transmission delays can be reliably reconstructed from MEG-data despite the spatial decay of magnetic fields and in the presence of simultaneously active cortical sources. These data support previous evidence from our group on the reconstruction of TC interactions in resting-state MEG-recordings and may have important implications for the assessment of transthalamic cortico-cortical coherence with MEG.

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Poster

088. Attentional Networks

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Title: Whole brain dynamic resting state functional connectivity of the basal nucleus of Meynert & ventral striatum

Authors: *S. ZHANG, S. HU, C.-S. R. LI, 06519;
Yale Univ. Sch. of Med., New Haven, CT

Abstract: Background: The basal nucleus of Meynert (BNM) provides the primary cholinergic inputs to the cerebral cortex and has been implicated in the etiology of Alzheimer's and Parkinson's disease. Our previous study (Li et al, 2014, NeuroImage) examined resting state functional connectivity (rsFC) of the BNM and ventral striatum (VS). Recent studies suggest that rsFC is dynamic, whereby distinct network connectivities characterize individual time periods in transition. The dynamic nature of rsFC provides additional information on network connectivities and may shed new light on cerebral functions in health and illness. Here, we examined the dynamic rsFC of the BNM and VS.
Methods: In resting state fMRI data (3T, 10 minutes, eye closed) of 80 healthy adults, we computed seed-region based correlation with a sliding window approach to obtain a series of correlation maps for each individual subject. The correlation maps were converted to z score maps (by Fisher's z transform), which were pooled across all subjects and clustered using a K-means algorithm. On the basis of the clustering results, images in one group (state) were averaged to generate a state "z map" for each individual subject, and these state "z maps" were used for group comparisons.
Results: The results of 1000 runs of K-means clustering suggested an optimal cluster number of 4 according to Bayesian Information Criterion (BIC) for both BNM and VS seeds. Group analysis showed that these 4 states dynamic networks provided important additional information comparing to traditional rsFC networks. For instance, the BNM is characterized by dynamic connectivity to a vmPFC-pCingC (default mode, state I), mPFC-striatal-cerebellar (executive control, state II), mPFC-thalamic-insular (saliency, state III) and mPFC-striatal-motor cortical (action, state IV) network. Furthermore, the transitions between these four states were all observed, but some of them were more frequent than others. For example, transitions from state I

to II were much more than transitions from state I to III and state I to IV (all p's = 0.002). **Conclusions**: The findings support dynamic rsFC of the BNM and VS. These dynamic rsFC networks provide additional information on cerebral dynamics that elude traditional analytics. Supported by NIH grants K25DA040032, DA023248, AA021449.

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Poster

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Support: Washington University Institute of Clinical and Translational Sciences grant UL1TR000448, sub-award TL1TR000449

Title: Delta phase-amplitude coupling in cued-attention task reflects task behavior

Authors: *R. CHACKO[1], E. C. LEUTHARDT[2];
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Abstract: Introduction: Recent studies indicate that there are interactions between oscillations of different frequencies within local brain regions and across distant locations. One theory suggest this activity is epiphenomena of synchronized spike timing. This theory holds that phase-amplitude coupling PAC reduces the potential information represented in an electrode. We investigate this possibility by comparing changes in PAC between task and rest conditions.
Methods: We recorded brain activity from 6 subjects with implanted ECoG arrays. We define electrodes that encode task conditions as task-relevant. We evaluate PAC by comparing differences in modulation index (MI) during task and rest conditions using a monte-carlo resampling procedure. We compare the number of significant changes in PAC for task-relevant and task-irrelevant electrodes using a chi-squared test.
Results: On average we find a reduction in PAC during the task. Exceptions show increases in delta-beta and delta-gamma phase-amplitude coupling. We find task-relevant electrodes are more likely to exhibit changes in phase amplitude. However, this effect did not reach statistical significance in a single patient analysis.
Discussion: The general reduction in PAC in task-relevant electrodes during the task conditions fits the theory that a reduction in PAC represents an increase in information content. However, exceptions suggest this theory is too simple to account for the diversity of PAC behavior.

Disclosures: **R. Chacko:** None. **E.C. Leuthardt:** None.

Poster

088. Attentional Networks

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Program#/Poster#: 88.04/III29

Topic: H.02. Human Cognition and Behavior

Support: US Army Research Laboratory, Cooperative Agreement Number W911NF-10-2-0022

Title: Rapid network reconfigurations within the alpha band following single pulses of TMS

Authors: ***J. O. GARCIA**[1], Q. K. TELESFORD[2,1], A. ASHOURVAN[2,1], D. S. BASSETT[2], J. M. VETTEL[1,2,3];
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Abstract: Alpha band activity (8-12Hz) is an intrinsic brain oscillation of prominent study due to its robustness in both resting state and task-dependent EEG paradigms. Hypothesized functions of this oscillation range from priming the brain for incoming information in a 'brain state' of *inactivity* (Adrian & Matthews, 1934) or *cortical idling* (Pfurtscheller et al., 1996), to a *gate* of perceptual information (Jensen and Mazaheri, 2010), or even an *access controller* to a knowledge system (Klimesch et al., 2012). Collectively, alpha activity consistently represents a diffuse, communicative signal with multiple functions (Basar et al., 1999), but the parameters that govern these functions remains elusive. Recent analyses on neural measures have used a more hodological approach of *interacting systems* rather than a topological perspective of *important brain areas* (Bortolleto et al., 2015), leveraging methods in network science to investigate system interactions (e.g., Bassett & Bullmore, 2006). We hypothesize that these multiple functions in alpha activity may be parameterized via metrics of modularity within a network science framework. In this study, we employ single-pulse transcranial magnetic stimulation (TMS) to measure the rapid network reconfigurations following stimulation of four entry points in right and left occipital and parietal cortices. We compute connectivity across pairs of estimated brain sources using debiased weighted phase lag index (dwPLI, Vinck et al., 2011), and we examine dynamic network communities by optimizing a modularity quality function using a Louvain-like greedy algorithm (Blondel et al., 2008; Jutla et al., 2011). Dynamic communities from non-TMS, *resting-state* intervals reveals distinct communities from coordinated activity within bilateral frontal and posterior regions as well as lateralized temporal regions. We find that TMS to (1) parietal regions increases the network allegiance between the

posterior and frontal communities, revealing that regional node pairs from these cortices consistently change network communities together, (2) the left occipital region decreases node allegiance within the frontal community, suggesting independent node change, and (3) overall nodal changes in community assignment after TMS cohere into a more complex organization than the resting-state community assignments. This research extends the resonant frequency account (Rosanova et al., 2009) of single pulse TMS to networks outside of the 'natural frequency' of the stimulation site and extends previous findings of local-global interactions from the temporal domain (Garcia et al., 2011) to the spatial domain.

Disclosures: **J.O. Garcia:** None. **Q.K. Telesford:** None. **A. Ashourvan:** None. **D.S. Bassett:** None. **J.M. Vettel:** None.

Poster

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Title: Attention-related spatially-selective alpha suppression is correlated with high frequency broadband power in the human visual system

Authors: ***A. B. MARTIN**[1], L. WANG[1,2,3], Y. B. SAALMANN[1,2,4], A. SHESTYUK[5], N. E. CRONE[7], J. PARVIZI[8], R. T. KNIGHT[5,6,9], S. KASTNER[1,2];
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Hosp., Baltimore, MD; [8]Dept. of Neurol. and Neurolog. Sci., Stanford Univ., Stanford, CA; [9]Dept. of Neurolog. Surgery, Univ. of California, San Francisco, San Francisco, CA

Abstract: Across the visual attention network, classical effects of attention on low frequency (alpha) suppression have been characterized in human EEG, and high frequency spiking activity has been investigated in many animal neurophysiology studies. However, detailed electrophysiological analyses on spatial selectivity and the effects of attention across these frequency domains in the human brain have not been reported. Here, we analyzed ECoG signals recorded from 8 epilepsy patients performing an Eriksen flanker task variant. Following a spatial cue and variable delay interval, subjects differentiated between two shapes at the cued location in an array of distracters, allowing us to measure selectivity within 25 degrees of visual angle. Using our probabilistic atlas of the human visual system (Wang et al., 2014), we localized electrodes to visual topographic areas and identified those with cue-evoked spatially-selective high-frequency broadband power (HFB). In the V3 complex (V3A/B), posterior intraparietal cortex (IPS0-2), and the lateral occipital complex (LO1/2), HFB power signaled the location of the up-coming target throughout the delay, and attention-related spatially-specific modulation persisted in response to the target. In these areas, we also found that alpha suppression was spatially selective in these intervals. Further, the strength of the HFB selectivity was strongly correlated with the selectivity of the alpha suppression. This parallel representation of space in low and high frequency bands suggests a coordinated functionality of processing that may integrate information transfer across these cortical areas.
Wang L, Mruczek RE, Arcaro MJ, Kastner S (2014) Probabilistic Maps of Visual Topography in Human Cortex. Cerebral Cortex:bhu277.

Disclosures: A.B. Martin: None. **L. Wang:** None. **Y.B. Saalmann:** None. **A. Shestyuk:** None. **N.E. Crone:** None. **J. Parvizi:** None. **R.T. Knight:** None. **S. Kastner:** None.

Poster

088. Attentional Networks

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Topic: H.02. Human Cognition and Behavior

Title: Changes in lateral prefrontal cortex oxygenation as a function of oddball task difficulty: A near-infrared spectroscopy study

Authors: *W. RIZER, J. ADAY, S. CONGER, J. CARLSON;
Psychology, Northern Michigan Univ., Marquette, MI

Abstract: The detection of goal-relevant stimuli in the environment is important for survival, but not all stimuli are easy to detect. Research on the P300 event-related potential indicates that as targets become more difficult to detect the posterior P300 attenuates. Functional MRI research suggests that target detection involves attention processes supported by the prefrontal cortex. However, the extent to which target difficulty differently activates the prefrontal cortex is unknown. We addressed this knowledge gap by collecting oxygenated (HbO) and deoxygenated hemoglobin (HbR) concentrations during an oddball task, which contained easy and difficult conditions, using a near-infrared spectroscopy (NIRS) optode array affixed above 10-20 coordinates Fp1 and Fp2 (N = 20). Reaction times were faster in the easy (M = 484.06) compared to difficult condition (M = 558.63), additionally participants missed fewer targets in the easy (M = 1.55) compared to difficult condition (M = 7). NIRS data was time locked to target onset and data from 5-10s post-target was averaged and analyzed. Both HbO and HbR had main effects for optode with lateral increases in HbO ($p = 0.04$) and lateral decreases in HbR ($p = 0.012$). An interaction between optode and condition approached significance for HbO ($p = 0.082$) and was significant for HbR ($p = 0.001$) with lateral optodes in the difficult condition recording the lowest HbR. The data indicates that lateral prefrontal regions (covered by the array) use more oxygen than medial prefrontal regions during both oddball tasks, but to a greater extent in the difficult condition.

Disclosures: **W. Rizer:** None. **J. Aday:** None. **S. Conger:** None. **J. Carlson:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: NSF BCS-1439188

Title: Decoding attentional states using multi-voxel pattern analysis.

Authors: ***S. MEYYAPPAN**[1], A. RAJAN[1], Y. LIU[2], R. SITARAM[3], G. R. MANGUN[2], M. DING[1];
[1]Univ. of Florida, Gainesville, FL; [2]Univ. of California, Davis, CA; [3]Pontifical Catholic Univ. of Chile, Santiago, Chile

Abstract: Functional magnetic resonance imaging (fMRI) data are traditionally analyzed using univariate approaches in which each voxel is treated independent of all the other voxels. Rich information contained in the multi-voxel patterns of brain activation is ignored. Advances in the

field of machine learning, pattern recognition, high resolution brain mapping, and associated computational techniques have begun to change that. In this study we applied multi-voxel pattern analysis (MVPA) to examine the neuronal substrate underlying the control of visual spatial attention. Functional MRI data were recorded from thirteen healthy subjects performing a cued visual spatial attention task. Each trial began with a cue, instructing the subject to either attend the left or the right hemi-field. After a random delay, a grating (Gabor patch) was presented in one of the two hemi-fields, and the subject was asked to discriminate the spatial frequency of the grating in the cued hemi-field and ignore the grating appearing in the un-cued hemi-field. Trial-by-trial BOLD response to the cue was estimated using the beta-series method. Linear support vector machine classifiers were applied to these beta values in multiple ROIs located in the occipital, parietal and frontal cortices. The following results were found: Decoding accuracies were significantly above chance level in ROIs in the parietal and occipital cortices. Multi-voxel patterns in visual ROIs along the dorsal pathway were more predictive of spatial orienting than those along the ventral pathway. Importantly, a traditional univariate analysis was also carried out using general linear model (GLM), and it revealed no differential activations between the two attention conditions. These findings demonstrate the value of MVPA analysis in uncovering neuronal mechanisms of cognitive functions.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: 4R01MH095984-04

T32-GM007507

MH064498

Title: Informational connectivity reveals representation-specific gating of information in object-based attention

Authors: ***A. D. SHELDON**[1], E. SAAD[2], B. R. POSTLE[3];
[1]Psychology, Univ. of Wisconsin-Madison, Madison, WI; [2]Psychiatry, Univ. of Wisconsin - Madison, Madison, WI; [3]Psychology, Univ. of Wiscosin-Madison, Madison, WI

Abstract: What are the consequences, for the neural representation of visual objects, of the selection of one from among multiple objects that are simultaneously present in the visual field? To assess this, we scanned subjects with functional magnetic resonance imaging (fMRI) while they performed a change-detection task in which the stimulus (one of three types -- a face; a doughnut; or an abacus) flickered with a cycle of 750 msec on/250 msec off, and subjects counted the number of "state changes" that the stimulus undergoes during a block. (The face shifted between two expressions; the doughnut between whole and with a bite-sized chunk removed, and the abacus between two configurations of beads.) The experimental task began with the 500 msec presentation of a search target (drawn from the set of three stimulus types) followed by a 7-sec delay, followed by a "search array" comprising the target plus a second stimulus, both flickering and periodically changing state. Multivariate pattern analysis (MVPA) at the level of ventral occipitotemporal cortex revealed evidence for biased competition between the two representations. The onset of the search array was associated with an initial decline in MVPA evidence for the Target stimulus (relative to Target-alone), followed a gradual strengthening. For the Nontarget, in contrast, the initial decline in MVPA evidence was steeper than that of the Target, and Nontarget evidence remained suppressed throughout the presentation of the search array. To assess possible downstream consequences of selection, we next applied an "informational connectivity" (IC; Coutanche and Thompson-Schill, 2013) analysis. IC measures the covariance of multivariate pattern discriminability between anatomically disparate regions of the brain, thus serving as an informational analogue of functional connectivity measures that assess covariation in signal intensity across regions. Importantly, IC can vary independent of the strength of information assessed in a local region. We employed a roving searchlight to assess IC between ventral occipitotemporal cortex (the seed region) and the rest of the brain, independently for both Target and Nontarget items. The results revealed markedly stronger IC for Target than Nontarget stimuli with several regions of dorsal frontoparietal cortex that are associated with endogenous shifts of spatial attention. This implies that one consequence of top-down object-based attention may be the privileged transmission of sensory information to downstream regions associated with the representation of attentional salience, and with the control of behavior.

Disclosures: **A.D. Sheldon:** None. **E. Saad:** None. **B.R. Postle:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: MH005286

Title: Do brain regions activated by unexpected outcomes represent the content of predictions?

Authors: *K. R. VELNOSKEY, G. MCCARTHY;
Psychology, Yale Univ., New Haven, CT

Abstract: Prior research on attention reorienting has identified a fronto-parietal network that responds to unexpected stimuli (Kim, 2014). However, it remains unknown whether the brain regions composing this network represent these predictions. We used a novel fMRI task in which the prediction and outcome were separated temporally to examine each process individually. We were particularly interested in temporal-parietal cortex (TPC) and dorsolateral prefrontal cortex (DLPFC), as TPC is implicated in context updating (Lee & McCarthy, 2016) and DLPFC is involved in working memory (McCarthy et al., 1994). Both of these processes may be related to making and updating predictions.
Participants (N=20, 12 female) used a color cue, which indicated the statistical likelihood of two possible outcomes, to predict whether an upcoming stimulus would be a face or house. Participants indicated their predictions via button press while the cue was on the screen, and were then shown either a face or house outcome. We first identified regions that responded to unpredicted stimuli by contrasting low probability to high probability outcomes using a whole-brain univariate general linear model (GLM). This contrast revealed activity in the following 13 regions: left and right DLPFC, paracingulate cortex, left and right middle temporal gyrus, left frontal pole, left insula, right frontal pole and insula, posterior cingulate cortex, precuneus, left and right TPC, and occipitotemporal cortex. We then used a Gaussian naïve Bayes pattern classifier within each region to determine whether the participant's prediction was represented there before the outcome occurred. We classified predictions above chance (50%) in three regions: left DLPFC (M=53.59, $S.E.$=0.77, t=4.75, p<0.001, one-tailed), paracingulate cortex (M=52.67, $S.E.$=1.09, t=2.51, p=0.011, one-tailed), and the right middle temporal gyrus (M=52.55, $S.E.$=1.11, t=1.87, p=0.04, one-tailed). However, our analyses did not reveal a representation of the prediction in the right TPC. We found that only some of the regions involved in responding to unpredicted stimuli represent content about held predictions. Others may be involved in signaling a mismatch between prediction and outcome. Analyses of task dependent connectivity between perceptual regions and regions responding to unpredicted stimuli are being conducted to clarify activity in response to unexpected events. This experiment reveals that the fronto-parietal network responding to unexpected events does so in a heterogeneous way, and that future experiments are needed to clarify the roles that individual regions play in making and updating predictions.

Disclosures: K.R. Velnoskey: None. **G. McCarthy:** None.

Poster

088. Attentional Networks

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Topic: H.02. Human Cognition and Behavior

Title: Difference in phase synchrony pattern between spatial and non-spatial attention based on electrocorticography

Authors: *Y. PARK[1], T. KIM[1], J. PARK[1], J. KANG[2], D. JANG[1];
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Abstract: In recent past, the functional role of inferior parietal lobe and other regions in the spatial attention and non-spatial attention has been studied using fMRI and electrocorticography (ECoG). However functional connectivity for spatial attention and non-spatial attention among the regions has not been observed from ECoG signals. In this study, we observed the phase synchrony of ECoG signals between each electrodes and showed different patterns of phase synchrony between spatial and non-spatial attention.
ECoG signals were recorded during the spatial and non-spatial tasks using grid electrodes covered frontal, temporal and parietal regions. The spatial and non-spatial attention tasks were adopted from a paradigm that examined the spatial and non-spatial attributed of the same pattern stimuli (Malhotra et al., 2009). To remove line noise, a 60Hz notch filter was used. MATLAB and HERMES toolbox were used to process ECoG signals. ECoG signals were segmented into 2000 msec epochs from 400 msec before the stimulus to 1600 msec after the stimulus. To calculate instantaneous phase of each electrode signal, a FIR band-pass filter from 4 to 8 Hz (theta band) and Hilbert transform were processed on each 2000 msec epoch. The averaged phase difference of every 400 msec interval between two electrodes was used to calculated phase locking value (PLV) and we obtained PLV for five time intervals (-400~0, 0~400, 400~800, 800~1200, 1200~1600 msec). Finally we calculated PLV differences between baseline (-400~0 msec) and each interval to estimate task dependent changes of phase synchrony.
As results of this study, we observed different PLV pattern in baseline and different increasing pattern of PLV between spatial and non-spatial attention.

Disclosures: **Y. Park:** None. **T. Kim:** None. **J. Park:** None. **J. Kang:** None. **D. Jang:** None.

Poster

088. Attentional Networks

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Topic: H.02. Human Cognition and Behavior

Support: NSF grant BCS-1439188

Title: Neural mechanisms of attentional control in spatial and feature attention

Authors: ***A. RAJAN**[1], S. MEYYAPPAN[1], H. WALKER[1], Y. LIU[2], G. MANGUN[2], M. DING[1];
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Abstract: Attention can be directed to spatial locations or to object features. Relative to feature attention the control mechanisms of spatial attention have been more extensively studied. The longstanding question is whether attention control mechanisms are specific to the type of information being selectively attended to. To further investigate this question we recorded fMRI data while the subject performed a novel cued spatial/feature attention task. Each trial started with an auditory cue directing the subject to attend either to a spatial location (left or right) or a color (red or green). Following a random time delay, two stimuli in the form of colored (one red and one green) rectangles were presented in the left and right visual fields (valid trials). The subject discriminated the orientation of the target rectangle (horizontal or vertical) and ignored the distractor rectangle. For spatial attention the target rectangle was the rectangle appearing in the cued visual field. For feature attention the target rectangle was the rectangle appearing in the cued color. On 8% of the trials (invalid trials), only one rectangle appeared, which was either in the uncued location or in the uncued color. We found the following results. First, the behavioural performance was similar for the spatial and the color conditions. Second, the reaction time for invalidly cued trials were significantly longer than validly cued trials for both spatial and color conditions, demonstrating the behavioural benefit of attentional cuing. Third, spatial and colour cues evoked a common set of dorsal frontal-parietal regions. Fourth, contrasting color-cue evoked BOLD activation with spatial-cue evoked BOLD activation revealed that (1) regions within the dorsal frontoparietal network, namely, frontal eye field (FEF), intraparietal sulcus (IPS), superior parietal lobule (SPL) was more activated in the spatial condition and (2) the default mode network regions, including the medial prefrontal cortex (MPFC), posterior cingulate cortex (PCC)/ventral precuneus, angular gyrus was more activated in the color condition. Multivoxel pattern analysis was further applied to examine the similarities and differences in the neural representations of spatial and feature attentional signals. Key words: Cued Attention, Feature Attention, Frontoparietal Network, EEG, fMRI

Disclosures: **A. Rajan:** None. **S. Meyyappan:** None. **H. Walker:** None. **Y. Liu:** None. **G. Mangun:** None. **M. Ding:** None.

Poster

088. Attentional Networks

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Topic: H.02. Human Cognition and Behavior

Title: Orienting of spatial attention with event-related functional magnetic resonance imaging

Authors: *T. S. CONTENÇAS[1,2], M. N. SILVA[3], L. RIBEIRO-DO-VALLE[3], R. M. AZEVEDO, Neto[3], E. AMARO, Júnior[3];
[1]UNIP, Santos, Brazil; [2]Icb, [3]USP, São Paulo, Brazil

Abstract: Introduction: Previous research has identified two major ways in wich attention can be directed to an object: voluntary or automatic. The purpose of this study is to identify the brain networks involved in voluntary attention using event-related functional magnetic resonance integrated with eye-tracking. **Methods:** We present the results of 20 young healthy (mean age 21,7±3,4). Each trial began with a Fixation Point (FP) and one white ring on each side. The prime stimulus (S1) was represented by a central arrow pointed to the ring on the left or right side of the FP. Fifty milliseconds after of the S1 a target stimulus (S2) appeared represented by a vertical line. In the valid condition, the S2 appeared in the same position of the S1. In the invalid condition, the S2 appeared in the opposite position of the S1. In the neutral condition, the S1 did not appear. The conditions were intermixed in an order optimized to produce maximal signal discriminability using a genetic optimization algorithm. Data was acquired in three runs. BOLD acquisitions were performed on a 3T scanner (Philips Achieva) with integrated MRI compatible Eye Tracker (Magconcept). TR was 2s, TE=30ms, voxels dim of 3x3x3mm. Images were analyzed using FSL software. Preprocessing included motion correction, spatial smoothing and transformation of image into standard. The activation group maps (by using the general linear model) were thresholded at Z-voxel>2.3 and cluster-wise corrected p-value<0.05. **Results:** Reaction time (RT) was shorter when the S2 appeared on the valid condition as the S1 compared to the invalid condition (405 ± 18 ms and 488 ± 19 ms, $p < 0.001$). The attentional effect was 83 ± 11 ms. The RT of the neutral condition was of 525 ± 19 ms. In general, we could detect areas similar to those described in the literature. Valid versus Neutral Trials: This contrast showed bilateral activations in fusiform gyrus, left superior frontal gyrus, left medial orbitofrontal gyrus, left precuneus. Invalid versus Neutral Trials: This contrast showed bilateral activations in the superior frontal gyrus and occipital lobe, left inferior frontal gyrus, lateral orbitofrontal gyrus,

left temporal pole. Invalid versus Valid Trials: This contrast showed bilateral activations in the lateral orbitofrontal gyrus, middle frontal gyrus, intraparietal sulcus and supramarginal gyrus, right superior frontal gyrus, left inferior frontal gyrus, left angular gyrus. **Conclusions:** These findings show participation of the temporal-parietal junction during endogenous orienting with symbolic cues. This finding may imply that the same neural networks mediating automatic and voluntary control of visuospatial selective attention.

Disclosures: T.S. Contenças: None. **M.N. Silva:** None. **L. Ribeiro-do-Valle:** None. **R.M. Azevedo:** None. **E. Amaro:** None.

Poster

088. Attentional Networks

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Support: National Institute on Aging Training Grant T35 AG029793-07

Advancing a Healthier Wisconsin (AHW) FP00005822 (MS)

Title: Suppression impairment in aging during bimodal selective attention

Authors: *A. ATHREYA[1], C. HUMPHRIES[1], M. T. KASSEL[1,2], K. A. ALTONJI[1], M. SABRI[1];
[1]Dept. of Neurol., Med. Col. of Wisconsin, Milwaukee, WI; [2]Psychology, Univ. of Wisconsin Milwaukee, Milwaukee, WI

Abstract: We investigated the effects of aging on brain activation in the auditory cortex and the frontal-parietal (FPN) and cingulo-opercular (CON) executive control networks during bimodal selective attention using functional magnetic resonance imaging (fMRI). FMRI was acquired in 18 younger (mean=26.1, SD=5.7) and 18 older (mean=62.4, SD=4.9) adults with normal hearing thresholds and cognitive function. In five task runs, two independent streams of consonant-vowel (CV) syllables were presented simultaneously in the visual and auditory modalities. Subjects attended to either the auditory (AA) or visual (AV) modalities and performed a two-alternative forced-choice (ABX) discrimination task, with the other modality serving as a distractor. Distractor syllables were either fixed (Fixed) or randomly changing (Rand). A passive listening/viewing (PLV) condition served as control. ANOVAs on reaction time (RT) and d' scores with group, distractor and modality as repeated measures revealed main-effects of distractor, with Fixed faster in RT and higher in d' than Rand. Modality by group interaction

indicated slower RT in AA than AV, in the young. A main-effect of modality revealed overall greater d' in AV than AA. Voxel-wise multiple linear regression, focusing on the encode period of the trial (i.e., AB) was applied to the individual time series. Analyses showed: left superior temporal gyrus (STG) enhancement (AA: Rand>PLV) during AA only in older adults; right STG suppression (AV: PLV>Rand) during AV only in young adults, with older adults showing an increase in signal, bilaterally (lack of suppression). In addition, right lateral occipital cortex enhancement (AV: Rand>PLV) was observed during AV only in older adults, with no sign of visual suppression (AA: PLV>Rand) in either group. Effects of distraction observed only for AV (Rand>Fixed) showed greater activation in CON (bilateral lateral and medial prefrontal cortices, bilateral superior frontal gyri, right anterior insula) and FPN (bilateral middle frontal gyri, MFG) for older adults during randomly presented distraction, while activity decreased in the right MFG for younger adults. Overall these results indicate that older compared to younger adults have greater difficulty suppressing specifically task-irrelevant auditory information when faced with bimodal stimulation.

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Poster

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Support: National Institute on Aging Training Grant T35 AG029793-07

Advancing a Healthier Wisconsin (AHW) FP00005822 (MS)

Title: White matter integrity of executive control networks predicts selective attention performance in healthy aging

Authors: ***M. T. KASSEL**[1,2], C. HUMPHRIES[2], K. A. ALTONJI[2], D. C. OSMON[1], M. SABRI[2];
[1]Psychology, Univ. of Wisconsin - Milwaukee, Milwaukee, WI; [2]Neurol., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: The present study investigated the impact of white matter microstructural changes in healthy aging on cross-modal selective attention (SA) performance. Eighteen younger (mean=26.1, SD=5.7) and 18 older (mean=62.4, SD=4.9) healthy adults with normal hearing

(audiometric thresholds ≤25 dB HL 500 - 4,000 Hz) underwent structural MRI (T1- and diffusion-weighted scans) and performed a two-alternative forced choice task employing distraction manipulation (random vs. fixed) during functional MRI (activation not reported here). Reaction times (RT) for correct responses were computed for each of the distraction conditions (random, fixed). The distraction manipulation was quantified using the RT Distraction Index [(RTDI; random minus fixed distraction; 6.38 ms for young (p=.445), 19.20 ms for old (p=.026); group differences (n.s.)]. Fiber tracts connecting the cingulo-opercular (CON) and frontoparietal (FPN) executive control networks were identified using probabilistic white-matter tractography using seed regions defined based on Power et al. (2011) meta-analysis. Mean radial diffusivity (RD), an indicator of myelin degradation, was computed for right and left CON and FPN white matter tracks. Separate multiple regression analyses revealed a relationship between FPN (p=.009), CON (p=.005), and RTDI, indicating that greater slowing of RT due to increased distraction (random > fixed) was associated with decreased white matter integrity. Between group ANOVA with network and hemisphere as repeated measures revealed a main effect of group (p<.001), illustrating that older adults have decreased integrity (higher RD) compared to young. Within-group comparisons indicated a main effect of network (p<.0001), such that the CON displayed greater overall integrity compared to the FPN. A network by hemisphere interaction was also evident (p=.003), demonstrating that the left FPN has lower integrity than the right FPN, however there was no difference in laterality apparent in the CON. In sum, while group differences in performance were not observed, older adults did display a decrease in white matter integrity compared to young. Collectively, the present results suggest that decreased SA performance can be explained by greater demyelination in executive control networks in healthy aging.

Disclosures: **M.T. Kassel:** None. **C. Humphries:** None. **K.A. Altonji:** None. **D.C. Osmon:** None. **M. Sabri:** None.

Poster

088. Attentional Networks

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 88.15/III40

Topic: H.02. Human Cognition and Behavior

Title: Control of spatial attention by alpha and gamma neurofeedback in human cortex

Authors: ***Y. BAGHERZADEH BIOKI**, D. BALDAUF, D. PANTAZIS, R. DESIMONE; McGovern Inst. For Brain Res. at MIT, Cambridge, MA

Abstract: Studies in people and animals have shown that enhanced alpha synchrony is associated with inattention and distracting information, whereas enhanced gamma synchrony is associated with attention to targets. To test whether alpha and gamma synchrony play a causal role in the control of attention, we used MEG neurofeedback to train subjects to manipulate alpha or gamma power over parietal cortex. We tested the effects of these oscillatory changes on both behavioral performance in an attention task and on visual evoked potentials recorded from visual cortex. The study consisted of three phases: (1) a pre-training session with a classic Posner paradigm, (2) neurofeedback phase with one or multiple sessions of feedback training to increase an asymmetry of oscillatory power between the left and right visual hemisphere (alpha in Exp1 or gamma in Exp2) and (3) a post-training session with the same Posner paradigm as in the pre-training session. The evoked responses elicited by the target stimuli in the Posner paradigm served as an index of attention being deployed in valid, invalid and neutral trials, respectively. During feedback trials a Gabor pattern was presented in the center of the screen, the contrast of which was modulated according to a real time measure of the hemispheric asymmetry in the alpha (or gamma) range. We found that subjects were able to control the asymmetry between their left and right hemisphere in the frequency range of interest, both within and between feedback sessions. Increasing alpha in one hemisphere lead to reduced visually evoked responses (Exp1) while increasing gamma in one hemisphere lead to enhanced evoked responses (Exp2) . The results support the idea that alpha and gamma synchrony play reciprocal roles in the control of spatially directed attention, even in the absence of explicit instructions.

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Poster

088. Attentional Networks

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Program#/Poster#: 88.16/III41

Topic: H.02. Human Cognition and Behavior

Support: Sidney R. Baer. Jr. Research Foundation

Title: Modulating performance on a sustained attention task with network targeted brain stimulation

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Abstract: Background Recent studies of human functional connectivity implicate a direct relationship between resting network activity and cognitive performance (c.f. Rosenberg et al 2016). In sustained attention tasks, changes in activity within the default network (DN) and dorsal attention network (DAN) correspond to changes in ongoing performance (Esterman et al. 2014). These same networks can be modulated with transcranial magnetic stimulation (Eldaief et al 2011; Halko et al 2014). The cerebellar nodes of these networks may offer unique access points for driving entire network activity by virtue of their anatomical connectivity to cerebral cortex via the thalamus. We hypothesized that targeted brain stimulation applied to discrete cerebellar functional connectivity nodes involved in attention would correlate with both changes in network connectivity and performance on a sustained attention task. Methods Resting-state functional connectivity was acquired on normal healthy subjects and used to precisely locate individualized network nodes of two networks (DN and DAN) within their cerebellum. Before and after stimulation, fMRI was collected during resting-state and task-state. During the 10-minute task-state fMRI, subjects performed a continuous performance task (gradCPT) for the entire sequence (Esterman et al. 2014). Subjects then received one session of targeted brain stimulation to either the DAN or DN using an intermittent theta-burst (iTBS) protocol. Commission errors, omission errors, reaction times, and reaction time variability during the task were assessed. Subject-level changes in functional connectivity within the DAN and DN (post vs. pre) were correlated to changes in behavioral measures and stimulation site. Results: Attentional performance - as measured by changes in commission errors on the gradCPT- was modulated by site-specific stimulation of the cerebellum. Preliminary data in 6 subjects suggest that the within network increases in connectivity in the default network corresponds to decreased commission errors on the sustained attention task. Conclusion Targeted brain stimulation to discrete networks involved in attention modulates both functional connectivity within the network and performance on a sustained attention task.

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Poster

088. Attentional Networks

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Topic: H.02. Human Cognition and Behavior

Support: Natural Science Foundation of China (31371127)

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Guangdong Province Universities and Colleges Pearl River Scholar Funded Scheme (2014)

Scientific Research Foundation of Graduate School of South China Normal University

Title: Neural practice effect during cross-modal selective attention: general and modality-specific effects

Authors: J. XIA, Y. LI, Y. JIANG, L. SHEN, *Q. CHEN;
South China Normal Univ., Guangdong, China

Abstract: Practice and experiences gradually modify the central nervous system, from the synaptic level to large-scale neural networks. Studying the effect of practice on cortical plasticity is crucial for us to understand the neural mechanisms of cortical reorganization as well as the mechanisms of functional recovery after brain injuries. It remains unknown, however, the supra-modal and modality-specific effects of practice during cross-modal selective attention. In two fMRI studies, we adopted a cross-modal interference paradigm in conjunction with the fMRI hybrid design. In alternating task blocks, participants attended to either the visual or the auditory component of the audiovisual stimuli and ignored information from the unattended modality. The temporal position of each and every trial in a given block was included as a covariate to calculate how neural activity changed as the first and second order function of the time of practice. Results from the two fMRI studies consistently showed that with the progress of practice, neural activity linearly decreased in the frontoparietal central executive network (CEN) while increased in the default mode network (DMN). In addition, neural activity in the supplementary motor area (SMA) changed as the second order function of practice by showing increased transient neural activity at the onset and offset of a block while sustained during the block. Critically, the practice effect in the CEN and the DMN occurred independent of the modality attended, indicating the general mechanisms of selective attention. On the other hand, increasing extent of functional decoupling between the auditory and the visual systems was observed with the progress of practice, and varied as a function of the modality attended. During auditory attention, the auditory system was functionally decoupled with both the dorsal and ventral visual stream with the practice; during visual attention, the auditory system was decoupled with the ventral visual stream only. Taken together, both supra-modal mechanisms in the CEN and DMN and modality-specific mechanisms in the sensory systems associated with practice were revealed during cross-modal attention.

Disclosures: **J. Xia:** None. **Y. Li:** None. **Y. Jiang:** None. **L. Shen:** None. **Q. Chen:** None.

Poster

088. Attentional Networks

Location: Halls B-H

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Program#/Poster#: 88.18/III43

Topic: H.02. Human Cognition and Behavior

Title: Functional changes in early latency medial prefrontal cortex activity following attention bias modification training: A near-infrared spectroscopy study

Authors: *J. ADAY, W. RIZER, J. CARLSON;
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Abstract: Attention bias modification (ABM) is a computerized cognitive training regimen that attempts to reduce attentional biases towards threat by continuously directing participants' attention away from threatening stimuli. fMRI studies utilizing ABM have found training-related changes in prefrontal cortex (PFC) activity and previous research from our lab has shown structural changes in PFC volume. Near-infrared spectroscopy (NIRS) is a noninvasive method of measuring oxygenated (HbO) and deoxygenated (HbR) hemoglobin, which are indirect measures of neural activity. Relative to fMRI, NIRS has several advantages including mobility, affordability, ease of use, and temporal resolution. Although ABM has been shown to modulate prefrontal activity using fMRI, the temporal resolution of these ABM effects on prefrontal activity remain poorly understood. To explore this, we had participants undergo 6 weeks of at-home ABM training using a cellphone app. Before and after training, participants performed an event-related dot-probe task while their brain activity was recorded using NIRS. There was an optode x session interaction 2-5 seconds post-stimulus presentation, such that optodes measuring more medial prefrontal activity showed decreases in HbR from pre to post-training. ABM training also resulted in greater HbR for congruent trials relative to incongruent and baseline trials in the medial prefrontal cortex. These results are consistent with previous fMRI studies implicating the PFC in ABM training and indicate that training-related changes in the PFC can be recorded using NIRS, suggesting early (2-5 sec) ABM-related effects on PFC activity.

Disclosures: **J. Aday:** None. **W. Rizer:** None. **J. Carlson:** None.

Poster

088. Attentional Networks

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant NS79698

Title: Effects of attention state regulation training on resting-state functional connectivity

Authors: *A. EICHENBAUM[1], S. M. YOUSEF[2,3], C. GALLEN[1], E. S. POOL[1,5], A. J. W. CHEN[1,4,5], M. A. SILVER[1,2,3], M. D'ESPOSITO[1];
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Abstract: Attention state regulation training has been shown to enhance cognitive functioning. There is some evidence to suggest that the resulting improvements in cognition are associated with changes in activity and connectivity in the Default Mode Network (DMN) and frontal-parietal (FP) networks; however, these findings have been inconsistent. Additionally, a critical component of successful paradigms is training across different modalities and contexts. Therefore, to better understand the neural bases by which training can enhance cognitive functioning, we examined how intrinsic brain networks derived from resting-state fMRI data are affected by an intensive multi-domain attention state regulation training paradigm.
During training, subjects (n=8, mean age = 23.8) attended classes where they learned about and practiced attention state regulation techniques applied to game scenarios and personal life goals. State regulation techniques were applied in both simple and complex cognitive contexts, with an overarching emphasis on using these techniques to manage distractions. The seven-week training paradigm incorporated classroom and individualized coaching with aspects of experiential skill application. Before and after the seven-week course of training, a 5-minute resting-state fMRI scan was collected to examine training-induced changes in connectivity in intrinsic networks. A battery of behavioral tasks measuring attention and working memory was also administered before and after training. The fMRI data were parcellated into regions of interest (Power et al., 2011), focusing on the DMN and FP networks.
Training resulted in improved performance on the SART (sustained attention to response task) ($p = .04$), a measure of sustained attention and inhibitory control. Training also increased within-network connectivity in the DMN ($p = .02$) and connectivity between the DMN and FP networks ($p = .02$) but did not alter connectivity within the FP network ($p = .12$). These findings may indicate that attention state regulation training enhanced subjects' ability to filter out and ignore distractions during an internally-oriented state (i.e., at rest). Further experiments will examine

how the specific components of this training paradigm (e.g., classroom versus game play) contribute to behavioral and neural changes, as well as the relationships between changes in behavior and functional connectivity.

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Poster

088. Attentional Networks

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Topic: H.02. Human Cognition and Behavior

Support: DGAPA PAPIIT IN219516 to AERC

DGAPA PAPIIT IN218316 to OPG

DGAPA PAPIIT IA207416 to MMD

Title: Association study of the genetic variations of the CNR1 gene and executive attention

Authors: *E. I. ORTEGA MORA[1], U. CABALLERO-SANCHEZ[1], C. B. ROSAS-ESCOBAR[1], T. V. ROMÁN-LÓPEZ[1], J. A. GONZALEZ-BARRIOS[2], S. ROMERO-HIDALGO[3], F. VADILLO-ORTEGA[4], M. MENDEZ-DIAZ[5], O. PROSPÉRO-GARCÍA[5], A. E. RUIZ-CONTRERAS[1];
[1]Univ. Nacional Autónoma De México; Fac. Psi, Ciudad De México, Mexico; [2]Lab. Medicina Genómica, Hosp. Regional "Primero de Octubre", Inst. de Seguridad y Servicios Sociales de los Trabajadores del Estado (ISSSTE), Cd. Mexico, Mexico; [3]Dept. de Genómica Computacional, Insituto Nacional de Medicina Genomica (INMEGEN), Cd. Mexico, Mexico; [4]Unidad de Vinculación Científica, Facultad de Medicina, UNAM, INMEGEN, Cd. Mexico, Mexico; [5]Gpo. Neurociencias: Lab. Canabinoides, Depto. Fisiologia, Fac. Medicina, Univ. Nacional Autonoma de Mexico, Cd. Mexico, Mexico

Abstract: Attention is the ability to select relevant information and, at the same time, inhibit irrelevant stimuli in a goal-directed behavior. Attentional Network Task (ANT) is useful to evaluate different systems of attention, such as alert, orientation and executive attention. For executive attention, participants indicate the orientation of the central target arrow, among four distractor flanker arrows. There were congruent (where the target and the flanker arrows point to the same orientation) and incongruent (where a conflict occurs, because the target arrow points to a different orientation from the flanker arrows) trials. With this task, a lower accuracy and

longer reaction times for incongruent vs. congruent trials are observed. Individual differences have been reported on the executive system, specifically associated to a dopamine receptor. In our workgroup, we are interested in evaluating the genetic association of the cannabinoid receptor 1 (CB1) because it is widely distributed in brain regions involved in attention and memory functions. The CNR1 gene codes for the CB1 receptor. We are interested in two polymorphisms of a single nucleotide in this gene: rs2023239 (T>C), which has been associated with addiction and impulsive behavior, as well as a larger CB1 expression at the prefrontal cortex; and rs1406977 (A>G), which has been associated with less prefrontal mRNA levels for CB1 and reduced working memory behavioral accuracy. Therefore, we could consider the C and G allele as risky ones for rs2023239 and rs1406977, respectively. The aim of this study was to test whether efficiency in the ANT is associated with the polymorphisms rs2023239 and rs1406977 of the CNR1 gene. Our results showed a main effect of genotype: C carriers of rs2023239 and G carriers of rs1406977 showed a lower percentage of correct responses in the whole task compared to the carriers of the complementary alleles, regardless of the trial type. These results show that the alternative genotypes of rs2023239 and rs1406977, previously associated with lower presence of CB1 protein or mRNA, are also associated with the ability to score higher on the executive system of the ANT, which suggest a potential function of the CB1 receptor and endocannabinoids to modulate this cognitive function.

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Poster

088. Attentional Networks

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 88.21/DP07 (Dynamic Poster)

Topic: H.02. Human Cognition and Behavior

Support: NIH/NIMH R33 MH096967

Title: The AttentionTrip: A game-based assessment of attention networks in autism

Authors: L. E. MASH[1], *J. TOWNSEND[2], Q. LUO[3], R. KLEIN[5], L. CHUKOSKIE[4]; [1]SDSU/UCSD Jt. Program in Clin. Psychology, San Diego, CA; [2]Dept Of Neurosci, [3]Dept Of Biol. Sci., [4]Inst. for Neural Computation, UCSD, La Jolla, CA; [5]Psychology & Neurosci., Dalhousie Univ., Halifax, NS, Canada

Abstract: A well-accepted model of attention specifies three distinct functional networks: alerting, orienting, and executive control (Posner & Petersen, 1990). These network constructs can be measured using the Attention Network Test (ANT; Fan et al, 2002) in which participants make responses to a target stimulus in the presence of both informative and uninformative cues and flankers. However, this task is necessarily long and repetitive, may yield invalid data in participants with low motivation or poor engagement. Klein and colleagues have developed a more engaging game-based assessment of the attention networks measured by the ANT. In this instrument (the AttentionTrip), participants use a driving wheel to navigate a small space ship through a wormhole or blood vessel. Players pilot the ship while responding to target stimuli. The game incorporates spatial and temporal cues as well as congruent and incongruent target flankers. In tests of typical adults, the AttentionTrip yielded robust indices for each of the three target attention networks (Klein & Wilson, 2013). A more enjoyable assessment of attention networks could particularly benefit individuals with ASD (e.g., poor motivation may affect results on or willingness to complete the traditional ANT). Attention is of high interest in ASD and attentional deficits may be among the earliest foundational features of the disorder (Keehn et al, 2013). A previous study in children with ASD found that the ANT can identify attention impairment in this group (Keehn et al, 2010). The current study examined the AttentionTrip for suitability of use with individuals on the autism spectrum. In a sample of teens and young adults with ASD, the AttentionTrip produced reliable indices for the three target attention networks (alerting, orienting and executive function).The goal of the second phase of this study is to examine teens and young adults with ASD and a matched typical sample using the ANT and AttentionTrip to: 1) directly compare results from the two instruments and 2) to replicate results from Keehn et al, 2010. There is increasing interest in the use of video games for assessment and intervention. Games have a distinct advantage in that they are intrinsically motivating, and offer opportunities to improve data quality by improving task compliance. Games such as the AttentionTrip that are based on theoretical models and specifically designed to target well-defined cognitive processes provide an important and exciting advance in neuropsychological evaluation.

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Poster

088. Attentional Networks

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Topic: H.02. Human Cognition and Behavior

Support: NIH Grant 1R01EY026701-01

COBRE P20GM103650

Title: Greater flanker effects for real vs. images of action-based stimuli

Authors: *M. A. GOMEZ[1], R. SKIBA[2], J. C. SNOW[2];
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Abstract: Object affordances are understood to bias visuo-spatial attention. Surprisingly, however, previous studies have relied predominantly on images, rather than real world graspable objects, to examine affordance-related effects. In the present study, we examined whether real graspable tool objects elicit stronger flanker effects than matched two-dimensional (2D) and three- dimensional (3D) image displays of the same items. Using an adaptation of the Eriksen flanker paradigm, we asked participants to indicate the left/right orientation of a centrally positioned target (a spoon) while ignoring two identical flankers positioned above and below the central target. The flankers were oriented so that their handles would elicit either a congruent, or incongruent, response to that required for the target. Critically, we manipulated the display format of the target and flanker stimuli. Using a block design, on half of the trials participants viewed real object displays, and on the remaining trials participants viewed high-resolution computerized images of the same stimuli that were presented on an LCD monitor. The images were matched to the real objects for size, apparent distance, viewpoint and background. In Experiment 1 the target and flankers were 2D planar images of objects. We found that reaction times (RTs) were longer overall for real objects compared to the 2D image arrays. Critically, we found that real flankers elicited greater interference effects than in the matched image arrays. To determine whether the results for Experiment 1 were attributable to additional stereo cues in the real objects (versus the 2D images) we ran a follow-up Experiment comparing flanker interference effects for real objects versus 3D stereo arrays. Again, we found that RTs were longer for real objects compared to 2D images and that real flankers caused greater interference compared to 3D image stimuli.

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Poster

088. Attentional Networks

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Topic: H.02. Human Cognition and Behavior

Support: NSERC Alexander Graham Bell CGS

Title: Investigating the influence of expertise and familiarity on segmentation of dance movements and working memory

Authors: *P. M. DI NOTA[1,2,3], M. P. OLSHANSKY[1,2], J. F. X. DESOUZA[1,2,3,4,5];
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Abstract: Several cognitive and behavioral improvements have been demonstrated following exercise in the short-term (Di Noto et al., 2013) and with long-term expertise, especially in attention and working memory (WM). Attention can be measured by event segmentation - a perceptual process by which observed movement is partitioned into discrete meaningful units, and facilitates learning of these actions (Zacks et al., 2007; 2009). When segmenting dance, expertise in the observed repertoire has been shown to result in significantly fewer event borders (Bläsing, 2015), but generalized expertise effects in segmenting unfamiliar genres of dance or non-dance movements have yet to be shown. The present study sought to investigate differences in segmentation of a ballet dance, a Bharatanatyam dance, and a non-dance acting sequence among experts from six groups (total N=93): ballet and Bharatanatyam dancers, dancers from other genres (i.e., contemporary), athletes, musicians, as well as non-experts. Each video sequence was 1-minute long and was presented 10 times randomized across three blocks for a total of 30 segmentation trials. Additionally, WM was assessed by having subjects perform a Retrieval-Induced Forgetting (RIF) task between blocks of event segmentation. In part one, subjects were shown 60 category-exemplar word pairs from 10 categories (i.e., FRUIT – apple), including Dance, Instrument, and Sport categories that were domain-specific to our expert groups. Practice recall (Pr) was performed immediately following presentation of the items, and final recall of practiced words (Rp+), non-practiced words from practiced categories (Rp-), and non-practiced words from non-practiced categories (Nrp) was assessed between blocks 2 and 3 of event segmentation. Results show significantly fewer event borders among Bharatanatyam dancers when segmenting a familiar dance sequence relative to dancers from other genres during early trials (trials 1 to 5, $P<0.05$), providing novel evidence for genre-specific familiarity effects during dance segmentation. Consistent with previous studies, practice effects for strong and weak items and RIF were shown among all groups ($P_{Bonf}=0.000$). However, we provide novel evidence for significantly enhanced recall of words from experientially-primed categories (i.e., Dance and Instruments) among ballet dancers relative to musicians, athletes ($P_{Bonf}=0.000$), and non-experts ($P_{Bonf}=0.001$). Together these findings provide further evidence for the modulation of observed action by the specificity of one's motor repertoire, and the translational benefits of dance expertise in improving WM for domain-specific items.

Disclosures: P.M. Di Nota: None. **M.P. Olshansky:** None. **J.F.X. DeSouza:** None.

Poster

088. Attentional Networks

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Topic: H.02. Human Cognition and Behavior

Support: Grant from the National Science Centre of Poland (2013/09/N/HS6/02947)

Title: How crossing hands modulates neural processes involved in directing attention to painful and tactile stimuli

Authors: *K. J. SWIDER[1,2], E. WRONKA[1], C. VAN RIJN[2], J. OOSTERMAN[2];
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Abstract: Presentation of an attention-directing cue elicits a lateralized ERPs components: Early Directing Attention Negativity (EDAN) at posterior electrodes contralateral to the direction of attentional shifts (200-300 ms after cue onset), Anterior Directing Attention Negativity (ADAN) at frontal cortex contralateral to the cue direction (300-500 ms after cue onset) and Late Directing Attention Positivity (LDAP) recorded in visual cortex contralateral to the cue direction (300-600 ms after cue onset). Those components are suggested to reflect different stages in the control of attention. Interestingly, crossing hands over the body midline can bring into conflict the internal somatosensory and visual frame of reference. Such manipulation can modify early stages of orienting shifts. Thus, the main goal of the present study was to verify whether the same neuronal processes are involved in directing attention to tactile and painful stimuli in crossed and uncrossed hand position. The participants (23 right-handed volunteers) crossed and uncrossed their hands at the beginning of each of 8 painful (4 crossed & 4 uncrossed) and 8 tactile (4 crossed & 4 uncrossed) blocks of the experiment. Each of the electric stimuli was preceded by a visual cue (rightward and leftward arrows) presented in the center of a computer screen. In 80% of trials the cues indicated correctly the side of the stimuli application. There were 400 trials in total where 100 painful and 100 tactile stimuli were delivered to each hand. The participants' task was to rate their sensation after each stimuli on Numerical Rating Scale. ERPs were recorded with 64-channels EEG in response to cues which directed attention to either side. Mean amplitudes for EDAN, ADAN and LDAP were compared with a repeated measures ANOVA. The negative (EDAN, ADAN) and positive (LDAP) deflections were found over the hemisphere contralateral to the hypothesized direction of shifts of attention. The ERPs showed double dissociation of stimuli intensity and hand position effects. When hands were kept crossed the EDAN and ADAN were stronger in painful compered to tactile condition. In tactile condition, EDAN and ADAN were found only in uncrossed condition. In painful condition no such difference for neither neurocorelatas was found. Higher activation was found in uncrossed

condition compared to crossed one for LDAP but only for painful stimuli. Simultaneously, the activation was more pronounced in painful condition for all lateralized ERPs components. Summing up, our results indicate that when we change the frame of reference we can manipulate early stages of orienting shifts and such effect is stronger for tactile stimuli.

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Poster

088. Attentional Networks

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Topic: H.02. Human Cognition and Behavior

Support: TKF Foundation Grant

Title: Investigating the scale invariance and global connectivity of BOLD when viewing natural versus man made scenes

Authors: ***O. KARDAN**[1], M. G. BERMAN[1], J. JONIDES[2];
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Abstract: Free viewing of pictures of nature vs. urban scenes have been shown to have restorative effects on cognition and affect, specifically improving performance in directed attention tasks [1,2]. Attention restoration theory (ART) posits that unlike man-made scenes, processing nature *modestly* grabs attention in a bottom-up fashion, allowing top-down directed-attention abilities a chance to replenish. Additionally, cognitive effort has been shown to be inversely related to fractal scaling of brain activity [3]. Thus, we hypothesized that global scale-invariance of Blood Oxygenation Level Dependent (BOLD) fMRI as indexed by the Hurst exponent of the signal might increase when processing nature scenes compared to when viewing man-made scenes. Eighteen participants viewed blocks of 50 images of nature and 50 images of urban scenes in the MRI scanner for 200 seconds in each block. For our analysis we parcellated the brain into 116 regions using the Automated Anatomical Labeling (AAL) scheme. We performed a Partial Least Squares (PLS) regression to statistically compare the scale-invariance and connectivity of these AAL brain regions for the two conditions. Global scale-invariance of BOLD dynamics over all regions significantly increased in nature blocks compared to urban blocks in 14 out of the 18 participants ($p_{adjusted} < 0.05$). However, the regions that drove these differences were idiosyncratic to participants, indicating that the multivariate results did not show a generalizable pattern of regions distinguishing nature and urban blocks ($p = 0.4611$). Additionally, global connectivity significantly increased for eight of the participants when

viewing the nature blocks, while it decreased significantly for two participants ($p_{adjusted} < 0.05$). Again, the spatial patterns were idiosyncratic to participants. The only consistent connectivity pattern that was found across participants was higher connectivity between right VI of the cerebellum and the right fusiform gyrus when viewing urban blocks ($Z_{bootstrap\text{-}ratio} > 3$). [1] Berman, M. G., Kross, E., Krpan, K. M., Askren, M. K., Burson, A., Deldin, P. J., . . . Jonides, J. (2012). Interacting with nature improves cognition and affect for individuals with depression. *Journal of Affective Disorders, 140*(3), 300-305. doi: 10.1016/j.jad.2012.03.012 [2] Berman, M. G., Jonides, J., & Kaplan, S. (2008). The cognitive benefits of interacting with nature. *Psychological science*, *19*(12), 1207-1212. [3] Churchil, N. W., et al., in-revision. The suppression of global scale-free brain dynamics across three different sources of effort: effects of aging, task novelty and task difficulty

Disclosures: O. Kardan: None. **M.G. Berman:** None. **J. Jonides:** None.

Poster

088. Attentional Networks

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 88.26/III50

Topic: H.02. Human Cognition and Behavior

Title: Implicit discrimination of natural versus built environments as evidenced by p3 elicitation

Authors: *S. P. MAHAMANE[1], A. PORTER[3], A. HANCOCK[2], N. WAN[1], K. E. JORDAN[1]; [1]Psychology, [2]Utah State Univ., Logan, UT; [3]Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Attention Restoration Theory (ART) posits that settings with certain psycho-environmental characteristics (most often natural environments) improve directed attention by aiding in the replenishment of cognitive resources. Evidence shows that natural environments, compared to built, improve people's ability to sustain focused attention and delay gratification, and increase positive affect. However, are these effects only attributable to how these environments engage visual attention or is environmental semantic category itself – being "nature" – important? To begin to address this question, we used hundreds of natural and built environment scenes in a passive looking task while recording EEG data from participants at 14 channel locations. Sixty participants viewed two blocks of trials with block order counterbalanced across participants. Stimuli for the task were selected based on data from a previous explicit categorization task performed by a separate sample of participants. In one block (nature-standard) 80 out of 100 images were nature scenes (categorized as "nature" by 60% or more of the previous sample) and 20 were built (categorized as "nature" by 40% or less). In the other block (built-standard), these category frequencies were reversed. Results showed that, in

the nature-standard block, P3 peak amplitude averaged across frontal channels was significantly higher for the low frequency built images than for the high frequency nature images. This finding suggests that, even without instruction that the task included different environmental scene categories with respect to content, participants implicitly discriminated natural from built environments using P3 as an index of stimulus novelty. However, the converse result was not significant in the built-standard block (p = .094). This inconsistency may be a result of the nature images, and thus the nature-standard block, being more restorative than the built images. These findings offer further justification to explore the role of environmental semantic content in attention restoration.

Disclosures: S.P. Mahamane: None. **A. Porter:** None. **A. Hancock:** None. **N. Wan:** None. **K.E. Jordan:** None.

Poster

088. Attentional Networks

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 88.27/III51

Topic: H.02. Human Cognition and Behavior

Title: Acute effects of a proprietary spearmint extract on cognition in healthy men and women

Authors: *K. A. HERRLINGER[1], B. J. LEWIS[1], J. A. LASRADO[1], K. D. SANOSHY[2], J. M. BALDWIN[2], E. MAH[2], B. A. FONSECA[1];
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Abstract: A proprietary spearmint extract, Neumentix™ Phenolic Complex K110-42, has shown positive effects on cognition following chronic supplementation in healthy adults with memory impairment. Two randomized, double-blind, placebo-controlled, crossover studies, (n=6/study) evaluating the acute effects of Neumentix on cognitive function were completed in full-time college students (18-24 y) and adults aged 30-50 y. Subjects completed a computerized cognitive function test battery and a Subjective Global Improvement (SGI) questionnaire 1 h before and 2 h after intake of either placebo (PL) or 900 mg Neumentix (Neu). After a 13-day washout period subjects crossed over to the opposite study product and repeated these procedures. Data (mean±SEM) were evaluated as the change from pre to post-supplementation, with P≤0.1 considered significant.
Objective measures of attention increased in both groups two hours following supplementation with Neu. In adults aged 30-50 y, scores for Feature Match (attention) were improved following acute Neu supplementation compared to PL (P=0.067). In college students, acute supplementation resulted in differences between Neu and PL for a measure of attention (Spatial

Rotations, P=0.088) and spatial working memory (Monkey Ladder, P=0.061). Within group scores for Spatial Rotations were increased following Neu (P=0.099), but were unchanged following PL. Scores for Monkey Ladder decreased following consumption of PL (P=0.041), while Neu prevented this decrease. Total SGI scores of college students were different between Neu vs. PL (P=0.093), suggesting that subjects noticed a subjective improvement in cognition with Neu vs. PL. Evaluation of pooled data (n=12) from both groups showed differences between Neu and PL for a measure of attention (Interlocking Polygons, P=0.0728), with improvements following Neu. Furthermore, pooled data from both groups showed differences between Neu and PL for subjective evaluation of memory, speed of thinking and Total SGI (P=0.027, P=0.086, P=0.057, respectively), with improvements following Neu.
These preliminary data show that consumption of Neu improved subjective assessment of cognitive improvements and objective measures of attention in both age groups. Furthermore, college students were able to maintain spatial working memory performance after consumption of Neu but not after PL. In conclusion, Neumentix, which was previously shown to have improved working memory benefits following chronic supplementation, may also have acute benefits on cognitive function which individuals were able to recognize themselves within two hours following supplementation.

Disclosures: **K.A. Herrlinger:** A. Employment/Salary (full or part-time): Kemin Foods, LC. **B.J. Lewis:** A. Employment/Salary (full or part-time): Kemin Foods, L.C. **J.A. Lasrado:** A. Employment/Salary (full or part-time): Kemin Foods, L.C. **K.D. Sanoshy:** A. Employment/Salary (full or part-time): Biofortis Inc. **J.M. Baldwin:** A. Employment/Salary (full or part-time): Biofortis Inc. **E. Mah:** A. Employment/Salary (full or part-time): Biofortis Inc. **B.A. Fonseca:** A. Employment/Salary (full or part-time): Kemin Foods, L.C..

Poster

089. Decision Making: Avoidance and Uncertainty

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 89.01/III52

Topic: H.02. Human Cognition and Behavior

Title: Effects of unilateral lesions of the insula and of the medial temporal lobe on taking risky decision: a study of epileptic patients

Authors: *Z. VON SIEBENTHAL[1], O. BOUCHER[2], I. ROULEAU[3], M. LASSONDE[4], F. LEPORE[5], D. K. NGUYEN[6];
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QC, Canada; [5]Univ. de Montréal, Montréal, QC, Canada; [6]Ctr. Hospitalier de l'Université de Montréal, Montréal, QC, Canada

Abstract: According to the somatic marker hypothesis, the insula and medial temporal lobe structures - especially the amygdala and hippocampus - are actively involved in risky decision making. However, the specific effects of damage to these structures remain unclear, partly due to the low prevalence of isolated lesions affecting either of these brain structures. In this study, adult patients who underwent partial or complete resection of the insula (n = 13) or of the medial temporal lobe (n = 13) as part of their epilepsy surgery, and a group of healthy volunteers (n = 20), were assessed using two tasks of decision making: the Iowa Gambling task (IGT), which assesses the ability to learn to select economically advantageous options in the long term, and Cups Task, which assesses the abilitiy to adjust to the expected value when taking risk, in terms of gain and loss separately. Group performance was compared using nonparametric tests. On the IGT, temporal lobe patients performed significantly worse than both the insular and the healthy control groups, as they failed to learn which decks were advantageous on the long term. On the Cups Task, both groups of patients showed impaired risk adjustment when facing a potential loss, when compared to healthy controls. These results suggest that the insula and medial temporal lobe structures play a crucial role in risky decision making when facing a potential loss, and that the temporal structures are additionally involved in learning the association between one's behavior and its outcome.

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Poster

089. Decision Making: Avoidance and Uncertainty

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 89.02/JJJ1

Topic: H.02. Human Cognition and Behavior

Support: FWO G.OC44.13N

Title: Context and outcome uncertainty in anterior insula

Authors: *W. H. ALEXANDER, E. VASSENA;
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Abstract: Risk and uncertainty are inherent to all decisions - choosing whether to eat at an Italian or Mexican restaurant, for example, entails uncertainty as to whether the food will be

acceptable or not, i.e., outcome uncertainty. Uncertainty of this sort may be compounded by uncertainty regarding the context in which such choices are made - if a person is unaware whether they are in Rome or San Diego when making a choice about restaurants, they may lack critical information needed to choose wisely ("context uncertainty"). Frequently these two types of uncertainty are confounded - uncertainty about the context in which one is operating leads to increased uncertainty about the ultimate outcome of one's choices. Anterior insula has previously been identified as a region involved in signaling predictions and errors regarding the uncertainty of an outcome. However, it remains an open question as to whether the region might also signal context uncertainty. We investigate this question using fMRI while subjects perform a task to earn money by guessing whether one of two playing cards will be higher or lower than the other (outcome uncertainty) while simultaneously trying to determine from which of two decks the cards they observe are being drawn (context uncertainty). The design of the experiment allows dissociation of these two forms of uncertainty by holding one type of uncertainty constant while manipulating the other. We find distinct signals in anterior insula related to both types of uncertainty, suggesting that anterior insula engages in processing risk at multiple levels.

Disclosures: W.H. Alexander: None. **E. Vassena:** None.

Poster

089. Decision Making: Avoidance and Uncertainty

Location: Halls B-H

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Program#/Poster#: 89.03/JJJ2

Topic: H.02. Human Cognition and Behavior

Support: Medical Research Council

European Research Council, Grant 260424

Title: Noradrenergic manipulations of dynamic uncertainty computations

Authors: *L. MARSHALL[1], A. O. DE BERKER[1,2], R. SOUTHWELL[3], G. QUATTROCCHI[1], S. LITTLE[1], S. BESTMANN[1];
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Abstract: Learning in uncertain environments depends on our ability to flexibly update our beliefs about the world. By tracking the environment’s underlying regularities and their changes over time, one can learn to predict, from sensory cues, the likelihood of future events. Noradrenaline (NA) has been proposed to play an important role in learning under uncertainty

that arises from environmental volatility (Yu & Dayan, 2005; Payzan-LeNestour et al., 2013; Marshall et al., submitted). Parallel lines of work have linked subjective uncertainty computations to changes in pupil diameter (Preuschoff et al., 2011; Nassar et al., 2012; de Gee et al., 2014; de Berker et al., 2016), and pupil dilatation to noradrenergic neuronal activity in the locus coeruleus (Aston-Jones & Cohen, 2005; Varazzani et al., 2015; Joshi et al., 2016).
Here we manipulated NA pharmacologically to assess the neuromodulator's causal impact on learning in dynamic environments and on pupillary responses to uncertainty. In a double-blind between-subjects design, 90 healthy human volunteers were administered a NA reuptake inhibitor (reboxetine; NA+ group), a NA antagonist (prazosin; NA- group), or a placebo before undertaking a probabilistic learning task with concurrent pupillometry. On each trial, subjects were presented with one of two auditory cues and asked to predict which one of two auditory outcomes would follow. Crucially, the contingencies between cues and outcomes shifted unpredictably over time, introducing uncertainty and requiring subjects to constantly update their estimates for each stimulus.
Using a hierarchical Bayesian learning model (Mathys et al., 2011), we quantified the relationship between the different forms of subjective task uncertainty and pupil diameter, and contrasted each NA drug manipulation to placebo. Both NA interventions disrupted task learning. Across groups, baseline pupil diameter reflected subjects' trial-wise estimates of the current cue-outcome contingencies. Individual uncertainty estimates predicted the magnitude of pupil dilatation responses to trial outcomes. The magnitude of pupil evoked responses to outcome differed across groups, driven by a significant increase in pupil responsivity under our NA+ manipulation. Collectively, our results provide empirical support for the involvement of NA in pupillary responses to dynamic uncertainty computations.

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Poster

089. Decision Making: Avoidance and Uncertainty

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Program#/Poster#: 89.04/JJJ3

Topic: B.08. Synaptic Plasticity

Support: BMBF grant "Netzwerk psychische Erkrankungen", grant 01EE1403C

Title: Acute and chronic effects of noradrenergic enhancement on transcranial direct current stimulation-induced neuroplasticity in humans

Authors: *M. A. NITSCHE[1], A. JAMIL[1], W. PAULUS[2], G. BATSIKADZE[2], M.-F. KUO[1], L. KUO[1];
[1]Leibniz Res. Ctr. For Working Envrn. An, Dortmund, Germany; [2]Univ. Med. Ctr., Goettingen, Germany

Abstract: Noradrenaline affects cognition and motor learning processes via its impact on long-term potentiation (LTP) and depression (LTD). We aimed to explore the impact of single dose and chronic application of the selective noradrenaline reuptake inhibitor (NRI) reboxetine (RBX) on plasticity induced by transcranial direct current stimulation (tDCS) in healthy humans via a double-blinded, placebo-controlled, randomized crossover study. 16 healthy volunteers received placebo or single dose RBX (8mg) before anodal or cathodal tDCS of the primary motor cortex. Afterwards, the same subjects took RBX (8 mg/day) consecutively for 21 days. During this period, two additional interventions were performed (RBX with anodal or cathodal tDCS), to explore the impact of chronic RBX treatment on plasticity. Plasticity was monitored by motor evoked potential amplitudes elicited by transcranial magnetic stimulation. Chronic application of RBX increased and prolonged the LTP-like plasticity induced by anodal tDCS for over 24 hours. Chronic RBX significantly converted cathodal tDCS-induced LTD-like plasticity into facilitation, as compared to the single dose condition, for 120 minutes after stimulation. The results show a prominent impact of noradrenaline receptor enhancement on plasticity of the human brain which might partially explain the delayed therapeutic impact of selective NRI in depression and other neuropsychiatric diseases.

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Poster

089. Decision Making: Avoidance and Uncertainty

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Topic: H.02. Human Cognition and Behavior

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Title: Serotonin depletion impairs instrumental avoidance

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Abstract: Serotonin (5HT) has long been implicated in the motivational control of behavior, particularly aversive processing1 and behavioral inhibition[2]. One popular recent idea is that 5HT has a specific role in driving instrumental inhibition in aversive contexts[3–5], while others postulate of particular importance the role of 5HT in valence-independent control of impulsive responding[6].
To pit these theories against each other, we independently varied instrumental demands (active approach/avoid, passive avoid) and motivational value (appetitive/aversive), while manipulating 5HT levels using acute tryptophan depletion (ATD) in healthy humans (N=25, randomised double blind within-subject). On each trial, a cue informed subjects whether they were playing to get a reward or avoid a punishment. To get the desired outcome, they had to learn whether to approach (mouse-click on a box in the location of the cue), avoid (mouse-click on a box presented contra-laterally to the cue) or inhibit responding (NoGo).
As expected, participants showed a motivational bias in their response tendencies: they were more likely to approach appetitive cues, and to inhibit responding to aversive cues. This effect was paralleled by their reaction times (RT): participants were faster to respond to appetitive than to aversive cues. However, ATD did not affect these motivational valence biases.
Surprisingly, motivational valence did not alter 'avoid' responses. Avoid responses were slower than approach responses, but this was unaffected by motivational valence. Critically, ATD reduced the number of avoid responses, while also increasing the proportion of inaccurate approach responses for fast RTs, again irrespective of motivational valence. These results support a selective role for 5HT in instrumental avoidance.
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Disclosures: **H.E. Den Ouden:** F. Consulting Fees (e.g., advisory boards); Consultant Eleusis Benefit Corp.. **K. Schmidt:** None. **J.C. Swart:** None. **D.E.M. Geurts:** None. **N.D. Daw:** None. **R. Cools:** None.

Poster

089. Decision Making: Avoidance and Uncertainty

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Program#/Poster#: 89.06/JJJ5

Topic: H.02. Human Cognition and Behavior

Support: McDonnell Foundation MH099078

R01 NIH NS065046

Title: Neural systems supporting demand avoidance.

Authors: *C. Z. SAYALI, N. HAMZAH, B. CIULLO, D. BADRE;
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Abstract: Performing difficult tasks results in an experience of effort. This “cognitive effort” is usually aversive such that people will tend to avoid cognitively difficult tasks in favor of the easier ones when given the option. One influential hypothesis is that effort costs are experienced to the degree that cognitive control systems are engaged during a task. However, it has not been established what neural systems drive cognitive effort avoidance, with separate studies yielding sometimes contradictory results. The differences across studies might partly stem from design limitations in prior experiments that relied primarily on categorical comparisons. To overcome these limitations, we manipulated the level of effort during fMRI scanning by parametrically increasing cognitive demands during a demand-selection paradigm. During a “choice phase”, participants chose between two context symbols that were each associated with a block of a different difficulty level. Then, during an “execution phase”, participants executed a block of the corresponding task difficulty level. Specifically, participants completed thirteen simple number categorizations, either magnitude or parity judgments based on the color of the number. Task difficulty was manipulated by increasing the likelihood that the judgment type switched from trial-to-trial within a block. A higher frequency of switching required more cognitive control and so more cognitive effort. The association of a given difficulty level and context symbol was acquired through experience. Of 36 participants, 20 were 'demand avoiders' who tended to avoid choosing the more difficult task context, and 16 were 'demand seekers' who did not avoid or even sought the harder task. Demand avoiders showed a linearly increasing avoidance with greater task difficulty. Both groups showed comparable behavioral performance and neural activity during task execution. Likewise, executing increasingly more difficult tasks yielded linearly greater activity in the frontoparietal cognitive control network and greater deactivation in the task-negative default mode network in both groups. Preliminary analyses did not find evidence of a relationship between activation of the fronto-parietal control system across levels during execution and avoidance behavior during choice. Rather, deactivation of task-negative brain regions during execution diminished avoidance behavior in ‘demand avoiders’. These results

suggest that cognitive effort may be related to ineffectively engaging task-negative "down states", as opposed to intensely engaging cognitive control-related "up states".

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Poster

089. Decision Making: Avoidance and Uncertainty

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Program#/Poster#: 89.07/JJJ6

Topic: H.02. Human Cognition and Behavior

Title: Neural mechanisms of avoidance behavior for high cognitive demands

Authors: ***A. M. NAGASE**[1], K. MORITA[3], K. ONODA[2], J. C. FOO[3], T. HAJI[4], S. YAMAGUCHI[2], K. SAKAI[5];
[1]Grad. Sch. of Med. Res., [2]Dept. of Neurol., Shimane Univ., Izumo, Japan; [3]Physical and Hlth. Educ., Grad. Sch. of Education, The Univ. of Tokyo, Tokyo, Japan; [4]Brain Activity Imaging Ctr., ATR-Promotions, Osaka, Japan; [5]Tamagawa Univ., Brain Sci. Inst., Tokyo, Japan

Abstract: Humans tend to choose options to minimize not only physical but also mental cost. However, it remains unclear how human choice reflects predicted cognitive demand that changes over time. Neural mechanisms of decision making to avoid high cognitive demand also remain elusive. To address these issues, we developed two choice tasks that implicitly urge subjects to predict the upcoming cognitive demands, which changed over time. We conducted two fMRI experiments using either of the tasks. In Exp. 1, two problem-related cues appeared at the beginning of each trial, and subjects were asked to choose one of the cues. After choosing a cue, subjects were given a mental division problem. There were two types of problems with low or high cognitive demand, either one of which was presented in a trial. The presentation probabilities of low- or high-demand problems were determined by the problem-related cue that was chosen at the beginning of a trial, and were varied dynamically over time. Exp. 2 was similar to Exp. 1, but used "folding the cube" problems instead of division problems. In Exp. 1 and 2, 24 (75.0%) and 17 (89.5%) subjects, respectively, showed avoidance behavior for high cognitive demands (Chi-square test, $p<0.01$). We fitted the choices of subjects who showed demand-avoidance, via maximum likelihood estimation with limitations of parameter ranges, by 9 types of models: 6 types of prediction error-based (PE), 2 types of Win-Stay/Lose-Switch (WSLS), and one other models. These models, with 1, 2, or 3 parameters, were fitted to the choice data of the subjects who showed avoidance significantly. Based on Bayesian information criteria, we determined the best-fit PE model out of the 6 PE models and the best-fit WSLS model out of the 2 WSLS models. Both of the best-fit models had 2 parameters. We then compared the best-fit PE

and WSLS models. The PE and WSLS models fitted the data of Exp. 1 comparably. On the other hand, in Exp. 2, the WSLS model gave a better fit than the PE model in 76.5% of subjects. By using the PE model, we conducted model-based fMRI analysis for the subjects who showed avoidance. This fMRI analysis revealed that signals in the right inferior frontal gyrus (IFG, Brodmann area 47) were correlated with the predicted amount of cognitive demand of chosen option commonly in both experiments (peak & cluster-level FWE, $p<0.05$). These results suggest that humans could potentially learn to avoid high cognitive demand through mechanisms approximated by the PE model, and the right IFG (BA47) represents the predicted amount of mental cost across different types of cognitive demands. Additional analysis of the neural mechanism based on the best-fit WSLS model is planned to be conducted.

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Poster

089. Decision Making: Avoidance and Uncertainty

Location: Halls B-H

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Topic: H.02. Human Cognition and Behavior

Support: John D. and Catherine T. MacArthur Foundation to Vanderbilt University

NSF GRFP (AOC)

Title: Behavioral and neural correlates of cognitive control during out-group encounters under threat

Authors: *E. RUBIEN-THOMAS[1], A. O. COHEN[2], A. LI[2], D. V. DELLARCO[2], M. RHEINSCHMIDT-SAME[3], N. M. DAUMEYER[1], N. CAMP[4], B. L. HUGHES[4], D. A. FAIR[5], K. A. TAYLOR-THOMPSON[6], J. L. EBERHARDT[4], J. A. RICHESON[1], B. J. CASEY[1,2]; [1]Psychology, Yale Univ., New Haven, CT; [2]Sackler Institute, Weill Cornell Med. Col., New York, NY; [3]Psychology, Northwestern Univ., Chicago, IL; [4]Psychology, Stanford Univ., Stanford, CA; [5]Behavioral Neurosci. and Psychiatry, Oregon Hlth. and Sci. Univ., Portland, OR; [6]NYU Sch. of Law, New York, NY

Abstract: The recent string of deaths of unarmed men of color at the hands of police have prompted questions regarding what factors contribute to these outcomes. A key question is whether there is a relative loss in cognitive control during interracial encounters under conditions of threat. The current study used a novel functional magnetic resonance imaging (fMRI)

paradigm to examine how brief and sustained states of arousal modulate cognitive control during out-group encounters in 54 black and white adult participants (22 black, 28 female). Participants performed a variation of the Cognitive Control Under Emotional Influences (CCUE) task (Cohen et al., 2016) that consisted of positive, negative, and neutral facial expressions as targets and rare non-targets, under sustained states of threat (anticipation of hearing an aversive noise), excitement (anticipation of winning money), or a neutral state. The current task differed from the original in that stimuli were composed of 45% male black faces and 45% male white faces, to allow for analysis of out-group encounters, and an additional 10% male Asian or Hispanic faces. Under threat, both white and black participants show increased false alarms to calm African-American faces compared to Caucasian faces ($p<0.001$). Within the threat state, differences in false alarm rates between Caucasian and African-American cues were greatest for fearful no-go trials ($p=0.018$). Pilot fMRI data from the original CCUE task (N=72, 27 black, 40 female) with fewer minority faces showed a significant interaction between sustained state and target race in the ventromedial prefrontal cortex (vmPFC) for correct trials (186 voxels, $p=0.05$, $\alpha<0.01$) and anterior cingulate cortex (ACC) for all trials (43 voxels, $p=0.01$,$\alpha<0.01$). Compared to the neutral state, activity under the threat state in both the vmPFC (correct trials, $p=0.0005$) and ACC (all trials, $p<0.001$) increased to white faces and decreased for black faces. Together, these findings suggest a loss in impulse control, specific to the race of the stimulus presented, that is modulated by both sustained states of threat and cues of potential threat. This work may provide new insights into police-citizen interracial encounters under threat and offer new approaches for intervening.

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Poster

089. Decision Making: Avoidance and Uncertainty

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Program#/Poster#: 89.09/JJJ8

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant MH098023

Title: Neural basis of social learning under incomplete payoff information

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Abstract: You're going to a bar in hopes of meeting a romantic partner. You want to invite a friend who will not compete, but help you get a date. However, a friend's intentions are not entirely known, so you need to separate good from bad wingmen. A wide array of social behavior can be understood from a game theoretic perspective where individuals seek to gain information about others' reward structures. A useful way of organizing this information is through social categorization. Making such distinctions is ubiquitous, but little work has been done exploring this topic behaviorally or neurally. Here, we focus on the social categorization of cooperative and competitive counterparts.

Participants played a series of games with a known 2x2 payoff matrix. Before each game, participants were told if their counterpart (selected randomly from a large pool of participants) was red or green. The counterpart's payoff matrix was unknown. Participants and red players could cooperatively maximize points, whereas green players were competitive.

Behavior across many strategic learning tasks is well characterized by two types of learning: simple reinforcement learning based on rewards from trial and error, and belief-based learning about the likely actions of others. We used the Experience Weighted Attraction (EWA) model, which describes how equilibria arise in interactive games through combining both types of learning.

We fit choice behavior of each participant using the EWA model, and compared models where red and green counterparts were modeled separately or together. The dual counterpart model fit better ($t(51) = 5.04$, $p < 10^{-5}$), suggesting people tend to differentiate counterpart types. A multiple linear regression revealed that participants earn more money if they distinguish counterparts ($t(49) = 3.34$, $p = 0.0016$), and if they employ belief-based learning ($t(49) = 2.55$, $p = 0.014$).

To analyze fMRI data, we fit a general linear model to look for main effects of expected reward (ER) and reward predictions errors (RPE), and found ventromedial prefrontal cortex (vmPFC) activation for both. Next, we identified areas involved in distinguishing counterparts. First, we subtracted the residual sum of squares (RSS) from the previous model from the RSS when ER and RPE were fit from the single counterpart model. Second, we performed a linear regression on how well participants distinguish counterparts from this difference in RSS to measure which voxels use ER and/or RPE representations to encode counterpart distinctions. Our findings indicate that ER and RPE representations in the vmPFC and ER representations in premotor cortex distinguish cooperative from competitive counterparts.

Disclosures: P. Krueger: None. **P. Karashchuk:** None. **I. Sáez:** None. **M. Hsu:** None.

Poster

089. Decision Making: Avoidance and Uncertainty

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 89.10/JJJ9

Topic: H.02. Human Cognition and Behavior

Support: NIMH Grant R21MH102634

Clinical Neurosciences Division of the U.S. Department of Veterans Affairs National Center for PTSD

Title: The neural correlates of trauma-related symptom severity in combat veterans: a neuroeconomic approach

Authors: L. RUDERMAN, R. JIA, D. B. EHRLICH, P. SALHOTRA, I. HARPAZ-ROTEM, *I. LEVY;
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Abstract: DSM categorical diagnoses for psychiatric disorders are insufficient in guiding treatment models. There is need of alternative classification models, based on the underlying basic mechanisms of psychopathological symptoms. We have recently demonstrated the usefulness of behavioral economics to psychiatric research as a framework for identifying behavioral markers using measures *independent of the pathology itself* (Ruderman et al., 2016). We focused on a neglected aspect of trauma symptoms – individual differences in uncertainty attitudes – and found that veterans with PTSD were more averse to ambiguity (unknown outcome probabilities), but not risk (known probabilities), compared to veterans without PTSD, when making choices between possible losses, but not gains. Here, we explore the neural markers of trauma-based symptomology using the same task, thereby eliminating potential biases related to self-report or re-experiencing of trauma.
We studied 39 combat veterans (16 with PTSD and 23 combat controls) to assess their attitudes towards risk and ambiguity in a monetary decision making task. Subjects made choices between a guaranteed win (or loss) of a fixed monetary amount or playing a lottery, which offered a larger gain (or loss) but also some chance of zero outcome. In half of the trials outcome probabilities for the lotteries were precisely known, while in the other half these probabilities were ambiguous. Functional MRI was used to track neural activation while subjects completed a total of 240 decisions. One choice was randomly picked for payment at the end of the experiment. Behaviorally, we replicated our recent results and found that veterans with PTSD were more averse to ambiguity under losses. Neurally, we found that overall activation in brain areas traditionally involved in value-based decision making, including the vmPFC and striatum, is negatively correlated with PTSD symptom severity (assessed by Clinician-Administered PTSD Scale [CAPS]; $p < 0.05$ under risk and under ambiguity, in both the gain and loss domains),

whereas activation in the insula is positively correlated with CAPS scores ($p < 0.01$ in both gains and losses). Interestingly, those regions are also implicated in trauma-related or fear learning PTSD studies, while the task employed here is utterly neutral and does not relate to any traumatic experiences. Our results demonstrate the potential of neuroeconomic and behavioral economic techniques for devising objective and incentive-compatible diagnostic tools, and investigating the etiology of psychiatric disorders.

Disclosures: **L. Ruderman:** None. **R. Jia:** None. **D.B. Ehrlich:** None. **P. Salhotra:** None. **I. Harpaz-Rotem:** None. **I. Levy:** None.

Poster

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Program#/Poster#: 89.11/JJJ10

Topic: H.02. Human Cognition and Behavior

Support: NIH-NINDS-NS054775

Title: Neuroanatomical correlates of loss of economic rationality in aging- Testing Generalized Axiom of Revealed Preference

Authors: *H.-K. CHUNG[1], P. GLIMCHER[2,3], A. TYMULA[4,3];
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Abstract: The population of people above 65 years old is growing and work investigating their cognitive function and decision making is of increasing importance. A previous study (Tymula, et al., 2013) found that age is a crucial factor in decision making, with older individuals making more stochastic choices. However, there is insufficient evidence on the effects of neuroanatomical aging on rationality in decision making. In our study, we used whole brain voxel based morphometry (VBM) analysis to determine where gray matter (GM) volume correlates with the behavioral measure of rationality in choice. We recruited 31 (12 male) healthy, right-handed adults over the age of 65. All subjects were functionally normal with mini-mental state examination scores ranging between 26 and 30. To quantify the degree of irrationality, we adopted the behavioral paradigm designed by Harbaugh and colleagues (2001) that estimates the number and severity of violations of the generalized axiom of revealed preference, which is a necessary and sufficient condition for utility maximisation. In individual trials, subjects chose their preferred bundle of snacks and beverages from a larger set of bundles. Assessing many such choices, we can infer whether the subject obeys transitivity and has

monotonic (within reason) preferences. High-resolution T1-weighted anatomical images were acquired with an MPRAGE pulse sequence using a 3T Siemens Allegra scanner equipped with a custom RF. We then conducted a VBM analysis relating our behavioral measure of rationality to gray matter volume. Multiple regression analysis was performed. The global GM volume, age, gender, extreme preference, numeracy skill, digit span score, Shipley vocabulary score, and education level were regressed out by including them as covariates of no interest. Our results indicate that irrationality in choice (the number of choices violating GARP and their severity) increases as GM volumes in anterior prefrontal cortex decreases. GARP violations and their severity were not correlated with the variables of no interest. In addition, the absence of correlation with mean reaction time suggests that our results cannot be explained by attentional or motivational confounds. Many studies have observed that global GM volume shrinks with age, with particularly local loss of frontal areas (see review in Matsuda, 2016). Our results show that a specific reduction in anterior prefrontal lobe GM correlates with economic irrationality. These findings point towards a neuroanatomic locus for economic rationality in the aging brain, and highlight the importance of understanding both anatomy and function in the study of aging and decision making.

Disclosures: H. Chung: None. **P. Glimcher:** None. **A. Tymula:** None.

Poster

089. Decision Making: Avoidance and Uncertainty

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Program#/Poster#: 89.12/JJJ11

Topic: H.02. Human Cognition and Behavior

Support: DFG SFB134

Title: Endocrine modulation of value representations in mesocorticolimbic circuits following short-term fasting

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Abstract: Dopaminergic neurons in the substantia nigra and ventral tegmental area (SN/VTA) are activated in response to reward-predicting cues. SN/VTA neurons also express growth hormone segretagogue receptors for the stomach-derived orexigenic hormone ghrelin. Subjective reward values during value-based decisions are represented in the ventromedial prefrontal cortex

(vmPFC) and the orbitofrontal cortex. After short-term fasting of 4 hours, subjective values of snack foods correlate with vmPFC activity. However, it is unclear if this correlation between neural activity and subjective values after short-term fasting is influenced by homeostatic hormones such as ghrelin. We investigated whether brain activity in mesolimbic and medial frontal regions in response to visual snack food cues is modulated by plasma ghrelin levels. Therefore, we took blood samples from 29 lean, healthy participants immediately before they underwent an fMRI food decision making task after short-term fasting of 4 or 8 hours in a randomized, within-subject design. We found that plasma levels of ghrelin as well as subjective hunger ratings were significantly increased after 8 hours compared with 4 hours of fasting. Functional MRI revealed that subjective value representations of snack foods in the SN/VTA were correlated with ghrelin levels and in the vmPFC with the intra-individual increase in ghrelin levels between the 4- and 8-hour fasting conditions. These findings demonstrate that reward in response to food cues is influenced by homeostatic hormone levels, highlighting the close link between reward circuits and homeostatic circuits. Furthermore, our results could help to understand dysfunctional reward processing in people suffering from homeostatic dysregulation due to obesity.

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Poster

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Topic: H.02. Human Cognition and Behavior

Title: Neural mechanisms of risky choice framing effects: A test of predictions by prospect theory versus fuzzy-trace theory

Authors: *C. CHICK[1], V. REYNA[2], R. WELDON[2], D. BLANSKY[2];
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Abstract: Although prior studies have described brain activation during risky choice framing effects, no study to date has used neural data to test competing theoretical explanations of framing effects in humans. Using a cognitive manipulation of framing effects, we tested competing hypotheses of prospect theory versus fuzzy-trace theory. In previous behavioral studies using this manipulation, fuzzy-trace theory has outpredicted prospect theory; here, we tested whether the same cognitive manipulation could also distinguish between these theories at the level of the brain. During framing effects, we observed increased activation in the amygdala, dorsal striatum, inferior parietal lobule, and ventral prefrontal cortex. In a subset of these regions,

including the caudate, angular gyrus, and supramarginal gyrus, activation mirrored the behavioral effect of our cognitive manipulation—increasing in the condition that enhanced framing effects, and decreasing in the condition that decreased framing effects. Psychophysiological interaction analyses revealed increased connectivity among the caudate and inferior parietal lobule, as well as between the inferior parietal lobule and inferior frontal gyrus, during framing effects. Our results support the prediction by fuzzy-trace theory that framing effects are driven by simplified numerical comparisons, rather than by precise valuation, and that this categorical numerical processing is subserved by a network of frontal, parietal and subcortical regions.

Disclosures: **C. Chick:** None. **V. Reyna:** None. **R. Weldon:** None. **D. Blansky:** None.

Poster

089. Decision Making: Avoidance and Uncertainty

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Program#/Poster#: 89.14/JJJ13

Topic: H.02. Human Cognition and Behavior

Support: NSF CRCNS grant BCS-1309346

Title: A Bayesian model-based fMRI investigation of temporal expectancy in inhibitory control

Authors: *O. RACCAH[1], J. S. IDE[2], N. MA[1], S. HU[3], C.-S. LI[3], A. J. YU[1];
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Abstract: In this work, we examine the neural basis of temporal expectancy using the stop-signal task, a classical inhibitory control task in which subjects inhibit a prepotent go response upon detecting a rare stop signal. Previously, we showed that Bayes-like learning of the need to stop, P(stop), based on recently experienced frequency of stop trials, can explain sequential effects in this task, e.g. an increase of go response time (RT) and decrease in stop error rate (ER) in response to the frequency and recency of experienced stop trials (Shenoy, Rao, & Yu, 2010). We also showed that BOLD response in the dorsal anterior cingulate cortex encodes an unsigned prediction error associated with P(stop) (Ide et al, 2013). More recently, we showed that trial-by-trial adjustment in behavioral response is not only modulated by sequential learning of P(stop), but also by Kalman filter-like learning of the expected temporal onset of the stop signal, or the expected duration of the stop-signal delay, E[SSD] (Ma & Yu, 2015). Here, we examine the neural correlates of the representation and computation of temporal expectancy, by using E[SSD] as a parametric regressor in a fMRI GLM analysis (n=80). We find that a number of cortical

regions have BOLD responses negatively correlated with E[SSD]; in contrast, we do not find any cluster that positively correlated with E[SSD]. These results begin to elucidate the neural basis of the modulation of top-down attention associated with timing-specific aspects of proactive inhibitory control.

Disclosures: **O. Raccah:** None. **J.S. Ide:** None. **N. Ma:** None. **S. Hu:** None. **C. Li:** None. **A.J. Yu:** None.

Poster

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Program#/Poster#: 89.15/JJJ14

Topic: H.02. Human Cognition and Behavior

Support: NIH R01 MH104251

Title: Can dynamic normalization models with asymmetric inhibition account for the "attraction" effect in choice?

Authors: ***C. XU**, J. ZIMMERMANN, K. LOUIE, P. W. GLIMCHER;
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Abstract: Given two isopreferent multi-attribute choice options, the addition of a new but inferior option will shift subject preference towards the option closer to the new option. For example, if a high quality high price option and a low quality low price option are equally preferred, adding a high quality option with higher price into the option set will increase the probability of choosing the high quality high price option. This phenomenon has been termed the attraction effect (Huber et al., 1982; Simonson, 1989), and is supported by numerous empirical animal and human choice experiments.

Multiple previous models have proposed mechanistic explanations for the preference shifts produced by the attraction effect. Early models (Russo et al., 1983;Wedell, 1991) assumed that subjects use a heuristic strategy, in which one attribute is evaluated at a given time and options are discarded on the basis of said attribute. A recent model (Soltani et al., 2012) proposes that the attraction effect arises from range normalization, where the internal representation of all option values is encoded within a fixed range. However, such models rely on tracking the minimum and maximum value of inputs and an explicit calculation of range, which may be difficult to implement in plausible biological circuits.

Here we propose a new class of biologically-inspired model based on divisive normalization that can account for the attraction effect. We built a simple rate network based on a large body of

existing work where local inhibition produces normalization between the values of different options on a given attribute dimension, while long-range inhibition governs normalization between different attribute pools. Neurons representing a given attribute are organized together within a sub-circuit that activates an inhibitory population, which in turn inhibits the encoding population. In this way, the network locally normalizes the value of different options in individual attribute dimensions. Normalization between different attributes is implemented by long-range inhibition from the inhibitory population of one attribute sub-circuit to the excitatory population of other attributes.
We find that this attribute-based normalization circuit reproduces the attraction effect. This form of context-dependence requires an asymmetric inhibitory balance, with long-range inhibition stronger than local inhibition. These findings suggest that an alternative, biologically inspired network based on a canonical neural computation can account for context specific phenomena such as the attraction effect.

Disclosures: **C. Xu:** None. **J. Zimmermann:** None. **K. Louie:** None. **P.W. Glimcher:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: NSERC

Alberta Gambling Research Institute

Title: EEG correlates of evidence accumulation during dynamic discrimination decisions across two spatial locations

Authors: ***N. J. WISPINSKI**[1], J. K. BERTRAND[2], A. SINGHAL[1], C. S. CHAPMAN[2];
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Abstract: Making a decision is theorized to involve the accumulation of evidence for available options over time. Neural signals supporting evidence accumulation have been observed in primate neurophysiology and human electroencephalography (EEG), but primarily in detection tasks with a single stimulus. However, most real-world decisions are discrimination decisions, where information needs to be compared relatively between multiple spatially distinct stimuli. Using EEG in a discrimination decision with two stimuli, we sought to separately characterize the dynamics of neural evidence accumulation and sensory information signals. Human

participants monitored two grey circles for a change in relative brightness between the left and right circle. On every trial, individual circles could change (increase or decrease in brightness), or stay the same. As a result, a single circle changing brightness did not necessarily mean a task-relevant difference in brightness (e.g., both circles becoming brighter). Therefore, sensory information (brightness changes), and decision evidence (relative differences), were manipulated independently. Circles flickered at two different rates in order to measure steady-state visually evoked potentials (SSVEP) independently from the left and right circles. We found that the centroparietal positivity signal exhibited accumulation patterns on trials with relative brightness differences, and rapidly fell post-response. The slope, speed of onset and responses, and amplitude at response, all increased with greater relative differences in stimuli brightness. In addition, SSVEP power from the independent flicker rates of both stimuli, measured at contralateral occipital sites, tracked the dynamic changes in brightness at both spatial locations. Overall, occipital oscillations reflected spatially distinct and dynamically changing sensory information while parietal positivity reflected the relative-brightness decision evidence. The findings of parietal amplitude at response for different levels of relative brightness have important implications for models of evidence accumulation, and suggest urgency signals at play during decision making. The current research also has practical implications for measuring decision signals using EEG in traditional two-alternative forced choice paradigms. In contrast with most previous research on human decision signals using detection tasks or a single stimulus location, our results open the door for extensions of decision signals to classic paradigms using discontinuous trials, and multiple spatial locations.

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Poster

089. Decision Making: Avoidance and Uncertainty

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Topic: H.02. Human Cognition and Behavior

Support: NIH R21AG044862

Title: pupil dynamics during prolonged performance of a cued stroop task

Authors: *I. BABU HENRY SAMUEL[1], S. BURKE[2], J. CAGLE[2], M. DING[2];
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Abstract: Prolonged performance of a cognitive task induces mental fatigue. Anecdotal evidence suggests that pupil size is an effective index of mental fatigue. To date no studies have been carried out to examine this issue systematically. We recorded pupillometry from participants performing a cued Stroop task continuously for two hours. At the beginning of each trial a cue instructed the subject to either read aloud the target word or name the font-color of the target word presented 1500ms after the cue. Subjects rated their fatigue levels every 20 minutes. Pupil size in the 200 ms time interval prior to the cue was extracted and averaged in a moving window of 30 minutes with 20-minute overlap. The following results were found. First, the subject's fatigue level increased as a function of time-on-task. Second, pupil size decreased as a function of time-on-task. Third, the slope of the pupil size decline is not predicted by the fatigue severity score (FSS) obtained prior to the start of the Stroop experiment. Fourth, the slope of the pupil size decline and the slope of the fatigue level increase were not correlated. These findings (1) confirm the previously suggested relation between pupil size and mental fatigue, (2) show that the rate of the pupil size decrease does not reflect the trait fatigue level, and may therefore provide a measure of objective fatigability, and (3) indicate that there may be multiple mechanisms underlying the increased subjective feelings of fatigue and objective decline of pupil size.

Disclosures: I. Babu Henry Samuel: None. **S. Burke:** None. **J. Cagle:** None. **M. Ding:** None.

Poster

089. Decision Making: Avoidance and Uncertainty

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Topic: H.02. Human Cognition and Behavior

Support: NRF Korea Grant No.2015R1D1A1A01056743

NST Korea Grant No. CRC-15-07-KIER

Title: Mental representations of covert intentions on 'like' and 'dislike' are differentiated in alpha-band neural synchronies

Authors: *J. CHOI, D. YEO, K. CHA, K. KIM;
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Abstract: The purpose of this study is to investigate spatiotemporal and spectral characteristics of neural activities based on electroencephalogram (EEG) during maintenance of the subjective preference, 'like' or 'dislike', in response to affective pictures.Fifteen healthy university male

students participated in the experiment. The pictures consisted of pleasant, unpleasant and neutral pictures selected from the International Affective Pictures System (IAPS). The subjects were required to decide whether they 'liked' or 'disliked' each picture, then maintain the preference in mind, and finally respond with button press. Multichannel EEGs were recorded during the task.EEGs during the 'preference in mind' period (before the button press) were segmented. We investigated local synchronies in neural activities within task-relevant cortical regions by analyzing event-related spectral perturbation (ERSP), along with conventional averaged event-related potential (ERP) analysis. In addition, inter-regional neural synchronies were explored by using the weighted phase lag index (wPLI).At the period of maintaining the preference, significantly greater alpha-band power was observed for 'like' at frontal regions for both emotional and neutral pictures. Furthermore, inter-regional neural synchronies in the alpha band were significantly different, but only for neutral picture, especially, frontal and parietal regions centered at parietal regions. The alpha-band fronto-parietal neural synchrony was significantly stronger for 'like'. In conclusion, local and global alpha-band neural synchronies during critical temporal epoch were significantly differentiated according to the contents of mental representation of subjective preference in response to affective pictures.

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Poster

089. Decision Making: Avoidance and Uncertainty

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Topic: H.02. Human Cognition and Behavior

Support: Taiwan National Science Council grant no. NSC 102-2410-H-002-004-MY3

Title: Midbrain to cerebral dopaminergic and serotonergic white-matter fiber tracts identified on the ntu-dsi-122 template

Authors: *U.-L. HSIEH[1], W.-Y. TSENG[1,2], Y.-C. LO[2], Y.-C. HSU[2], J. GOH[1,3,4];
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Abstract: The ventral tegmental area (VTA) and the dorsal and median raphe nuclei (DRN, MRN) of the midbrain consist of dopaminergic and serotonergic neurons, respectively, that project to various cortical and subcortical regions. Together, these two neurotransmitter systems play critical regulatory roles on cerebral functions that are implicated in various human mental

abilities including reward processing, motivation, learning, and memory. At present, it is difficult to assess the *in vivo* structural and functional contributions of these systems because the locations of the midbrain-to-cerebral connections have not yet been specified in human brain imaging templates. The aim of this study was to construct midbrain-to-cerebral dopaminergic/serotonergic white-matter fiber tracks that are known to subserve the above cognitive functions on a standard brain atlas. Fiber tracking analysis was performed on the NTU-DSI-122 (National Taiwan University, Diffusion Spectrum Imaging) template, which is an averaged white-matter template in MNI (Montreal Neurological Institute) space built on DSI data from 122 normal individuals (61 males; 61 females) with mean age (SD) of 28.0 (5.3) yrs with a range of 19-40 yrs. VTA, DRN, and MRN midbrain regions-of-interest (ROI) were defined in previous studies that targeted these areas using specialized functional magnetic resonance imaging (fMRI), positron emission tomography (PET), or manual tracing techniques. Frontal, medial temporal, and subcortical ROIs involved in the above cognitive processes were based on the Automated Anatomical Labeling atlas. Using DSI Studio, we applied whole-brain seeding to identify fiber tracts that spanned the midbrain source and cerebral target ROIs and filtered out tracts that did not pass both source and target ROIs. This analysis revealed dopaminergic tracts in the template consistent with the mesocortical (VTA to prefrontal region) and mesolimbic (VTA to anterior cingulate, ventral striatum, globus pallidus, and medial temporal regions) pathways. Also, serotonergic tracts were identified that included the ventrolateral (DRN to prefrontal, globus pallidus, and medial temporal regions) and ventromedial (MRN to prefrontal, anterior cingulate, caudate, putamen, hippocampal and entorhinal regions) bundles, as well as the dorsal raphe subcortical tract (DRN to caudate and putamen). Comparisons with structural studies in different mammalian species validated the reliability of our findings. Our findings contribute valuable imaging tools towards more specific studies investigating the roles of dopaminergic and serotonergic systems in human cognition.

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Poster

090. Cognitive Development

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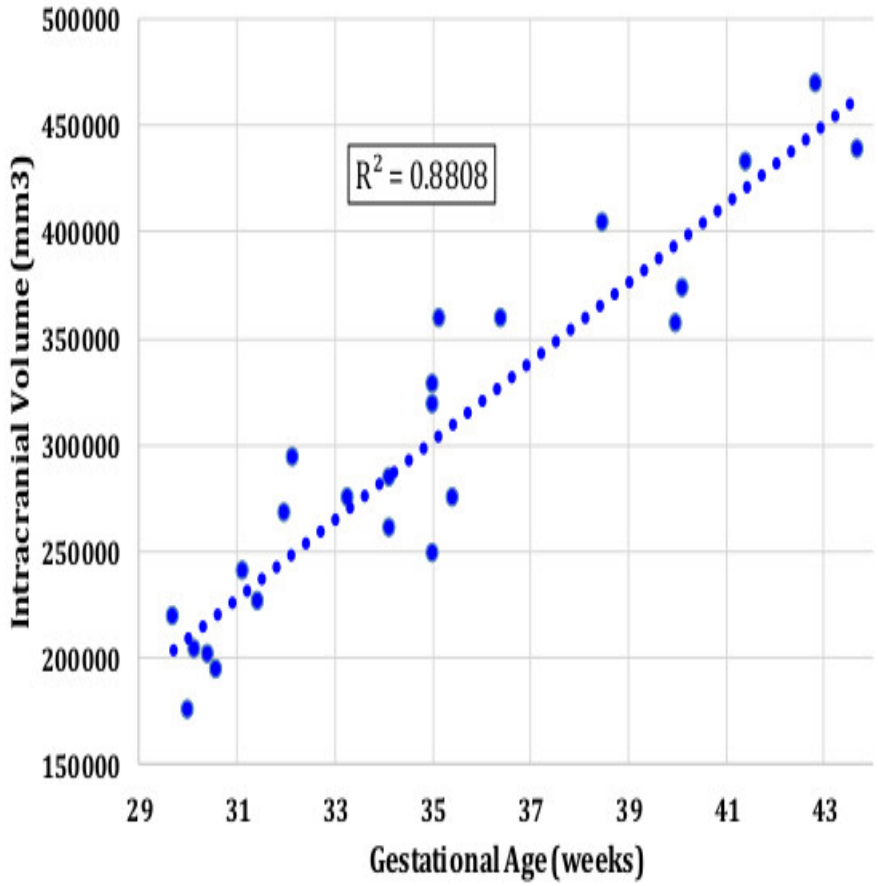
Topic: H.02. Human Cognition and Behavior

Support: The Gates Foundation OPP1119263

Title: Total Intracranial volume growth across the third trimester using longitudinal fetal MRI

Authors: S. COHEN, 06513[1], S. KWON[2], S. CROSS[2], C. LACADIE[2], G. SZE[2], T. CONSTABLE[2], *L. R. MENT[2], D. SCHEINOST, 06513[2];
[1]Yale Univ., New Haven, CT; [2]Yale Univ. Sch. Med., New Haven, CT

Abstract: The third trimester through the neonatal period is characterized by rapid brain growth. However, longitudinal magnetic resonance imaging (MRI) studies of fetal brain volume have been limited. Increased understanding of normal, fetal brain growth could help define patterns of abnormal brain development in clinical populations.
The objective of this study was to investigate fetal volumetric brain growth from the third trimester through the neonatal period using longitudinal volumetric MRI.
Ten typically-developing fetuses underwent longitudinal volumetric MRI at 30-32 weeks post-menstrual age (PMA), at 34-36 weeks PMA, and, after birth, at 40-44 weeks PMA. Imaging was performed on a 3.0 T scanner (Siemens Skyra) using a 32 channel body or head coil. Total intracranial volume (TIV) was estimated by manual segmentation using BioImage Suite. TIV included cerebrospinal fluid, gray and white matter, cerebellum, and brain stem. Linear regression was used to correlate PMA and TIV.
The average TIV was 230.8 ml for the first fetal scan (31.1±1.1 wks PMA, N=10), 304.3 ml for the second fetal scan (35.0±0.7 wks PMA, N=8), and 412.1 ml (41.1±1.9 wks PMA, N=6). TIV exhibited a significant linear association with PMA from 30 weeks to 44 weeks ($r=0.94$, $R^2=0.88$, $p<0.001$). TIV increased at an average rate of 8.0% per week and approximately doubled in size during this period.
These longitudinal data suggest that the brain grows linearly and doubles in size from the third trimester through the neonatal period. Future studies should continue to investigate fetal brain growth using longitudinal MRI, especially in clinical populations.



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Poster

090. Cognitive Development

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Program#/Poster#: 90.02/JJJ20

Topic: H.02. Human Cognition and Behavior

Support: MOP-84399

Title: Altered white matter development in very preterm children

Authors: *J. M. YOUNG[1,2], B. R. MORGAN[2], M. SMITH[1,2], M. J. TAYLOR[1,2];
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Abstract: Objective: Very preterm (VPT) children born less than 32 weeks gestational age (GA) are at-risk for altered brain maturation in early development. In the present study, diffusion tensor imaging was utilized to examine structural white matter differences and its relation to cognitive function between very preterm born and term-born children at 4 years of age.
Methods: Sixty-direction diffusion and T1 anatomical data were acquired in 31 VPT children (mean GA: 28.76 weeks, 18 males) and 28 term-born children (13 males) at 4 years of age on a 3T Siemens Trio MR scanner. Using FSL's tract-based spatial statistics (Smith et al., 2006), each subject's DTI data were non-linearly co-registered to create a cohort-specific template. A mean fractional anisotropy (FA) skeleton was generated on the average FA image and thresholded at 0.25 to only represent major white matter tracts, which was then applied to each subject's FA, mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD) data. Within group analyses were also performed to test associations between FA values and IQ and language measures. FSL Randomise (Anderson & Robunson, 2001) was used to determine significance of the above tests, using threshold-free cluster enhancement. Group connectivity differences were further investigated using connectometry analyses in DSI Studio (Yeh et al., 2015).
Results: In VPT born children, FA values were consistently reduced compared to term-born children at 4 years of age within major white matter tracts. Affected tracts included the corpus callosum as well as bilateral areas of the corona radiata, superior longitudinal fasciculus, internal and external capsule, inferior fronto-occipital fasciculus and the left optic radiation. Scores of IQ and language fell within average limits for both groups. Within group analyses revealed significant associations in term born children between FA and IQ within regions of the superior longitudinal fasciculus, inferior fronto-occipital fasciculus, forceps minor and forceps major. No associations were found between cognitive scores and FA in the VPT born children. Connectometry analyses also revealed similar group differences.
Conclusions: Widespread, significant reductions of FA were found in VPT born children compared to term-born children within major white matter tracts. Unlike the very preterm born children, term-born children had significant linear associations between FA measures and cognitive scores. This disrupted neurodevelopment occurring in early childhood contributes to our understanding of white matter development and its relation to cognitive function in VPT children.

Disclosures: J.M. Young: None. **B.R. Morgan:** None. **M. Smith:** None. **M.J. Taylor:** None.

Poster

090. Cognitive Development

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 90.03/JJJ21

Topic: H.02. Human Cognition and Behavior

Support: MRC Grant G0300117-65439

MRC Grant G1002276-98624

Title: Reduced grey matter concentrations in infants with transposition in the great arteries

Authors: *R. ELWARD[1,2], M. SAINI[1,2], D. GADIAN[1], D. CARMICHAEL[1], A. GIARDINI[2], M. DE HAAN[1], T. BALDEWEG[1], F. VARGHA-KHADEM[1,2];
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Abstract: Transposition of the great arteries (TGA) is a congenital heart defect with a prevalence of 0.2 per 1000 live births. This condition is associated with a risk of systemic hypoxic/ischaemic (HI) brain injury in the neonatal period. Depending on their severity, HI episodes can target the hippocampus and/or the basal ganglia, sometimes producing cerebral palsy. Children and adolescents with corrected TGA have been reported to show motor coordination and cognitive deficits, and increased incidence of psychiatric diagnoses in long term follow-up. We examined the ontogeny of cognitive and motor coordination deficits in relation to structural Magnetic Resonance Imaging (MRI) evidence of the integrity of the hippocampus and basal ganglia, by conducting a longitudinal, prospective study of groups of infants treated for TGA (N=24) and their age-matched healthy controls (NC) (N=25). All infants were free from any neurological diagnoses. Voxel-Based Morphometry (VBM) analyses were conducted on T1 and T2-weighted MRIs (mean age at scan = 12.5 weeks for TGA, and 11.6 weeks for NC). The VBM results indicate that infants with TGA have reduced grey-matter volume in the hippocampus relative to NC ($p < 0.001$; $k = 54$; peak Z score = 3.35). This finding is consistent with previous reports that older children with TGA have reduced hippocampal volumes. The VBM showed no evidence of grey matter volume reduction in the caudate nucleus, putamen, or the thalamus in the TGA group relative to NC. Magnetic resonance spectroscopy (MRS) was also used for the assessment of focal brain pathology in the caudate and the thalamus. The MRS analysis revealed no evidence of pathology in either structure. The Bayley Scales of Infant Development were used to assess cognitive, fine-motor, gross-motor, expressive language and receptive language skills at two time-points (six months and 12 months). A Group X Scale X age at test ANOVA showed a significant three-way interaction ($p < 0.005$). At six months of age, the TGA group was delayed relative to NC on the gross-motor scale only ($p < 0.05$). By 12 months of age, the TGA group showed significant delays on the cognitive ($p <$

0.005), expressive language ($p<0.05$) and fine motor ($p < 0.05$) scales. We conclude that infants with corrected TGA suffer HI damage to the hippocampus during the perinatal period which has significant consequences for subsequent development within the first year of life. These data provide insight into the emergence of cognitive and motor deficits in infants with TGA.

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Poster

090. Cognitive Development

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Program#/Poster#: 90.04/JJJ22

Topic: H.02. Human Cognition and Behavior

Support: Elizabeth H. Solomon Center for Neurodevelopmental Research

Rutgers University Board of Trustees Excellence in Research Award

Title: Cross-sectional comparison of spontaneous electrocortical alpha-gamma cross-frequency coupling in infancy

Authors: ***S. HEIM**[1], G. MUSACCHIA[2], S. PETERS[1], S. ORTIZ-MANTILLA[1], A. A. BENASICH[1];
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Abstract: Recent conceptual and modeling work emphasizes the role of oscillatory communication at the level of neural populations within and across brain regions. Accordingly, studies in animal models and using adult participants have provided evidence for a systematic dynamic relation between electrocortical oscillations at different frequencies. One of the most widely held hypotheses in this field has been that the amplitude of higher-frequency brain oscillations depends on the oscillatory phase of specific lower-frequency oscillations, subject to the participant's brain state or task. The presence and putative maturational trajectory of such relations have yet to be explored in human infants. The current study examined spontaneous phase-amplitude coupling cross-sectionally, over typical development, using dense-array electroencephalography (EEG) recordings from 31 infants at either 4 (n = 15) or 7 months of age (n = 16). Spectral power was calculated on EEG collected during quiet play. Differences between groups emerged in the 6-8 Hz range as well as the 22-28 Hz range. Further analysis of these group power differences showed that the phase in the emerging alpha band (around 7 Hz)

significantly predicted gamma-range oscillations in younger (4-month-old), but not older infants. These effects were seen in voltage space as well as in current density space, at centro-parietal sensors, where both alpha-like and gamma-band oscillations showed maximum power. The present results suggest that resting coupling between the alpha and gamma bands may constitute a mechanism for organizing spontaneous brain rhythms, which reorganizes as alpha emerges as a functionally relevant, distinct oscillatory phenomenon that varies with behavioral changes.

Disclosures: **S. Heim:** None. **G. Musacchia:** None. **S. Peters:** None. **S. Ortiz-Mantilla:** None. **A.A. Benasich:** None.

Poster

090. Cognitive Development

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 90.05/JJJ23

Topic: H.02. Human Cognition and Behavior

Title: Resting brain interactions during natural sleep in typically developing toddlers are biased to the left hemisphere

Authors: ***S. J. GOTTS**[1], E. REDCAY[2], S. C. MILLEVILLE[1], A. THURM[3], S. SHUMWAY-MANWARING[4], A. MARTIN[1];
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Abstract: Resting brain activity in typically developing adolescents and young adults exhibits two distinct forms of lateralized interactions, with left-hemisphere regions biased to interact more strongly within the same hemisphere in support of language and fine-motor functions and right-hemisphere regions biased to interact more strongly with both hemispheres in support of visuospatial processing (Gotts et al., 2013, PNAS, 110, E3435-44). Whether these patterns emerge as a result of experience or are present prior to behavioral competence is unknown. In the current study, we examined resting brain activity in 32 typically developing toddlers with fMRI [Age range = 12.91 to 39.69 mos; Mean (SD) Age = 24.64 (9.35)]. Data from 3 toddlers were excluded due to excessive head motion (mean Frame Displacement, FD > 0.3 mm/TR) and one for poor MPRAGE quality, retaining 28 toddlers for analyses [Mean (SD) FD = .087 (.043)]. Resting-state data (TR=2 sec, voxel size = 2x2x3 mm^3; 8-minute scan duration) were acquired during natural sleep and were processed using the ANATICOR approach (regressors: 6 motion, ventricle signal, local white matter signal within 15mm radius, Retroicor + RVT, 1st 3 PCs of combined white matter/ventricle mask). Lateralization metrics for resting-state data were

calculated by comparing the original data orientation with L/R flipped data after first non-linearly warping the flipped to the original MPRAGE scan for each participant in order to better align homotopic locations volumetrically. As in adolescents and adults, "Segregation" effects (a bias for within-hemisphere interactions) were left-lateralized in the planum temporale, Heschl's gyrus, STG, and premotor cortex (FWE-corrected to P<.05 by permutation). In contrast to adolescents and adults, toddlers exhibited no significant right-lateralized "Integration" effects (a bias to interact more strongly with both hemispheres). Integration was limited to one left-lateralized cluster in the dorsolateral prefrontal cortex (P<.05 corrected). Neither of these patterns changed systematically with Age [with Segregation: r(26) = .034, P>.8; with Integration: r(26) = .085, P>.6] and were unrelated to head motion or hemispheric differences in signal amplitude (all P's > 0.2). Follow-up whole-brain searches for Age-dependent lateralization effects yielded uncorrected Segregation effects in the left parietal cortex and ventral temporal cortex that grew stronger with Age. Left-lateralized Segregation effects in toddlers are present early and ontogenetically prior to right-hemisphere Integration effects, suggesting a potentially important role for left segregation in language learning.

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Poster

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Program#/Poster#: 90.06/JJJ24

Topic: H.02. Human Cognition and Behavior

Support: NIH Grant P50HD055784

NIH NIDA T90-DA022768

Title: Structural connectivity of language tracts in 6-week-old infants

Authors: ***J. LIU**[1], C. PONTING[2], T. TSANG[3], S. BOOKHEIMER[2], M. DAPRETTO[4]; [1]Neurosci., [3]Psychology, [4]Psychiatry and Biobehavioral Sci., [2]UCLA, Los Angeles, CA

Abstract: Altered structural connectivity has been reported in several developmental disorders that involve atypical language function such as specific language impairment, global developmental delay, dyslexia, and autism spectrum disorder, suggesting that atypical structural connectivity in the developing infant brain may provide a biomarker of future developmental outcome. White matter tracts connecting language areas in the brain are present very early in

development. These include the arcuate fasciculus (AF) and the superior longitudinal fasciculus (SLF), which are dorsal pathways connecting Wernicke's and Broca's areas, two brain regions which have been found to be activated during speech processing in infants during natural sleep. These early developing white matter pathways are thought to subserve the integration of sensory and motor representations that must precede more advanced language development; indeed, these early structural connections are in place to support language acquisition well before the onset of overt language production. While a number of studies have previously shown that these white matter tracts can be detected in infants as young as 2 days of age, very little is known about how early measures of structural connectivity might relate to later behavioral language outcome.

This study began to address this gap using diffusion tensor imaging (DTI), which provides an index of structural connectivity by measuring the diffusion of water molecules in the brain to reveal white matter anatomy. DTI data were collected in 6-week-old infants who underwent MRI during natural sleep. Deterministic and probabilistic tractography approaches were used to identify the AF and SLF connecting the superior temporal gyrus and the inferior frontal gyrus. Indices of white matter fiber integrity, including average fractional anisotropy and mean diffusivity, were extracted from probabilistic tractography and subsequently related to behavioral indices of language development (including both receptive and expressive language measures). These findings confirm that white matter tracts connecting language regions in the brain can be readily detected in 6-week-old infants using DTI during natural sleep. Furthermore, exploratory correlational analyses suggest that the structural integrity of white matter fibers connecting language regions at 6 weeks of age does relate to subsequent language outcome.

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Poster

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Title: Structural brain correlates of global motion sensitivity in typically developing children: Parietal surface area and TBSS measures of the superior longitudinal fasciculus

Authors: *O. J. BRADDICK[1], J. ATKINSON[2], N. AKSHOOMOFF[3], E. NEWMAN[3], L. B. CURLEY[3], A. M. DALE[3], T. L. JERNIGAN[3];
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Abstract: Impaired sensitivity to global visual motion, but not to global form, has been found as a signature of a range of neurodevelopmental disorders, often associated with spatial and attention deficits and poor mathematical skills ('Dorsal stream vulnerability' - Braddick et al, *Neuropsychologia*, 2003, 41:1769). We have previously reported (Atkinson et al, *J Vision* 2014 14(10): 1324) cerebral correlates of individual differences in global motion in typically developing children, high sensitivity being associated with a relatively enlarged surface area of the parietal lobe (especially around the intraparietal sulcus) and reduced occipital area.
Is this cortical correlate of global motion sensitivity linked to variations of organization in white matter fiber tracts? We analysed fractional anisotropy (FA) within major fiber tracts defined by tract-based spatial statistics (TBSS) in 125 typically developing children aged 5-12 years, from the PLING study (Pediatric Longitudinal Imaging Neurocognition and Genetics). The superior longitudinal fasciculus (SLF) was treated as the main candidate tract, given its connections to parietal areas previously associated with global motion performance.
We found an asymmetrical relation of global motion sensitivity to SLF structure, with higher FA in the right SLF showing a positive association ($p=0.003$) with high sensitivity, while the left SLF showed a negative association ($p=0.02$) (age taken out as a covariate). This relation was not found for overall FA or for other specific tracts, and did not occur for global form. In contrast, our data show that the associations of parietal surface area relative expansion, and occipital area contraction, associated with motion performance while in the same direction in both hemispheres, were stronger in the left than in the right hemisphere.
We conclude that (a) developmental variation in global motion sensitivity is linked to local white matter organization in SLF, as well as to regional differences in parietal area; (b) a complex pattern of hemispheric asymmetry in both fiber tracts and cortical area is associated with the phenotype of high global motion sensitivity. These findings provide pointers for the neuroanatomical investigation of the visual correlates of neurodevelopmental disabilities and of 'dorsal stream vulnerability'.
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Poster

090. Cognitive Development

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Support: UCL Grand Challenges studentship

MRC Programme Grant G1002276-98624

National Institute for Health Research Biomedical Research Centre Funding Scheme

Title: White matter damage associated with motor, attention and working memory deficits in children with congenital hyperinsulinism

Authors: *G. PITTS[1], K. HUSSAIN[1], A. KUMARAN[1], J. BULLOCK[2], D. GADIAN[1], F. VARGHA-KHADEM[1];
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Abstract: Neonates and infants with congenital hyperinsulinism (CHI) experience severe and recurrent episodes of hypoglycaemia. It is recognised that these infants are at risk of brain damage, and imaging studies of neonates have reported global white matter damage through visual inspection of MRI scans. However little is known about the extent of this damage and its adverse effects on cognition and behaviour during childhood and adolescence.
Twenty-four patients with CHI, but without neurological impairments (mean age 11.2yrs, sd= 2.6 yrs, 14 male) completed a comprehensive neuropsychological assessment (including IQ, academic attainments, memory, attention and motor coordination), and underwent structural MRI scans. Forty-two healthy controls (NC, mean age 11.8yrs, sd=2.5 yrs, 16 male) also underwent MRI scanning. Voxel Based Morphometry (VBM) and Tract-Based Spatial Statistics (TBSS) analyses were performed to assess differences in brain structure.
Compared to the standard population mean (X = 100; SD, 15), the CHI group scored lower on working memory, sustained attention, divided attention, fine motor skills and overall motor proficiency (all comparisons Bonferroni corrected, $p<0.002$), but not on full scale IQ or memory and learning.
Morphometric analyses showed a significant reduction in white matter volume in prefrontal regions ($p=0.05$, FWE corrected), and a reduction in total white matter volume. TBSS analysis revealed decreased Fractional Anisotropy (FA) across almost the entire white matter skeleton ($p=0.05$, corrected for multiple comparisons), and an increase in Mean Diffusivity (MD) across the corpus callosum, and in the left cingulum. In the CHI group, lower FA values were significantly correlated with poor attention and motor scores. Moreover, motor scores were

positively correlated with FA of tracts known to serve motor function, including the cortico-spinal tract, anterior and posterior thalamic radiations, anterior limb of internal capsule, and corpus callosum (p=0.05, corrected). No significant correlation was found between full scale IQ and FA.
Using quantitative neuroimaging analyses, we have shown that school-aged children with a history of neonatal hypoglycaemia show widespread damage to white matter. This is accompanied by specific impairments in working memory, attention and motor skills. These deficits appear to be a function of disruption in specific white matter tracts.

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Poster

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Topic: H.02. Human Cognition and Behavior

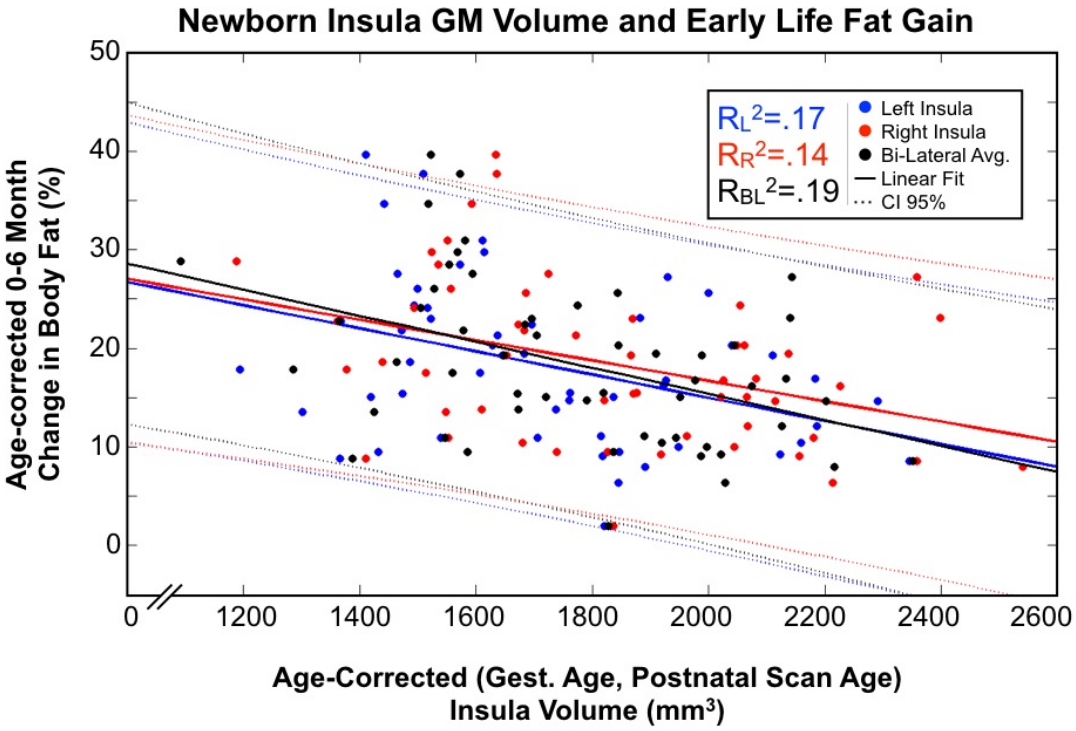
Support: NIMH R01 MH091351

Title: Newborn insula gray matter volume is prospectively associated with early life fat gain

Authors: ***J. RASMUSSEN**[1], S. ENTRINGER[2], F. KRUGGEL[3], D. COOPER[3], M. STYNER[4], J. H. GILMORE[4], S. G. POTKIN[3], P. D. WADHWA[3], C. BUSS[2];
[1]Univ. of California, Irvine, Long Beach, CA; [2]The Charité Berlin, Berlin, Germany; [3]Univ. of California Irvine, Irvine, CA; [4]Univ. of North Carolina, Chapel Hill, NC

Abstract: Childhood obesity is of great concern as obese children are more likely to be obese as adults, and develop obesity-related diseases at earlier ages and of greater severity. The importance of homeostatic brain circuitry, particularly the insula, is well established through structural and functional imaging in obese adults. In this work, we investigate the association between newborn insula gray matter (GM) volume and rate of fat accrual in the first six months of life, an outcome thought to be among the most reliable, valid, and strong predictors of childhood obesity. 52 neonates were assessed using structural MRI within the first month of life and longitudinal Dual X-Ray Absorptiometry shortly after birth and at six months of age. Bilateral insula gray matter (GM) volume was negatively associated with change in body fat percentage (BF%) from birth to six months postnatal age (Figure; R^2=19%; p=0.001). This finding was replicated using GM concentration (ratio of GM to total insula volume) within the insula, a relative measure of GM volume more independent of age and intracranial volume, as a

predictor of change in BF% (R^2=13%; p=0.009;). Furthermore, the observation was spatially consistent with known gustatory regions within the insula, the direction of effect was in concordance with adult findings, the magnitude of effect was substantial, and the results remained significant after post hoc testing of relevant confounding variables. Taken together, insula GM volume assessed at birth holds significant promise as a brain phenotype predicting childhood obesity risk.



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Poster

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Support: The Foundation of Hope for Research and Treatment of Mental Illness

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Title: Gut microbiome associated with cognitive and brain imaging outcomes in human infants

Authors: *A. L. CARLSON[1], K. XIA[2], M. A. AZCARATE-PERIL[3], B. D. GOLDMAN[4], M. A. STYNER[5], A. L. THOMPSON[6], X. GENG[7], J. H. GILMORE[2], R. C. KNICKMEYER[2]; [1]Neurobio. Curriculum, [2]Psychiatry, [3]Cell Biol. and Physiol., [4]NC Frank Porter Graham Child Develop. Inst., [5]Psychiatry and Computer Sci., [6]Anthrop., Univ. of North Carolina At Chapel Hill, Chapel Hill, NC; [7]Univ. of Hong Kong, Hong Kong, Hong Kong

Abstract: Microorganisms inhabiting the human gut play a vital role in health and likely influence neurodevelopment. Differences in microbial diversity impact anxious behavior and cognition in rodents and are associated with temperament in human children. Here we test the association of infant gut microbial composition with cognitive and brain imaging outcomes. 89 typically developing infants were recruited at 1 year of age for structural magnetic resonance imaging, diffusion tensor imaging, and resting state connectivity scans during unsedated sleep. Infant fecal samples were analyzed by 16s rRNA amplicon sequencing for identification and relative quantification of bacterial taxa. Distance metrics and cluster scoring methods were used to identify genus level enterotypes within the sample. We tested for differences in Mullen Scales of Early Learning scores and brain imaging outcomes between clusters. We also investigated associations between alpha diversity and these different outcomes. There was moderate support for clustering subjects into three enterotypes by the relative abundance of different bacterial genera. Many genera showed variation across clusters including *Faecalibacterium*, *Bacteroides*, and a genus of Clostridiales. Mullen cognitive scores at 2 years of age differed significantly between clusters and measures of alpha diversity were predictive of scores. The cluster characterized by Bacteroides abundance showed higher cognitive ability compared to the first cluster. The scores for this cluster were increased by one standard deviation compared to average ability shown in cluster 1. Measures of alpha diversity were negatively associated with cognitive measures. Alpha diversity was significantly different between clusters where higher performing clusters had lower alpha diversity. Analyses of regional grey matter volumes at 1 year of age suggest differences in visual processing and emotion regulation areas. This cross-sectional study shows associations between the gut microbiome and cognitive and brain imaging outcomes during a foundational and vulnerable period in neurodevelopment. Future research on microbiome modulation of the gut-brain axis may allow non-invasive intervention to adjust abnormal developmental trajectories during this critical period of human development.

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Poster

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Topic: H.02. Human Cognition and Behavior

Support: CNLM Grant ANGC1206

NICDH Grant HD055352

Title: Added sugar intake is inversely related to creativity among preadolescents

Authors: *K. M. HASSEVOORT[1], A. S. LIN[2], S. E. KHAZOUM[2], C. H. HILLMAN[3], N. A. KHAN[2], N. J. COHEN[2];
[1]The Beckman Inst. of Sci. and Technol., Urbana, IL; [2]Univ. of Illinois at Urbana-Champaign, Urbana, IL; [3]Northeastern Univ., Boston, MA

Abstract: Creativity depends on the ability to combine existing mental representations in new ways and depends, in part, on the hippocampus (Duff *et al.*, 2013). The hippocampus, in turn, is affected by a number of health factors, including aerobic fitness, obesity, and diet. Specifically, in rodent studies, diets high in saturated fatty acids and sugar - hallmarks of a western diet pattern - have been shown to negatively impact hippocampal function. Yet few studies have examined the effects of diet on hippocampal-dependent cognition in children. Therefore, the current study sought to explore the relationship of the intake of dietary lipids (saturated fatty acids and omega-3 fatty acids) and simple carbohydrates (added sugars) with creativity in preadolescent children. Participants (N = 36; mean age = 9.1 years) completed the Verbal Form of the Torrance Test of Creative Thinking (TTCT), a widely used test of creativity. Responses on this task were scored according to their fluency, flexibility, and originality. In addition to cognitive testing, participants completed a 3-day food record with the assistance of a parent. This food record was used to conduct nutrient-level analyses. While saturated fatty acid intake and Omega-3 fatty acid intake were not significantly related to creativity, added sugar intake was negatively related to performance across all three dimensions of the TTCT. These relationships were sustained even after controlling for important covariates including age, sex, socioeconomic status, IQ, aerobic fitness, and abdominal adiposity, factors that are also linked to hippocampal function in this age group. These findings are among the first to indicate a link between added

sugar consumption and cognitive performance during childhood, and implicate western diet patterns in poorer cognitive function among a pediatric population.

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Poster

090. Cognitive Development

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Program#/Poster#: 90.12/JJJ30

Topic: H.02. Human Cognition and Behavior

Title: Developmental and familial characteristics of top-down inhibitory control networks and performance strategies: ERPs to the Blue Man Stop-Response Task

Authors: *C. BOUCHARD[1], I. SOLIS[1], B. COFFMAN[2], B. SEAMAN[2], J. C. PESKO[3], K. R. CIESIELSKI[1,4];
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Abstract: RATIONALE**:** Deficits in top-down inhibitory control are one of the major foci of neurocognitive conceptual frameworks in the neuroscience of developmental psychopathological disorders. Since developmental disorders are associated with genetic risk factors, we ask whether the inhibitory performance strategies and activation of the Frontal-Parietal Network (FPN) that is implicated in effortful top-down inhibitory control of behavior, will demonstrate developmental and familial characteristics. Identification of such a marker may have a value for early diagnostics. METHOD: 18 typically developing children (TD) age 7-12, and their biological parents (BP) age 25-50, were examined using neuropsychological tests high on top-down inhibitory control, e.g. WCST and RO-CFT. Brain Event-Related Potentials (ERPs) were recorded to a visual-spatial N-Back stop-response task, The Blue Man T. (BMT, Ciesielski, 2007). BioSemi High Density 64-ch EEG system with a Polhemus digitizer was used. The amplitude, peak-latency and scalp distribution were examined for the frontal ERP P200 and the parietal-occipital N200 during the first 1000 ms of the response-delay window of the BMT

epoch. Spearman r was calculated for amplitude changes in frontal and parietal nodes of FPN within TD and BP. Between-group comparisons (amplitude, peak-latency) were performed using Kruskal-Wallace T. The effect size eta-squared were calculated. RESULTS**:** We predicted that the marker of familial relationship between TD and BP be displayed in similar amplitude changes in FPN and in performance strategies on BMT and neuropsychological tests**.** Some similarity in performance for recall tasks TD vs. BP were found, however the pattern of task-related activation between the FPN frontal P200 and parietal-occipital N200 was determined predominantly by developmental characteristics not by familial. The occipital-parietal N200 (amplitude, scalp distribution) was more similar between TD and BP, however the frontal P200 was not. The N200 amplitude was positively correlated with performance accuracy in BMT. The P200 increased with age to a stop-response task. The reduction in latency of P200 was correlated with reduction of RTs in BMT and neuropsychological tests. CONCLUSION**:** The top-down inhibitory control related FPN activation is determined more profoundly by developmental factors, and particularly by the prolonged development of the frontal brain systems, than by the commonality of familial characteristics. A valid examination of familial factors in brain activation associated with top-down inhibitory control requires a strict match in gender and age of compared family members.

Disclosures: C. Bouchard: None. **I. Solis:** None. **B. Coffman:** None. **B. Seaman:** None. **J.C. Pesko:** None. **K.R. Ciesielski:** None.

Poster

090. Cognitive Development

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 90.13/JJJ31

Topic: H.02. Human Cognition and Behavior

Support: FONDECYT 11140535

CONICYT PCHA/Doctorado Nacional/2014-21140043

FOCEDYT 1140268

Title: From joint attention to mentalization: beta oscillatory activity predicts theory of mind skill in typically developing children

Authors: P. SOTO-ICAZA[1], L. VARGAS-BECERRA[2], F. ABOITIZ[1], *P. BILLEKE[3]; [1]Psiquiatria, Pontificia Univ. Católica de Chile, Santiago, Chile; [2]Hosp. Luis Calvo Mackenna, Santiago, Chile; [3]Univ. del Desarrollo, Santiago, Chile

Abstract: Early social development involves the capacity to share with another person the perception of an object, namely joint attention. This skill precedes the development of the ability to attribute mental states to others or theory of mind. Nevertheless, the neural mechanism of development of these abilities is still unknown. Since temporo-parietal region in adults participates in both attention and theory of mind, we tested the hypothesis that functional specialization of this region participates in the development from shared attention to theory of mind. To assess this neural mechanism, we study 24 typically developing children between 3 and 4 years old (12 girls) undergone electroencephalographic (EEG) recording. In a real interactive paradigm, children watched a novel stimulus presented in a screen and looking for the interaction with the experimenter (joint attention condition, JA) or not (no joint attention, nJA).Since share attention with another person requires both uncoupling of the stimuli of interest and refocusing of that attention to the social partner, we expect that attentional networks will be involved. Moreover, if JA is a necessary previous step for the explicit theory of mind development, then it involves the activation of a neural network whose specialization leads to the appearance of theory of mind ability (i.e., mentalization network).Event related potential (ERP) analysis showed a significant difference between JA and nJA conditions in right frontal electrodes between 490ms and 560ms (p 0.01 Wilcoxon test, cluster based permutation test). This difference reveals the Nc component (mid-latency negative component) that suggests attentional orienting. Source analysis shows that this activity is placed in right intraparietal sulcus and right inferior frontal gyrus.Time frequency analysis demonstrated a significant difference in beta band (15Hz-25Hz) between JA and nJA conditions over right parietal channels in JA condition (p 0.01). The source of this oscillatory activity was placed in right temporo-parietal region. Interestingly beta activity only in temporo-parietal junction, and not in others attentional areas (e.g., intraparietal sulcus) discriminates children who already have theory of mind ability.The evidence presented here show that through the development of social interactions begin to recruit more specialized brain areas associated to the ability to understand other persons' perspective and preferences. This process coincides with the apparition of explicit theory of mind in children and could reveal neural mechanism of the acquisition of complex social abilities.

Disclosures: **P. Soto-Icaza:** None. **L. Vargas-Becerra:** None. **F. Aboitiz:** None. **P. Billeke:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: 1R21HD064983-01

Title: Independent components of neural activation during reading and their relationship to behavior

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Abstract: Visual word recognition is a complex cognitive skill that requires the precise coordination of multiple spatially remote networks in the brain. We used independent components analysis, a data-driven method of assessing functional connectivity, to explore the networks involved in visual word recognition and to investigate the relationship of these networks to observable behavior. Additionally, we analyzed the ability of task-related changes in these networks to predict reading ability compared to structural and functional markers associated with reading ability in previous research. We recruited 68 participants (aged 16-39 years, with mean of 22.4 years) with a wide range of reading abilities (from individuals with dyslexia to very good readers) and assessed their reading skills with a battery of behavioral tasks aimed at tapping constituent parts of the process of reading, such as phonological awareness and lexical processing. We also used functional MRI to estimate neural activity while they matched line orientations (basic visual task), matched letter strings (orthographic task), and determined whether two non-words rhymed (phonological task) (*Brain*, 119: 1221). We used independent component analysis to identify spatially independent networks of brain regions involved in the tasks. We then explored the relationship between task-related modulation of these networks and behavioral performance. The ICA analysis revealed both task-positive and task-negative components that were significantly modulated by the task demands, including networks not previously identified by standard fMRI analysis methods. Additionally, task-related dynamics in many of these networks were better predictors of both dyslexia diagnosis and of reading-related behavioral measures than were previously identified structural and functional markers.

Disclosures: **M. Simmonite:** None. **C. Weng:** None. **T. Polk:** None.

Poster

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Program#/Poster#: 90.15/JJJ33

Topic: H.02. Human Cognition and Behavior

Support: NRF-2014M3C7A1062893

Title: Altered functional connectivity of hippocampus with premotor area in internet gaming disorder

Authors: *J.-Y. KIM;
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Abstract: With the fact that American Psychiatric Association added Internet Gaming Disorder (IGD) to section 3 in DSM-5, it is serious problem that people not only enjoy the Internet gaming but they are addicted to it. So this study aimed to estimate the alteration of the resting-state functional connectivity (rsFC) of the hippocampus as priori region of interest (ROI) in subjects with Internet Gaming Disorder (IGD) compared with healthy control (HC). The sample was comprised of 23 male with IGD and 23 HC. The MRI data were acquired using a 3T MRI system (Siemens, MAGNETOM Verio). For the preprocessing, we use the statistical parametric mapping toolbox (SPM8). In the connectivity analysis, temporal bandpass filtering (0.009-0.08 Hz) to the smoothed data, detrending the globally increasing cerebral spinal fluid are applied. Then, we analyzed the functional connectivity from the bilateral hippocampus using seed based correlation approach. Finally, the differences of the functional connectivity were investigated by performing the two sample t-test between the IGD and HC. The results were considered statistically significant if they exceeded the uncorrected $p<.001$ with an extent threshold of 50 voxels. The rsFC from the bilateral hippocampus to the BA6 especially the premotor region was decreased in subjects with IGD. In addition, the IGD showed increased rsFC of left hippocampus with the right posterior cingulate cortex and also the subjects with IGD showed increased rsFC from right hippocampus to the right precuneus when compared with HC. The observation of decreased rsFC from bilateral hippocampus to premotor cortex may infer that excessive use of Internet gaming causes functional abnormality in spatial memory and movement related to surrounding stimuli. Further study is needed to verify the brain abnormality caused by obsessive use of Internet game. Acknowledgements This work was supported by the National Research Foundation of Korea Grant funded by the Korean Government (NRF-2014M3C7A1062893).

Disclosures: J. Kim: None.

Poster

090. Cognitive Development

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Program#/Poster#: 90.16/JJJ34

Topic: H.02. Human Cognition and Behavior

Support: NSF DRL 0929779

Title: Correlation of cognitive training gains and resting state functional connectivity

Authors: ***C. LEDBETTER**[1], M. FAISON[2], O. HILL[2], J. PATTERSON[1];
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Abstract: Background: Cognitive training interventions purport to improve overall cognitive ability by strengthening individual cognitive skills essential to learning. It is presumed that these gains are in part a result of the positive effects of neural plasticity. Functional MRI and analysis of resting state connectivity offers a non-invasive method for investigating underlying changes in functional connectivity associated with cognitive training. Objective: The purpose of this study analysis was to test the hypothesis that completion of an intensive cognitive training program would result in cognitive skill gains, and that changes in functional connectivity would correlate with measured gains. Methods: To test this hypothesis a group of high-school students (n=23) completed 15-weeks of intensive cognitive training. 11 completed the one-on-one ThinkRx program and 12 completed the BrainSkills program (a digital version of ThinkRx). These students, along with a control group (n=7) of students, underwent pre and post cognitive testing and functional MRI imaging. Cognitive tests measured processing speed, working memory, visual memory, auditory memory, short-term memory, long-term memory, visual processing, auditory analysis segmenting, auditory analysis drop, and logic and reasoning. MR imaging was performed on a 1.5T GE MR scanner and a high-resolution T1 anatomical scan and a 5 minute EPI-BOLD resting state functional scan were acquired. Resting state functional connectivity (rs-fc) was calculated and correlated with cognitive testing scores using SPM12 and the CONN toolbox. Results: The most significant gains associated with cognitive training were seen in measures of auditory processing (auditory analysis segmenting, $F=16.34$, $p=0.000$; auditory analysis drop, $F=13.56$, $p=0.001$). Group analysis demonstrated an association between participation in this intensive cognitive training intervention and changes in rs-fc with the auditory cortex that correlated with auditory processing gains (superior temporal gyrus, $p < 0.01$; middle inferior temporal gyrus, $p< 0.001$; inferior temporal gyrus, $p<0.001$). In addition, following cognitive training network global efficiency increased ($T=2.44$, $p=0.02$). Conclusion: Findings from this analysis provide support for the hypothesis that changes in network connectivity underlie gains in cognitive ability resulting from cognitive training intervention. Further, they provide causal support for the hypothesis that intensive cognitive training can positively affect neural plasticity.

Disclosures: **C. Ledbetter:** F. Consulting Fees (e.g., advisory boards); GIBSON INSTITUTE. **M. Faison:** None. **O. HIll:** None. **J. Patterson:** None.

Poster

090. Cognitive Development

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Program#/Poster#: 90.17/JJJ35

Topic: H.02. Human Cognition and Behavior

Support: LearningRx Research and Development Fund

Title: Intensive, metronome-based, one-on-one cognitive training improves cognitive skills in children

Authors: *A. L. MOORE[1], C. LEDBETTER[2], D. M. CARPENTER[3];
[1]Gibson Inst. of Cognitive Res., Colorado Springs, CO; [2]Hlth. Sci. Ctr., LSU, Shreveport, LA;
[3]Univ. of Colorado Colorado Springs, Colorado Springs, CO

Abstract: Cognitive skills associated with general intellectual ability predict reading ability, academic achievement, severity of mental health problems, social mobility, obesity, suicidality, early mortality, income potential, and occupational performance. Accelerating the development and remediation of cognitive skills can be accomplished with an intensive training such as ThinkRx, a comprehensive, trainer-delivered intervention that targets multiple cognitive skills. OBJECTIVE: In a 2-phase randomized controlled trial, we examined the differences between two methods of delivering an intensive, metronome-based cognitive training program on IQ, memory, visual & auditory processing, processing speed, reasoning, and attention for children ages 8-14 in a center setting. METHODS: In phase 1, participants (n = 39) were randomly assigned to either an experimental group (n = 20) to complete 60 hours of cognitive training or to a waitlist control group (n = 19). Pre and post training assessments included the Woodcock Johnson III - Tests of Cognitive Abilities. The experimental group attended 40 90-minute sessions delivered one-on-one by a cognitive trainer who modified the intensity of 24 mental tasks using a metronome, stopwatch, manipulatives, and deliberate distractions. In phase 2, the waitlist control group from phase 1 completed 60 hours of the same cognitive training program with 30 hours delivered 1-on-1 by a cognitive trainer and 30 hours delivered through a digital platform. The tasks in the digital version of the program mimicked the tasks in the 1-on-1 program but did not include feedback or modifications. RESULTS: Phase 1 training effects included significant pretest to post-test gains on all measures, and a mean gain of 21 points in IQ for the trained group. MANOVA results indicated an overall significant difference between treatment and control groups (F = 15.83, p = .00, partial η^2 = .83), with pairwise comparisons indicating significant differences on all measures except attention. Phase 2 training effects included significant pretest to post-test gains on all measures, and a mean gain of 22 points in IQ for the second trained group. Although MANOVA results revealed an overall significant difference between the 1-on-1 delivery group and the hybrid delivery group (F = 3.36, p = .008,

partial η^2 = .48), pairwise comparisons only indicated a significant difference on long-term memory. CONCLUSION: The generalized improvements across cognitive skills suggests that two methods of delivering ThinkRx, an intensive, metronome-based, cognitive training, may be viable options for enhancing general intellectual ability and cognition in a center setting.

Disclosures: **A.L. Moore:** A. Employment/Salary (full or part-time): Gibson Institute of Cognitive Research. **C. Ledbetter:** F. Consulting Fees (e.g., advisory boards); LearningRx Scientific Advisory Board. **D.M. Carpenter:** None.

Poster

090. Cognitive Development

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 90.18/JJJ36

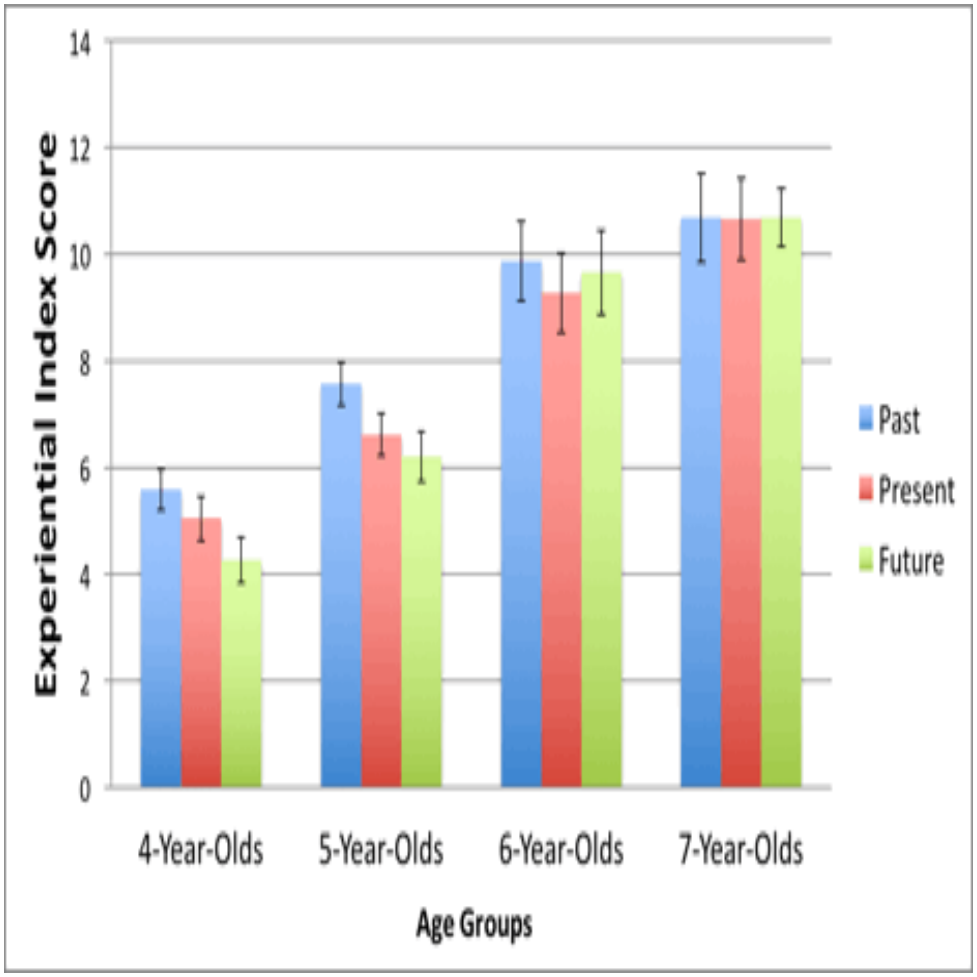
Topic: H.02. Human Cognition and Behavior

Title: Assessing the development of episodic memory and future thinking through verbal and behavioral tests

Authors: *K. L. DICKERSON, E. BRYANT, A. M. SEED, J. A. AINGE;
Psychology and Neurosci., Univ. of St Andrews, St Andrews, United Kingdom

Abstract: The ability to mentally recreate past experiences and the ability to imagine future episodes are supported by a common network of brain regions. The explanation for this link at a cognitive level is debated, but it has been suggested that both of these cognitive processes are supported by mental time travel. Through the mental representation and flexible evaluation of future scenarios, we are able to anticipate and plan for events that have not yet come to fruition. Episodic memory and episodic future thinking has been tested in the literature through behavioral and verbal tasks. However, the relationship between functional ability and reporting skill has not yet been evaluated. In order to explore this question, we verbally evaluated differences in memory, planning and imagination in children between the ages of four and seven years. We compared these results to an experimental test of memory and planning (a version of the so-called spoon test) and evaluated differences both within individuals and across development. In the interviews subjects were instructed to remember or create a scene based on word prompts. Trials fell into three language categories: past, present and future. Each interview was transcribed and coded based on the guidelines given by Hassabis, Kumaran and Maguire (2007) with modifications to allow for the decreased age of participants. Across all three conditions scores improved incrementally with age. For four and five-year-olds, the ability to construct rich scenes appears diminished in the future and present tenses as compared to the past tense. This indicates that these verbal tasks possess a unique developmental trajectory, possibly

due to the unique element of construction in present and future scene generation. We also completed a forced-choice task, which are more commonly used in this age group, and found that a correct choice on this task was correlated with higher performance in the future and present tenses during the interview assessment. This may help to validate item-choice tasks as a test of episodic future planning capabilities in non-verbal populations.



Disclosures: **K.L. Dickerson:** None. **E. Bryant:** None. **A.M. Seed:** None. **J.A. Ainge:** None.

Poster

090. Cognitive Development

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Topic: H.02. Human Cognition and Behavior

Support: LEGO Foundation

LEGO Education

Title: Effects of 6 weeks motor-enrichment-intervention to improve math performance in preadolescent children

Authors: *J. WIENECKE[1], M. BECK[2], R. LIND[2], J. LUNDBYE-JENSEN[2], S. GEERTSEN[2]; [1]Univ. of Copenhagen, Copenhanen, Denmark; [2]Univ. of Copenhagen, Copenhagen, Denmark

Abstract: Multisensory learning paradigms can positively affect learning of various cognitive skills (Shams & Seitz, 2008). Furthermore, integrating congruent motor movements (i.e. motor-enrichment) during acquisition can be superior to only audio-visual acquisition in academically related learning (Mayer et al., 2015).
We conducted a six-week cluster-randomized intervention study of motor-enriched mathematics for Danish schoolchildren (n= 148, age= 7.5 ± 0.02). We investigated whether low intensity motor activity congruently integrated during solving of math problems could enhance math performance. Three groups were included:
1) Control group with normal math teaching, CON (used pencil, paper but refrained from additional motor activity).
2) Fine-motor-enriched-group, FM (motor-manipulating LEGO bricks integrated in the lessons).
3) Gross-motor-enriched-group, GM (full-body movements integrated in the lessons).
In FM and GM, all math classes (six lessons pr. week) had motor activity integrated in the math lessons and the teachers of all groups followed a detailed description for the conduction of the lessons. This aimed at ensuring homogeneity between groups concerning the taught themes.
The children were tested pre- (T0) and post- (T1) intervention. We used a standardized 1st grade math test including 2nd grade questions to avoid a potential ceiling effect (50 math questions in total). Additionally, we conducted a phonological- and spatial working memory test and a test of executive functioning to investigate potential mediators of academic performance. Furthermore, electroencephalography (EEG) was measured during mental arithmetic at T1.
All groups improved their numbers of correct answers (CA) from T0 to T1. Interestingly, the improvement in GM from T0-T1 was significantly larger than FM 1.76 ± 0.70 CA ($P = 0.03$). Additionally, a subgroup analysis was performed, extracting data from children with learning difficulties, according to national standards (n= 44). In this subgroup no significant differences was observed in the improvements from T0-T1 between the three groups ($P > 0.05$). Conversely, in normal-achieving children, GM improved significantly more from T0-T1 than CON 1.78 ± 0.70 CA ($P = 0.04$) and FM 2.14 ± 0.69 CA ($P < 0.01$) from T0-T1.
We conclude that gross-motor enrichment can increase math performance and that this effect might be more pronounced for normal-achieving children compared to children with learning difficulties. Future work should seek to investigate the mechanisms behind the observed behavioral differences.

Disclosures: **J. Wienecke:** None. **M. Beck:** None. **R. Lind:** None. **J. Lundbye-Jensen:** None. **S. Geertsen:** None.

Poster

090. Cognitive Development

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Topic: H.02. Human Cognition and Behavior

Support: MOST Grant 103-2511-S-004-004

MOST Grant 104-2511-S-004 -004

Title: Development of neural stability for four basic arithmetic operations

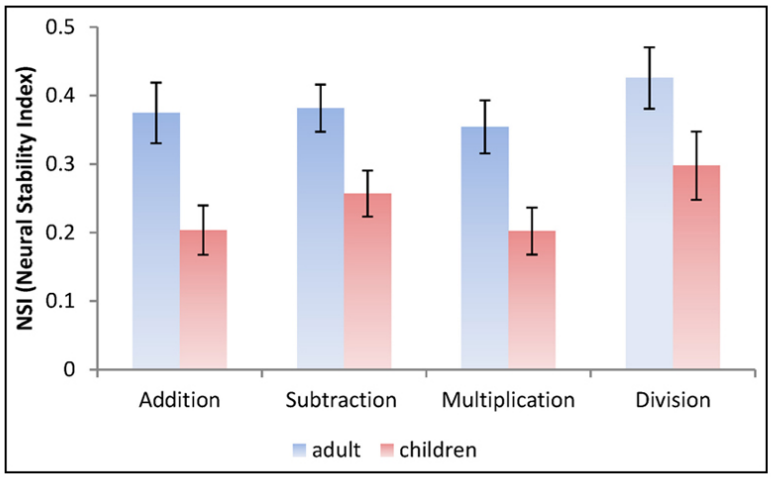
Authors: *T.-T. CHANG[1], P.-H. LEE[2], A. W. S. METCALFE[3];
[1]Natl. Chengchi University., Taipei, Taiwan; [2]Natl. Chengchi Univ., Taipei, Taiwan;
[3]Sunnybrook Res. Inst., Toronto, ON, Canada

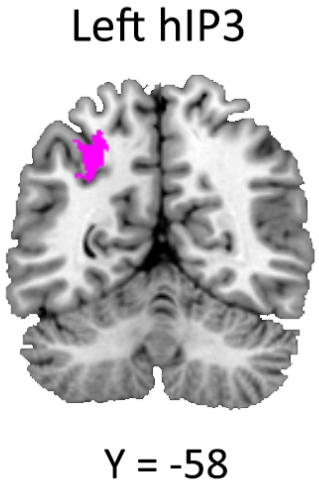
Abstract: Arithmetic problem solving skill is a cognitive domain crucial for academic development. Although neuroimaging studies have well characterized the neural engagement of distinct arithmetic problems, how these skills transit into more stable representations with learning and experience is yet unknown. Here we tackle this fundamental issue by implementing trial-wise multivoxel representational similarity (MRS) analyses (Kriegeskorte, Mur, & Bandettini, 2008) on a cross-sectional fMRI study.

We assessed brain activation of a group of 19-to-29-year-old adults (n=29) and a group of 8-to-10-year-old children (n=21) while they performed the four basic arithmetic operation problems - addition, subtraction, multiplication, and division. We first examined whether each participant demonstrated stable neural representations for each of the four operations by implementing trial-wise MRS analyses. Beta weights of each trial for each operation were extracted from voxels within anatomically-defined bilateral IPS, the regions specialized for numerical processing. For each participant, neural stability index (NSI) was calculated as an average of the pairwise correlation coefficient for each pair within each operation. NSIs were then entered into a 4 (operations)-by-2 (group) ANOVA, with operation as within-subject and group as between-subject factors.

As shown in Figure 1, regardless of age, subtraction and division engaged higher levels of NSIs than addition and multiplication ($F = 6.518$, $p < .001$). Relative to children, adults exhibited higher levels of neural stability in the left IPS when solving problems from all of the four operations ($F = 7.510$, $p = .009$).

Our findings suggest that the development of arithmetic problem solving skills from childhood to adulthood is characterized by more stable neural representations in brain regions crucial for representing numerical quantity. This pattern is more salient in operations associated with quantity manipulation, suggesting that the emergence of arithmetic problem skill has a function-specific developmental progression.





Disclosures: **T. Chang:** None. **P. Lee:** None. **A.W.S. Metcalfe:** None.

Poster

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Program#/Poster#: 90.21/JJJ39

Topic: H.02. Human Cognition and Behavior

Support: the Science Campus Tuebingen, project 8.4

Title: The Neural underpinnings of arithmetic learning in children are different from those in adults

Authors: ***M. SOLTANLOU**[1,2,3], C. ARTEMENKO[4], T. DRESLER[4,5], A.-C. EHLIS[5,4], A. J. FALLGATTER[5,4], H.-C. NUERK[1,3,4];
[1]Dept. of Psychology, Univ. of Tuebingen, Tuebingen, Germany; [2]Grad. Training Ctr. of Neuroscience/ IMPRS for Cognitive and Systems Neurosci., Tuebingen, Germany; [3]Leibniz-Institut für Wissensmedien, Tuebingen, Germany; [4]LEAD Grad. Sch. and Res. Network, Univ. of Tuebingen, Tuebingen, Germany; [5]Dept. of Psychiatry and Psychotherapy, Univ. Hosp. of Tuebingen, Tuebingen, Germany

Abstract: Arithmetic learning studies in adults suggested reduced activation in the fronto-parietal network and increased activation in the left angular gyrus after learning. This shift has been assumed to be associated to shift from procedural to retrieval strategies. However,

developmental studies indicate that findings are not easily transferable from adults to children. Therefore, in the present study, brain activation changes of arithmetic learning in children have been investigated.
20 fifth-graders received seven sessions of complex multiplication training by using a web-based learning platform. Their performance was tested before training, after one session of training, and after seven sessions of training via functional near-infrared spectroscopy (fNIRS), while children solved multiplication problem.
Behavioral data revealed significantly improved performance in trained complex problems after seven sessions of training, being accompanied by decreased activation of the left angular gyrus and medial temporal gyrus, and also of the right middle frontal gyrus. After one session of training, although no significant behavioral improvement was observed in trained multiplication problems, fNIRS data revealed decreased activation of the left angular gyrus and inferior parietal lobule, along with decreased activation in the right superior parietal lobule and intraparietal sulcus.
In line with multiplication learning in adults and training studies of other operations in children, our findings indicate a decreased fronto-parietal network activation after multiplication training. However, contradictory to the multiplication training in adults, decreased activation of the left angular gyrus and surrounding regions was found. We conclude that strategy shifts from procedural to retrieval strategies in children engage different neural patterns than in adults and that the role of angular gyrus activation changes over the course of development.

Disclosures: **M. Soltanlou:** None. **C. Artemenko:** None. **T. Dresler:** None. **A. Ehlis:** None. **A.J. Fallgatter:** None. **H. Nuerk:** None.

Poster

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Topic: H.02. Human Cognition and Behavior

Support: Korea NRF Grant 2014R1A1A3051034

Title: Number comparison efficiency mediates the relationship between the linearity of the mental number line and math achievement

Authors: ***N.-R. KIM**, S. CHO;
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Abstract: Numerical magnitude is believed to be mentally represented as a 'number line' that resembles a ruler, in which relatively smaller vs. larger magnitudes are represented towards the left vs. right side in the order of magnitude (Note, the direction of space-magnitude association may depend on reading direction or culture). Studies of children's number estimation consistently report overestimation of relatively smaller numbers and underestimation of relatively larger numbers manifesting as a logarithmic pattern of estimation. With development, there is a gradual logarithmic-to-linear transformation of number line estimation performance. Many previous studies report that measurements of the linearity of the mental number line correlates with math achievement. On the other hand, number comparison performance has also been reported to be correlated with math ability. Number comparison performance can be thought to reflect how efficiently one can accurately access the semantic meaning of numbers represented on the mental number line. In the present study, we investigated whether number comparison efficiency mediates the relationship between the linearity of the mental number line and math achievement.

Forty-nine first graders (aged 6 to 7, 25 females) participated in the present study. We measured each child's performance on number line estimation, Arabic number comparison and a comprehensive math achievement test (covering calculation, mental arithmetic, etc.). The number range for number line estimation was from 0 to 200 which is more expanded, compared to most previous studies which tested estimation up to 100. In the Arabic number comparison task, two Arabic numbers were presented side by side on each side of the screen. Participants were asked to indicate with a button press which number was larger in semantic magnitude.

A mediation analysis using a bootstrapping method (5000 resamples, 95% confidence interval) was employed. As a result, the relationship between the linearity of the mental number line and math ability was found to be fully mediated by number comparison performance (more specifically, response time). This result supports the idea that children's ability to efficiently access the semantic meaning of symbolic numbers mediates the previously reported relationship between the linearity of the mental number line and math achievement.

Disclosures: **N. Kim:** None. **S. Cho:** None.

Poster

090. Cognitive Development

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Program#/Poster#: 90.23/JJJ41

Topic: H.02. Human Cognition and Behavior

Support: CHRI Stanford CTSA UL1 TR000093 to M.S.

HPI-Stanford Hasso Plattner Design Thinking Research Program (HPDTRP) to A.L.R.

NIMH Career Development Award K99-MH104605 to M.S.

Title: Finding the neural correlates of middle childhood "slump" in creativity

Authors: *M. SAGGAR, A. STANKOV, M. SCHREIER, A. REISS;
Psychiatry and Behavioral Sci., Stanford Univ., Palo Alto, CA

Abstract: Little is known about the underlying neurodevelopmental processes that contribute to a child's creative capacity. Of particular interest is the decline in creativity during middle childhood (esp. 4th grade). This decline has been linked to underachievement and an increased risk for mental health problems. Longitudinal neuroimaging studies are, thus, required to understand whether decline in creativity is due to the burden of social norms, educational training, or typical brain development. Here, we present preliminary results from a cohort-sequential semi-longitudinal study using functional Near-infrared Spectroscopy (fNIRS). A total of 56 children (n=24 third-graders and n=32 fourth-graders) were assessed longitudinally at two time points. Creativity was evaluated using the standardized Torrance Test of Creative Thinking (TTCT). The 52-channel fNIRS data, covering bilateral prefrontal cortices, were collected while children engaged in the TTCT and a control-drawing task to connect dots on paper. Additionally, data were collected to assess general intelligence, response inhibition, temperamental characteristics, and family environment. As expected, the examination of average TTCT raw scores indicated a significant grade by time interaction ($p=0.044$), such that 3rd graders scored higher on TTCT during the end of their grade, while 4th graders had a decline in TTCT scores towards the end of their grade. Due to technical difficulties, only a subset of participants (n=15 fourth-graders and n=9 third-graders) had good quality fNIRS data at both assessments. Based on previous work, we hypothesized that the 4th-grade decline in creativity could be associated with a greater need for "conformity" from classroom expectations and peer pressure. We operationalized conformity in terms of variability in task-related functional connectivity (FC). Thus, less variability in FC would imply greater conformity. Significant grade by time interactions were observed for both TTCT and control conditions ($ps < 0.05$), such that 4th graders had reduced variability in FC towards the end of their grade, where as no changes in FC variability were observed for the 3rd graders. Taken together, our data suggest that longitudinal neuroimaging studies could provide better insights about the neural basis for 4th-grade decline in creativity.

Disclosures: **M. Saggar:** None. **A. Stankov:** None. **M. Schreier:** None. **A. Reiss:** None.

Poster

090. Cognitive Development

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 90.24/JJJ42

Topic: H.02. Human Cognition and Behavior

Support: K99HD078483

Title: The functional brain architecture of own- and other-race face processing in children and adults

Authors: *G. ANZURES[1], C. J. MONDLOCH[3], F. HAIST[2];
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Abstract: Differential experience with different races profoundly affects face perception in children and adults. Understanding this other-race effect can shed light on the development of racial stereotypes and prejudices. However, we know virtually nothing about the functional brain architecture for own- and other-race face processing in children. Here, we administered a passive and an active face perception task during FMRI testing (multi-echo simultaneous multi-slice [MESMS] acquisition with Multi-echo Independent Component Analysis [ME-ICA]) in Caucasian adults (N=16, 18-21 years) and children (N=14, 8-10 years). In the passive task, participants viewed Caucasian and Asian faces and houses (blocked design) and pressed a button indicating the color of the background. In the categorization task, participants pressed buttons to indicate Caucasian and Asian faces (event-related). Behaviorally, adult and child participants showed the classic other-race effect in face recognition ($p < .01$). In the simple viewing FMRI task, all adults produced a reliable fusiform face area (FFA) bilaterally (faces > houses), 12 of 14 children produced bilateral FFAs. Within the right FFAs, other-race faces produced a greater extent of activation than own-race faces in adults and children. Whole-brain analyses of the simple viewing task showed an other-race activation advantage primarily in posterior occipito-temporal regions, including bilateral fusiform gyrus (right > left), lingual gyrus, calcarine cortex, and cuneus. Adults, but not children, also showed greater other-race processing in left amygdala, left lateral inferior occipital gyrus (OFA region), and left inferior frontal gyrus (BA47). We found striking developmental differences in the categorization task. Adults showed greater other-race activation in left insula, left superior frontal gyrus, and crucially, left anterior cingulate gyrus. In contrast, children produced greater other-race activation throughout all regions of cortex with the important exception of the anterior cingulate gyrus. During simple viewing, adults showed greater processing for other-race faces in core and extended face regions that were not observed in children. When categorizing faces by race, children produced widespread hyperactivation to other-race faces throughout cortex, but the anterior cingulate cortex, thought

to be crucial for monitoring and controlling automatic thoughts of prejudice, stereotype, and bias, is not mature during this period of childhood.

Disclosures: **G. Anzures:** None. **C.J. Mondloch:** None. **F. Haist:** None.

Poster

090. Cognitive Development

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 90.25/JJJ43

Topic: H.02. Human Cognition and Behavior

Title: The impact of emotional cues on short-term and long-term memory during adolescence

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Abstract: Emotional information pervades daily life and can influence memory, which affects learning and performance of everyday cognitive tasks. Adolescence is a time of heightened sensitivity to emotional and social inputs, as dynamic changes in brain structure and function take place. In adults, emotional information typically enhances subsequent memory, via consolidation mechanisms involving the amygdala (Beyler et al., 2016); however, the impact of emotional and social content on memory across development, as neural circuitries continues to mature (Casey et al., 2016), remains unclear. The present study implements an emotional n-back task using happy, fearful, and calm faces as well as places in individuals ages 9 to 29 (n = 90, 50 females). Participants are tested for their implicit memory of the stimuli used in the task either immediately after completing the task or 24 hours later, to allow for memory consolidation. Adolescents show better memory for places than faces in the immediate recall condition. However, after 24 hours, we observe linear improvement in corrected recognition memory (correct hits minus incorrect false alarms) for neutral faces across age and non-linear changes in memory for happy and fearful cues. Late adolescence also shows evidence of a novelty bias to faces after 24-hour consolidation. These preliminary results suggest that emotional and social information influence short-term and long-term memory capacity across development. These findings may have implications for learning and for observed developmental differences in behavior.

Disclosures: **A. Cohen:** None. **D.V. Dellarco:** None. **M.I. Conley:** None. **B. Casey:** None.

Poster

090. Cognitive Development

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 90.26/JJJ44

Topic: H.02. Human Cognition and Behavior

Support: Royal Society Grant SJ Blakemore

Title: Neural predictors of educational attainment in adolescence

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[2]Exptl. Psychology, [3]Inst. of Cognitive Neurosci., Univ. Col. London, London, United Kingdom

Abstract: The developmental mismatch hypothesis proposes that, in humans, striatal structures involved in reward processing mature earlier than prefrontal areas involved in cognitive control, leading to a mismatch in maturation in adolescence. Structural mismatch in adolescence has mainly been investigated in relation to risk and sensation seeking behaviors but it may also affect other processes relying on the interplay between reward processing and self-control. One such measure is grit, the ability to sustain efforts towards long-term goals. Grit has been shown to predict changes in grades better than IQ. Here we used magnetic resonance imaging to investigate whether connectivity and activation of frontal and striatal regions as an indicator of structural mismatch predicts grit during adolescence. We scanned adolescent girls to assess fronto-striatal connectivity strength and frontal and striatal activation in an emotional go-no go task. We correlated these neural predictors with grit to assess the validity of structural mismatch in adolescence as a predictor of educational outcomes.

Disclosures: D. Fuhrmann: None. **M. Speekenbrink:** None. **S. Blakemore:** None.

Poster

091. Schizophrenia: Behavior and Symptoms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 91.01/JJJ45

Topic: H.03. Schizophrenia

Support: JSPS KAKENHI 25240019

Title: Cognitive functions negatively correlate to the angular gray matter volume in schizophrenia

Authors: ***T. UENO**[1], H. KUGA[1], N. ORIBE[1], K. TAKAO[1], R. HAYASHIDA[1], N. NAKAYAMA[1], H. MIZUHARA[2], T. YUZURIHA[1];
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Abstract: Background; Several Studies showed that impairment of cognitive function in schizophrenia patients. (Hedman, van Haren et al. 2012) However, little is known about the exact intelligence quotients (IQ) using Wechsler Adult Intelligence Scale (WAIS) in schizophrenia patients. Moreover, there are very few studies about the relationship of brain structure and IQ in schizophrenia. The current study shows the correlation between regional brain gray matter volume and IQ in schizophrenia patients.
Methods; Thirty schizophrenia patients were recruited from Hizen Psychiatric Center. They met to DSM-4 criteria diagnosed by two independent psychiatrists (age 20-65). All participants signed informed consent forms according to the ethical committee of the Hizen Psychiatric Center. The exclusion criteria were alcohol/drug abuse, brain hemorrhage /infarction, or thyroid dysfunction. Independent psychologist tested the patients using WAIS to determine the exact cognitive function. All participants were scanned by 1.5 T MRI machine (Phillips) to get the T1 weighted structural brain images in 6 minutes. Resolution was 1mm x 1mm x 1.2 mm. All the images were segmented to gay matter images and converted to normalized images with the canonical brain image in MNI coordinate with the method of Dartel method in SPM software (Ashburner 2007). General linear model was used to investigate correlation between the regional gray matter volume and the full IQ of WAIS with covariate of age and sex. Cutoff was under 0.001 (P value) of each point of brain, and 0.001 (P value) of spatial extent with Gaussian random field model to exclude the type 1 error.
Result; We found the negative correlation between the regional gray matter volume and full IQ in right angular cortex. No voxel was survived in positive correlation.
Discussion; The angular gyrus was considered to have functions of attention, arithmetic ability, and spatial cognition. Interestingly low volume of this area was negatively correlated to high IQ. A paper showed that more intelligent children demonstrated a particularly plastic cortex. (Shaw, Greenstein et al. 2006) The result might suggest dynamical change of angular cortex along a curing course of schizophrenia.
Ashburner, J. (2007). Neuroimage **38**(1): 95-113.
Hedman, A. M., N. E. van Haren, et al. (2012). Psychol Med **42**(12): 2535-2541.
Shaw, P., D. Greenstein, et al. (2006). Nature **440**(7084): 676-679.

Disclosures: **T. Ueno:** A. Employment/Salary (full or part-time): full, Hizen Psychiatric Center. **H. Kuga:** None. **N. Oribe:** None. **K. Takao:** None. **R. Hayashida:** None. **N. Nakayama:** None. **H. Mizuhara:** None. **T. Yuzuriha:** None.

Poster

091. Schizophrenia: Behavior and Symptoms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 91.02/JJJ46

Topic: H.03. Schizophrenia

Title: Hemispheric silencing of phospholipase C-β1 in the right anterior cingulate cortical neurons leads to impaired observational fear learning in mice

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Abstract: Patients with schizophrenia exhibit significant deficiencies in empathic behaviors, resulting in social dysfunction. Decreased expression of phospholipase C-β1 (PLC-β1) has been detected in some brain regions of patients with schizophrenia. Nonetheless, a causal link between this biochemical change and impaired empathic behaviors has not been demonstrated. In this study, we report that mice with a specific knockdown of PLC-β1 in the anterior cingulate cortex (ACC), but not in the prelimbic or infralimbic cortex, exhibited impaired observational fear learning in mice. On slice recording experiment, neuronal excitability of ACC was decreased in the mutant mice compared with wild-type. Furthermore, a knockdown of PLC-β1 in the right ACC, but not in the left ACC, decreased observational fear learning. These results indicate that PLC-β1 signaling in the right ACC is critical for observational fear learning. Importantly, these results support the notion that the decrease in PLC-β1 expression in the brains of patients with schizophrenia is a pathogenically relevant molecular marker of the disorder.

Disclosures: S. Kim: None. **M. Kim:** None. **J. Byun:** None. **T. Cho:** None. **Y. Kim:** None. **H. Shin:** None.

Poster

091. Schizophrenia: Behavior and Symptoms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 91.03/JJJ47

Topic: H.03. Schizophrenia

Support: PhD fellowship from PSL Research University

Title: Perceptual rivalry as bayesian inference : Accumulating evidences towards circular inferences in schizophrenia

Authors: *P. LEPTOURGOS[1], C.- . NOTREDAME[2], R. JARDRI[2], S. DENEVE[1];
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Abstract: Probabilistic inference is considered as one of the most promising candidate frameworks to explain
brain function. Similarly, impairments in the mechanics of those inferences have been suggested as the
generative mechanism of various psychiatric illnesses, including schizophrenia. According to the Circular
Inference Framework (CIF), aberrant inferences (circular inferences) implemented in cortical networks
generate a system that overinterprets weak sensory evidence (and / or priors) and might cause psychotic symptoms (Jardri and Denève, 2013). In this project, we used bistable perception to probe
those mechanisms and validate experimentally the CIF in the domain of visual perception.
The Necker Cube is an ambiguous figure, known to induce bistability. We continuously presented such
figures to healthy participants (in a preliminary study) and schizophrenia patients (in an ongoing study)
during 15 consecutive runs. We manipulated sensory evidence by adding shades to the stimuli (in the
last 3 runs) and prior expectations by giving different instructions to 3 different groups (15 – 15 – 20
participants), concerning the presence of an implicit preference. Participants' responses were discretely
and pseudo-regularly collected (according to Mamassian and Goutcher, 2005).
In the preliminary study conducted on healthy participants (N=50), we confirmed the existence of an
implicit prior ($Z=-4.613$, $p<0.001$) and we showed that manipulation of this prior had significant opposite
effects on relative predominance (Kruskal Wallis, $H(2)=9.35$, $p=0.009$), either by exacerbating or
cancelling the intrinsic bias of the system, but also significantly reduced reaction times ($H(2)=10.74$,
$p=0.0047$). The effect of sensory evidence was even stronger ($Z=-3.364$, $p<0.001$), and induced a significant bias corresponding to the direction of the cue. Moreover, we found trends in the correlations

between bistability and psychotic tendencies (measured with the LSHS score). At the theoretical level, a
simple multiplicative rule produced good fits and first order markovian statistics could account very well
for the dynamics of bistability. Finally, we extended the above study by recruiting schizophrenia patients
with prominent psychotic symptoms (ongoing study). Our first results suggest that patients exhibit
important deviations from optimality and their behavior is well predicted by the CIF (higher alternation
rate, weaker effects of the visual cues, instructions).

Disclosures: **P. Leptourgos:** None. **C. Notredame:** None. **R. Jardri:** None. **S. Deneve:** None.

Poster

091. Schizophrenia: Behavior and Symptoms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 91.04/JJJ48

Topic: H.03. Schizophrenia

Support: BMBF Grant 01ZX1404D

Title: Lower resilience against surprising information predicts hallucination proneness in healthy individuals

Authors: ***H. STUKE**[1], H. STUKE[2], V. WEILNHAMMER[1], K. SCHMACK[1];
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Abstract: Introduction: Sensory information is intrinsically ambiguous and a stable interpretation of the world is only possible through a suppression of unlikely interpretations by prior beliefs. A weakening of the impact of prior beliefs might thus lead to a tendency towards unlikely interpretations and hence contribute to the formation of hallucinations in psychosis. Here, we empirically tested this suggested link between a weakened impact of prior beliefs and psychosis-like symptoms. Methods: Eighty-two healthy human participants with varying hallucination proneness performed a probabilistic reasoning task, in which carps and trouts were sequentially angled from one of two possible lakes. On each trial, participants had to guess from which lake fishes were being angled based on two sources of information: Firstly, the proportion of fishes differed between the two lakes and participants could infer the lake on the basis of the

number of already angled carps and trouts. Secondly, prior information was given in form of a tone that was - dependent on the pitch - more often associated with the one or the other lake. For data analysis, we developed a novel computational learning model that tracks the trajectory of the participants' beliefs about the correct lake. This model allowed us to quantify the impact of prior beliefs in two ways: (1) based on static information learned prior to the experiment (i.e., the tone pitch), and, (2) as the 'resilience against surprising information' of the belief generated during the course of the experiment: This resilience was captured by a non-linear relationship between prediction error and learning that reduces the impact of more surprising information. For cross-validation, participants performed a standard reversal learning task where the number of strategy changes served as a measure for belief instability. Results: The novel model proved to be superior to conventional learning models in explaining the participants' behavior. The parameter encoding the impact of the static information learned prior to the experiment was not associated with neither hallucination proneness nor belief instability. The non-linearity parameter encoding the strength of resilience against surprising information however correlated negatively with hallucination proneness and belief instability. Discussion: Our results suggest that a lowered resilience against surprising information, as implemented by a non-linear relationship between prediction error and learning, might be involved in the formation of hallucinations. Our current findings hence empirically substantiate theories that link psychosis to a weakened stability of prior beliefs.

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Poster

091. Schizophrenia: Behavior and Symptoms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 91.05/JJJ49

Topic: H.02. Human Cognition and Behavior

Support: Human Neurobiology Laboratory Support Contract with Pfizer, 55202665

Title: Test-retest reliability of the MATRICS Cognitive Consensus Battery (MCCB) in persons with schizophrenia and healthy volunteers: A non-interventional study

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Abstract: Objective. The MATRICS Cognitive Consensus Battery (MCCB) evaluates cognitive function and change due to treatment in schizophrenia (SCZ) patients. The goals of this study were: (1) Evaluate the Practice Effect (PE) of repeated MCCB scores in SCZ to establish test-retest reliability without intervention (2) Compare baseline scores and PEs between SCZ and healthy volunteers (HV).
Methods. This study, approved by the Boston University School of Medicine Institutional Review Board conformed to the Declaration of Helsinki. Thirty-one HV and thirty SCZ adults provided written consented and the MCCB battery twice (interval: 14±2 days).
Age-normed performance was assessed on all MCCB tests: Trail Making Test (TMT), Brief Assessment of Cognition in Schizophrenia Symbol Coding (BACS), Hopkins Verbal Learning Test-Revised (HVLT-R), Wechsler Memory Scale-III Spatial Span (SS), Letter Number Span (LNS), Neuropsychological Assessment Battery Mazes (NAB), Brief Visuospatial Memory Test-Revised (BVMT-R), Continuous Performance Task-Identical Pairs (CPT-IP); Composite scores: attention/vigilance, speed of processing, working memory, verbal learning, visual learning, reasoning and problem solving, and overall composite score.
Baseline and follow-up data were compared using paired-samples t-tests with PE considered statistically significant ($p<.05$, 2-tailed) with an effect size (Cohen's d) ≥ 0.2.
Results. Incomplete data from two HVs were excluded from CPT-IP, attention/vigilance, and overall composite analyses.
SCZ group displayed medium PEs for BVMT-R and visual learning (*p's < .05, d's =0.54)* and weak PEs for LNS and overall composite score (*p's < .05, d's =0.40-0.48)*. Scores on all other tests and composites remained unchanged.
HV group displayed strong PEs for BACS, speed of processing, working memory, and overall composite (*p's < .001, d's =0.81-1.34*); medium PEs for NAB, LNS, BVMT-R, HVLT-R, reasoning/problem solving, verbal learning, and visual learning (*p's < .05, d's =0.56-0.67)*; and weak PEs for TMT and CPT-IP (*p's < .05, d's =0.38-0.41)*.
Independent two-sample t-tests were used to compare SCZ and HV baseline scores. All tests and composite scores except NAB and reasoning and problem solving showed statistically significant differences (with at least $p<.05$).
Conclusion. In the SCZ group, MCCB had good test-retest reliability with limited PEs except for LNS, BVMT-R, and overall composite score. In contrast, the majority of tests and the composite scores showed small to very large practice effect in the HV group.
Acknowledgements. Funding was provided by the Neuroscience Research Unit of Pfizer, Inc. The authors declare no competing interests.

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Poster

091. Schizophrenia: Behavior and Symptoms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 91.06/JJJ50

Topic: H.03. Schizophrenia

Support: Stanley Center at the Broad Institute

MH-077851

MH 078113

MH 077945

MH 077852

MH 077862

MH MH100228

Title: Schizophrenia polygenic burden is significantly associated with cognitive performance in nonpsychotic individuals

Authors: *R. SHAFEE[1,3], P. NANDA[4], N. TANDON[5,7], J. PADMANABHAN[6,8], N. ALLIEY-RODRIGUEZ[9], E. GERSHON[10], B. CLEMENTZ[11], G. PEARLSON[12], C. TAMMINGA[13], M. KESHAVAN[2,6], S. MCCARROLL[1,3];
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Abstract: Psychiatric disorders such as schizophrenia are frequently accompanied by cognitive deficits. Probands as well as their unaffected relatives show decreased functioning in many cognitive domains. The Brief Assessment of Cognition in Schizophrenia (BACS) is a cognitive battery designed to capture the deficits that are seen frequently in schizophrenia. We used data from the Bipolar-Schizophrenia Network for Intermediate Phenotypes (BSNIP) consortium to investigate the effect of the polygenic burden of schizophrenia common variants (PRS_SZ) on BACS performance as well as on Wide Range Achievement Test (WRAT, often used as a proxy for premorbid IQ) in two groups: psychotic probands (N = 314) and nonpsychotic individuals consisting of healthy controls and non-psychotic relatives (N = 438). Our BACS score consisted

of six components: verbal memory, verbal fluency, digit sequencing, digit symbol coding, token motor task and Tower of London. We found that PRS_SZ is significantly negatively correlated with digit sequencing (DS) and digit symbol (DSC) coding scores in the nonpsychotic group (combined r = -0.21, p<10^-4) but not in the psychotic group (p>0.05). PRS_SZ showed no significant correlation with WRAT in either group. Additionally, the correlation between BACS and PRS_SZ in the nonpsychotic group remained unchanged after regressing out WRAT. We noted that while DS and DSC BACS scores differed significantly between the psychotic and nonpsychotic groups (p<10^-10), WRAT was only nominally different (p~0.05 after controlling for years of education). Additionally, a polygenic score for educational attainment showed significant positive correlation with WRAT (p<10^-3) but not with BACS (p>0.05). Together, these results indicate that in our sample, schizophrenia polygenic burden captures the variability in cognitive functions affected in schizophrenia much better than the variability in overall intelligence. The absence of significant correlation between PRS_SZ and BACS components in the probands could be due to the effects of medication or due to disease pathology altering these cognitive functions in a way that could not be captured by genetic predisposition to schizophrenia.

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Poster

091. Schizophrenia: Behavior and Symptoms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 91.07/JJJ51

Topic: H.03. Schizophrenia

Title: Behavioral response to LPS treatment in heterozygous Disc1 mutant mice

Authors: *Y.-F. CHANG[1], C.-C. LAI[1], L.-J. LEE[1,2,3];
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Abstract: Both genetic and environmental factors contribute to the pathogenesis of schizophrenia. To study the interaction of the two, we examined the effects of neuroinflammation as an environmental insult in a genetic mouse model of schizophrenia in which the Disc1 gene has been manipulated. In our Disc1 mutant mice, the normal Disc1 gene was replaced by 129S6/SvEv 25-bp deletion variant. In this study, adult male wildtype (WT) and heterozygous Disc1 mutant (Het) mice were used. Neuroinflammation paradigm was induced by

intraperitoneal injection of bacterial endotoxin lipopolysaccharide(LPS; 0.5 mg/kg). One day after LPS or saline injection, mice were subjected to behavioral examinations. The acoustic startle responses were equally reduced in both genotypes after LPS while the ratios of prepulse inhibition were not significantly altered by LPS treatment.In the open field arena, the locomotor activity and explorative behaviors were also largely reduced in both genotypes after LPS injection. The time spent and travelled distance in the central and peripheral area of the open field were further analyzed. No difference was noted between WT and Het mice in saline-treated groups. After LPS injection, the WT mice spent significantly less amount of time in the central region compared with those received saline treatment, indicating a LPS-induced anxiety-like behavioral change.However, in the Het mice, the time spent in the central region was not altered by LPS treatment. Our result indicated the LPS-induced anxiety-like behavior is reduced in heterozygous Disc1 mutant mice.

Disclosures: Y. Chang: None. **C. Lai:** None. **L. Lee:** None.

Poster

091. Schizophrenia: Behavior and Symptoms

Location: Halls B-H

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Program#/Poster#: 91.08/JJJ52

Topic: H.03. Schizophrenia

Title: DISC1 KO as model for bipolar disorders? Correlations of behavior and MR spectroscopy in mice.

Authors: *M. A. VOGT[1], A. SARTORIUS[2], W. WEBER-FAHR[2], A. SAWA[5], A. MEYER-LINDENBERG[3], P. GASS[4];
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Abstract: *Disrupted-in-Schizophrenia 1* (DISC1) was identified as a gene disrupted in a Scottish pedigree suffering of mental diseases as schizophrenia and depression. Although recent genome-wide association studies failed to identify DISC1 as a main genetic factor of schizophrenia, DISC1 is considered as a valid model used to understand the biological and pathophysiological processes underlying mental diseases. Consequently, a variety of DISC1 mutant mice was used in recent years to model a wide range of psychiatric diseases associated to the field of schizophrenia. Here we analyzed DISC1 mutant mice on a C57BL6 background and littermate

controls in a set of behavioral assays dedicated to locomotion, sensorimotor gating and executive functions. As a second step, the mice were analyzed in MR spectroscopy and fMRI for brain activity and metabolism. We could show that DISC1 mutant mice show a distinct behavioral phenotype with subtle hypo-locomotion, decreased acoustic startle response and prepulse inhibition. Referring to executive functions, the mutant mice demonstrated increased ability to problem solving in the puzzle box task. This behavioral phenotype was correlated to results obtained in the MR spectroscopy and fMRI. Although no basal alterations could be detected in the DISC1 mutants, the altered sensorimotor gating was highly correlated to e.g. levels of GABA in the hippocampus. This is, to our knowledge, the first time that a distinct behavioral phenotype could be clearly linked to a neurotransmitter level in a distinct brain region. The behavioral phenotype observed here is more associated towards bipolar disorders than to an endophenotype of schizophrenia. The findings of the spectroscopy studies and fMRI show that this can be linked to a single specific behavioral characteristic.

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Poster

091. Schizophrenia: Behavior and Symptoms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 91.09/JJJ53

Topic: H.03. Schizophrenia

Title: A pilot study to assess the effects of gamma neurofeedback on working memory in schizophrenia patients

Authors: F. SINGH[1], A. SMITH[2], N. DUDECK[2], R. CHENG[2], R. GOSLA[2], E. HERRERA[2], Y. QIU[2], Z. YANG[2], *J. A. PINEDA[2];
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Abstract: Deficits in working memory are a common and recalcitrant symptom of schizophrenia (SCZ) spectrum disorders and have been associated with reduced gamma (35-50Hz) synchrony in frontoparietal regions of the brain. Neurofeedback (NFB) is an operant conditioning methodology used to train individuals to volitionally control brain oscillatory activity. NFB influences frontoparietal neural circuitry and improves executive control in healthy populations. In this pilot study, we examined 1) whether NFB can be employed to improve gamma synchrony in SCZ patients and 2) if improved synchrony is associated with improvements in working memory (WM). We hypothesized that increased coherence in gamma oscillations over frontal

cortex would correlate positively with increased performance on a working memory task. Three subjects (two male and one female, ages 18-27), who met DSM-V criteria for SCZ were recruited from University of California, San Diego and Veterans' Administration Healthcare System for an open-label pilot NFB study. Subjects remained on their existing medications throughout the study. Pre-treatment assessments included EEG (5 minutes eyes closed, 5 minutes eyes open), performance on the n-back (a WM task), and neuropsychological testing using the Matrics Consensus Cognitive Battery (MATRICs). In a cross-over design, all 3 subjects completed 8 sessions of Gamma-NFB (G-NFB; 2 sessions/week, three 15-min trials/session) followed by 8 sessions of active-placebo (AP-NFB) training over frontal cortex. Assessments were also obtained at the conclusion of G-NFB and AP-NFB. Subjects showed 153% increase in threshold of training during G-NFB indicating training effects, and this training correlated with improved WM. Average threshold increased during AP-NFB by 66%, but there was no change in WM. Thus, the present study indicates that SCZ subjects can undergo NFB to enhance gamma frequency power over the frontal cortices of the brain. Additionally, enhancements in gamma frequency, but not other frequencies are correlated with improved WM. Therefore, the data from this pilot study suggest that NFB may be a successful way to enhance WM and has the potential to be used in a clinical setting to help treat some of the symptoms associated with schizophrenia spectrum disorders.

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Poster

091. Schizophrenia: Behavior and Symptoms

Location: Halls B-H

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Program#/Poster#: 91.10/JJJ54

Topic: H.03. Schizophrenia

Support: SFAz Bisgrove Scholarship

American Sleep Medicine Foundation, Focused Project Award

NIH F31NS090921

NIH R01MH097803

Title: Influence of schizophrenia-associated gene Egr3 on circadian rhythms in mice

Authors: ***A. M. MAPLE**[1], R. K. ROWE[3,2,4], J. L. HARRISON[3,2,5], J. LIFSHITZ[3,2,4,5], F. FERNANDEZ[6], A. L. GALLITANO[1];

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Abstract: Sleep abnormalities are a common symptom of psychiatric disorders. Upwards of 80% of patients with schizophrenia report abnormal sleep with potential negative effects on quality of life and cognitive function. Abnormal sleep patterns are often reported in the prodromal stages and prior to a psychotic break, suggesting they may be a predictive feature of the illness. Although sleep abnormalities are a significant feature of schizophrenia symptomatology, and may provide insight into its etiology, the biological mechanisms underlying disrupted sleep in schizophrenia are unknown. Changes to the timing and quality of sleep can result from dysfunction of: 1) circadian rhythms, which determine the time-of-day when sleep occurs and/or 2) sleep homeostasis, comprising mechanisms to a) determine and b) alleviate sleep need. The immediate early gene *early growth response 3* (*Egr3*) has been associated with schizophrenia risk in human populations and decreased levels of EGR3 protein have been found in postmortem patient brain tissue. *Egr3*-deficient mice display behaviors associated with schizophrenia pathophysiology including sleep abnormalities. We previously found that male and female *Egr3 -/-* mice slept less than wild type (WT) littermates and that these differences were most profound during light/dark transitions. Compared to WT mice after sleep deprivation, *Egr3-/-* mice also exhibit diminished compensatory responses in non-rapid eye movement sleep (NREM) EEG slow-wave activity during recovery sleep. The potential roles of *Egr3* in both schizophrenia and sleep homeostasis led us to examine the role of *Egr3* in circadian rhythms. Adult female *Egr3 -/-* and WT mice were monitored for 9 weeks including 3 weeks in normal light/dark (LD), constant dark (DD), and constant light (LL) conditions in non-invasive sleep monitoring cages. We found that *Egr3-/-* mice have no circadian disruption and actually display more robust circadian rhythms of locomotor activity compared to WT. For example, *Egr3-/-* mice show increased circadian amplitude and inter-daily stability during LD and DD conditions. Both *Egr3-/-* and WT mice show normal responses to constant light, such as activity suppression and lengthening of their free-running clock. These data suggest that loss of *Egr3* does not disrupt circadian function, but impairs sleep homeostasis. *Egr3* knockouts are a valuable tool to selectively investigate the mechanisms underlying sleep homeostasis independent of circadian rhythms. Elucidation of the mechanisms by which *Egr3* regulates sleep homeostasis should provide insights into a key feature of both the prodromal phase and precipitation of acute episodes of schizophrenia.

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Poster

091. Schizophrenia: Behavior and Symptoms

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 91.11/JJJ55

Topic: H.03. Schizophrenia

Support: Sidney R. Baer, Jr. Foundation

Title: Speech-in-noise perception deficits reflect central auditory dysfunction in schizophrenia.

Authors: S. RACKELMANN, 92093[1], *M. TARASENKO[2], A. SHILUK[1], W. ZHANG[1], S. T. PIANKA[3], A. W. BISMARK[2], M. L. THOMAS[1], J. SPROCK[1], C. KAUFFMAN[4], G. A. LIGHT[1];
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Abstract: Auditory Targeted Cognitive Training (ATCT) has shown great promise for remediating cognitive deficits in schizophrenia (SZ) by producing neuroplastic changes within the neural substrates underlying verbal memory. However, nearly half of chronic SZ patients fail to derive a meaningful benefit from the intervention, underscoring the need to identify early predictors of therapeutic response. Speech-in-noise (SIN) perception, or the ability to decipher a "target" speech signal in the presence of irrelevant background noise, is deficient in other disorders of central auditory processing, including autism and learning disorders, but no research has yet examined SIN perception in SZ. The present study thus utilized two established measures (Words-in-Noise [WIN] and Quick Speech-in-Noise [QuickSIN]) to determine whether SIN perception deficits are present in SZ, whether such deficits reflect illness-related central auditory dysfunction, and the potential utility of these measures to serve as predictive treatment biomarkers. Participants were 28 adults diagnosed with SZ at a residential transitional care facility. For both WIN and QuickSIN, speech stimuli were presented in varying intensities of background conversation noise (four-talker babble); WIN utilized one-word stimuli whereas QuickSIN utilized sentence stimuli. Participants were instructed to repeat the words or sentences aloud, and their responses were scored based on repetition accuracy of the single word (WIN) or five "key" words (QuickSIN). Cognitive functioning was assessed using the MATRICS Cognitive Consensus Battery (MCCB). On average, participants performed in the "mildly impaired" range on both the WIN (M = 5.3, SD = 2.6) and the QuickSIN (M = 3.9, SD = 3.8). WIN performance was significantly associated with MCCB Attention/Vigilance only (r = 0.43, p = 0.03). QuickSIN performance was significantly associated with MCCB Working Memory, Verbal Learning/Memory, and Global Cognition (r's $\geq$ 0.40, p's $\leq$ 0.04) and marginally associated with MCCB Reasoning/Problem Solving (r = 0.34, p = 0.08). For people with SZ, WIN performance appears to reflect peripheral auditory processing (i.e. auditory attention),

whereas QuickSIN indexes functioning of the central auditory processing network underlying the cognitive domains targeted by ATCT. QuickSIN thus appears to be a promising measure for predicting and monitoring ATCT response in SZ.

Disclosures: **S. Rackelmann:** None. **M. Tarasenko:** None. **A. Shiluk:** None. **W. Zhang:** None. **S.T. Pianka:** None. **A.W. Bismark:** None. **M.L. Thomas:** None. **J. Sprock:** None. **C. Kauffman:** None. **G.A. Light:** 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, Boehringer-Ingelheim, Merck, NeuroSig.

Poster

091. Schizophrenia: Behavior and Symptoms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 91.12/JJJ56

Topic: H.03. Schizophrenia

Title: Cognition predicts treatment engagement among inpatients with schizophrenia

Authors: ***A. SHILUK**[1], M. L. THOMAS[1], S. RACKELMANN[1], W. ZHANG[1], S. T. PIANKA[2], C. KAUFFMAN[3], J. SPROCK[1], A. W. BISMARK[4,1], M. TARASENKO[4,1], G. A. LIGHT[4,1];
[1]Psychiatry, UCSD, San Diego, CA; [2]UCLA, Los Angeles, CA; [3]Alpine Special Treatment Ctr., Alpine, CA; [4]VA San Diego Healthcare Syst., San Diego, CA

Abstract: Facilitating treatment engagement from people with schizophrenia has long been problematic for mental health providers, with treatment failures contributing to elevated rates of poverty, homelessness, and incarceration. Consequences of inadequate treatment include symptom exacerbation, rehospitalization, as well as the detrimental neural sequelae of disease progression. Inpatient settings present a valuable opportunity for intensive rehabilitation and thus continue to be an important locus of long-term care and support for many with psychotic acuity and chronic disability. It is not clear, however, which patient factors are associated with adherence to treatment. The aim of the present study was to determine whether neurocognition predicts treatment engagement in chronic schizophrenia.
Schizophrenia patients (n = 28) recruited from an ongoing longitudinal study within an inpatient transitional care facility were evaluated following acute stabilization. The MATRICS Consensus Cognitive Battery (MCCB) was administered to assess global cognition. Psychosocial treatment engagement was defined by the standardized total number of attended group therapies, structured social activities, self-care, and vocational rehabilitation sessions. Subjects were tracked over a

period of 20 weeks. Data were analyzed using linear mixed-effects models.
MCCB global scores significantly predicted baseline treatment engagement ($\beta = 0.49$; $p = .003$) but not change over time ($\beta = -0.01$; $p = .92$); patients with higher cognition showed greater engagement. Using stepwise regression with specific MCCB cognitive domains, the strongest predictor of engagement was MCCB Visual Learning (Brief Visuospatial Memory Test – Revised) followed, in order, by Working Memory, Attention and Vigilance, Reasoning and Problem Solving, Verbal Learning, and Speed of Processing.
Neurocognition is a robust predictor of treatment engagement in schizophrenia inpatients. These results suggest that cognitive impairments prevalent in this population interfere with patients' adherence to treatment and are therefore barriers to successful outcome. Findings from this study underscore the necessity of targeted interventions enhancing functioning within specific cognitive domains.

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Poster

091. Schizophrenia: Behavior and Symptoms

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Program#/Poster#: 91.13/JJJ57

Topic: H.03. Schizophrenia

Support: JSPS KAKENHI 25242078

MEXT Grant-in-Aid for Scientific Research on Innovative Areas 25116526

Title: Genome-wide gene expression analysis reveals molecular phenotypes related to schizophrenia in Neurogranin knockout mice

Authors: *S. HATTORI[1], H. HAGIHARA[1], T. KAMEYAMA[1], Y. OUCHI[1], H. INAGAKI[1], H. KURAHASHI[1], F. L. HUANG[2], K.-P. HUANG[2], T. MIYAKAWA[1];
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Abstract: Large-scale genome-wide association studies have identified susceptibility loci for schizophrenia in the gene encoding neurogranin (*NRGN*). Neurogranin is a neuron-specific calmodulin binding protein abundantly expressed in the hippocampus and cortex. Nrgn knockout (KO) mice exhibit a series of behavioral abnormalities relevant to schizophrenia, including

hyper-locomotor activity, cognitive deficits, and impaired sensorimotor gating (Hattori et al., abstract for SfN meeting 2015). We have reported that dentate gyrus (DG) granule cells in adult but not in young (<10 weeks old) mutants are in pseudo-immature state, which has been proposed as an endophenotype of schizophrenia, at molecular levels (Hagihara et al., abstract for SfN meeting 2015). In this study, we measured genome-wide gene expression levels in the DG and prefrontal cortex (PFC) of adult (>20 weeks old) *Nrgn* KO mice by mRNA sequencing (RNA-seq). Bioinformatics analyses of transcriptome data revealed that the gene expression patterns of the DG in adult mutants are significantly similar to those of several other strains of mutant mice, including *Schnurri-2* (*Shn-2*) KO mice, an animal model of schizophrenia with remarkable face validity (Takao et al., *Neuropsychopharmacology*, 2013). Based on the gene expression patterns of the DG, adult *Nrgn* KO mice could be divided in 2 groups: mutant mice with immature DG and those with mature DG, suggesting that deficiency of *Nrgn* results in progressive and age-dependent "dematuration". Additionally, the transcriptome pattern of the DG in *Nrgn* KO mice was similar to that of animal models of epilepsy, which is caused by excessive neuronal excitability implicated in the mechanism for induction of immature DG. The gene expression patterns of the mutant PFC showed slight but significant similarity to those of post-mortem cortex from patients with schizophrenia. Collectively, these data indicate that *Nrgn* KO mice have a high degree of face validity as an animal model for schizophrenia. Moreover, *Nrgn* KO mice might be an animal model recapitulating the fact that typical onset of schizophrenia occurs during late adolescence or early adulthood. The late onset of these phenotypes would provide unique opportunity for studying molecular mechanisms underlying the pathogenesis of schizophrenia and its related disorders.

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Poster

091. Schizophrenia: Behavior and Symptoms

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 91.14/JJJ58

Topic: H.03. Schizophrenia

Support: SD VA MIRECC PALA Pilot Grant

Title: Predicting cognition in schizophrenia patients using novel reverse-translated tasks

Authors: *A. W. BISMARK[1], A. SHILUK[2], S. RACKELMANN[2], W. ZHANG[2], S. T. PIANKA[3], C. KAUFFMAN[4], M. TARASENKO[1], M. L. THOMAS[2], J. W. YOUNG[2], G. A. LIGHT[2];
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Abstract: Despite a growing appreciation of the contribution of attention, motivation and reward learning impairments to the disabling global cognitive deficits in schizophrenia patients, there are no available medications that alleviate these deficits. This dearth of effective procognitive agents may be driven in part by a lack of reverse-translated cognitive tasks with domain pure metrics for screening promising compounds across preclinical and clinical investigations. Here, we address this translational gap by examining the relationships among novel reverse-translated tasks of attention, motivation, and reward learning to well-established cognitive tests used in schizophrenia trials.
Schizophrenia patients (n=30) recruited from an inpatient transitional care facility were tested in the 5-choice continuous performance task (5C-CPT: Attention), progressive ratio breakpoint (PRB: Motivation), and probabilistic learning (PL: Reward Learning) tasks. The MATRICS Consensus Cognitive Battery (MCCB) was administered to assess global cognition with the attention (CPT-IP) and working memory subtests used as benchmark comparisons for the 5C-CPT.
Initial correlation between the 5C-CPT and CPT-IP ($r=.65$, $p<.001$) indicate overlapping, but distinct measures of attentional functioning. Although both measures significantly correlated with global cognition (5C-CPT: $r=.72$, $p<.001$; CPT-IP: $r=.86$, $p<.001$), the CPT-IP displayed a much stronger relationship with working memory (5C-CPT: $r=.49$, $p<.010$; CPT-IP: $r=.68$, $p<.001$), suggesting the 5C-CPT is a more domain-pure measure of attention, likely driven by its lack of reliance on working memory. Multiple regression indicated attention (5C-CPT) ($\beta=.611$, $p<.001$) and motivation ($\beta=.334$, $p<.022$) significantly predicted global cognition and accounted for 51% and 10% of the variance respectively. In contrast, reward learning did not significantly predict global cognition ($\beta=-.012$, $p<$*ns*), accounting for <1% of variance.
Reverse-translated measures of attention and motivation are robust predictors of established measures of cognitive functioning in schizophrenia patients, demonstrating their convergent validity. Since these reverse-translated 5C-CPT and PRB appear to be more “domain-pure” tests of attention and motivation, their use in therapeutic development may facilitate translation from preclinical to clinical trials. Future studies will determine whether these novel tasks are sensitive to and predict pharmacologic and cognitive interventions in schizophrenia patients.

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Poster

091. Schizophrenia: Behavior and Symptoms

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NIMH Grant MH-077862

Title: Frontal neural dysfunction is implicated in executive inflexibility in psychosis.

Authors: *L.-Y. HUANG[1], D. A. PARKER[1], S. K. HILL[2], M. S. KESHAVAN[3], G. D. PEARLSON[4], C. A. TAMMINGA[5], J. A. SWEENEY[5], B. A. CLEMENTZ[1];
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Abstract: Difficulty to switch from an existing response set to a new one is called perseverative errors, which can be measured using the Wisconsin Card Sorting Test (WCST). Perseverative errors are consistently seen in animals with prefrontal lesions, indicating the critical role of the region in attention control, task switching and context updating. Using electroencephalogram simultaneously administered with WCST, studies with healthy human subjects captured weaker P300 evoked potential around the prefrontal area during error trials. This finding confirmed P300 as an endogenous task-switching marker in addition to its modality-independent salience detection properties, making it a powerful ERP component to assess the neural correlates of cognitive response in different populations. Psychosis cases are known to have elevated perseverative errors, but the electrophysiological profiles of psychotic probands in relation to task-switching are less understood. In the present study, 628 clinically stable subjects from the Bipolar and Schizophrenia Network on Intermediate Phenotypes consortium (B-SNIP) underwent Penn Conditional Exclusion Test (PCET), a computerized analog to WCST. These participants were divided into high, medium, and low-performing groups based on their perseverative error rates in PCET, then each group's ERP components to an auditory oddball task, a standard paradigm to consistently evoke P300, were examined. Principle component analysis of the pooled ERP recordings condensed the data into two components, accounting for

about 90% of P300 variability in the sample; 1-way ANOVA showed marked difference in P300 and N200 amplitudes between high, medium, and low performance groups (p=0.0047 and p=0.01, respectively). This analysis provided direct evidence that perseverative error rates are associated with strength of prefrontal response to novel information, regardless of disease load.

Disclosures: **L. Huang:** None. **D.A. Parker:** None. **S.K. Hill:** None. **M.S. Keshavan:** 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; Sunovion. F. Consulting Fees (e.g., advisory boards); Forum Pharmaceuticals. **G.D. Pearlson:** None. **C.A. Tamminga:** Other; American Journal of Psychiatry. **J.A. Sweeney:** F. Consulting Fees (e.g., advisory boards); Taketa, Roche, and Lilly. **B.A. Clementz:** None.

Poster

092. Optogenetic Approaches to Studying Neural Circuit Function

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 92.01/JJJ60

Topic: I.04. Physiological Methods

Title: Tapered and nano-patterned optical fiber for customized light delivery geometries *In vivo*

Authors: ***M. PISANELLO**[1,2], L. SILEO[1], A. DELLA PATRIA[1], B. L. SABATINI[3], M. DE VITTORIO[1,2], F. PISANELLO[1];
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Abstract: It is well recognized that important advances in optogenetic modulation of neural activity can be achieved using structured light illumination into the living mouse brain, with the goal of better matching spatio-temporal stimulation patterns with the complex structural topology of different brain areas. This can be obtained with sub-cellular resolution by using multiphoton microscopy in the superficial cortical layers, while structured illumination of deep-brain regions can be obtained through microendoscopes [Neuron 84, 1157 (2014)] or micrometer-sized light emitting diodes arrays [Neuron, 88, 1136 (2016)]. These approaches, however, can produce severe tissue damage due a to pronounced implant cross section or induce tissue heating when used for long stimulation periods [Front. Neurosci., 10:70 (2016)].
Here we exploit advanced nanostructuring of tapered and metal-coated optical fibers to allow for user-customized light delivery patterns in deep-brain regions. The technological process to realize such devices flow as follows. First, a low-angle taper profile is obtained by heat-and-pull

process, leading to sub-micrometer tips for reduced tissue damage during implantation. A thin metallic layer is then deposited by thermal evaporation, with the goal to shield light emission (otherwise arising almost all-along the taper). Next, focused ion beam milling is used to realize optical apertures to allow for out-of-axis light emission at well-defined sections along the taper. Apertures position, size and shape can be designed to match with the specific spatial distribution of the cellular groups to be controlled: small windows with size from 5μm to 50μm can allow for high accuracy and precision in spatial light delivery, while larger aperture of more than 100μm can be used to shine light over larger brain structures. The fabrication technology allows for the realization of emission sites along the whole conical surface of the taper, making possible to obtain two different light delivery patterns placed back-to-back around the waveguide or with a 360° rotational symmetry.
Moreover, a simple optical strategy can be used to tune the light injection angle of the laser beam into the optical fiber. This, combined with the modal demultiplexing properties of the taper, allows for the activation of a fraction of the light delivery sites [Neuron 82, 1245 (2014), Biomed. Opt. Express 6, 4014 (2015)]. Such approach can therefore be exploited to dynamically reconfigure the spatio-temporal light delivery pattern, paving the way toward structured light delivery in deep-brain regions with reduced invasiveness.

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Poster

092. Optogenetic Approaches to Studying Neural Circuit Function

Location: Halls B-H

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Program#/Poster#: 92.02/JJJ61

Topic: I.04. Physiological Methods

Support: DFG SPP1665

Title: In-vivo characterization of excitatory opsins in different regions of the mouse brain

Authors: ***J. TEGTMEIER**, K. JANITZKY, K. TAKAGAKI, F. W. OHL, M. T. LIPPERT; Leibniz Inst. For Neurobio., Magdeburg, Germany

Abstract: Since the discovery of ChR2 and its application in optogenetics, a large number of advanced excitatory optogenetic probes have been developed. However, most studies to date have used stimulation parameters derived from *in vitro* measurements, which cannot be directly translated to *in vivo* applications. It is therefore often unclear what exact neuronal effect a particular stimulation paradigm might have *in vivo*. For example, *in vivo*, opsins may not be able

to follow high stimulation frequencies, or inadvertent overstimulation might result in silencing rather than stimulation. We therefore characterized responses of two commonly used opsins in various brain structures in mice *in vivo*: the most commonly used excitatory opsin mutant ChR2(H134R) and the advanced fast opsin Chronos. We determined the attainable stimulation frequencies, necessary light levels, and effective pulse lengths in the neocortex, VTA and LC. We also compared the promoters hSyn and CamKIIa in terms of their influence on *in vivo* stimulation results and tested different expression durations. For the two monoaminergic areas, we employed cell type specific expression in Th-Cre and Dat-Cre mice to selectively stimulate dopaminergic and noradrenergic cells. In VTA and LC we found only small differences between opsins and expression schemes, indicating a direct limiting effect of the targeted cells. In cortex, however, we found both longer term expression of ChR2(H134R) as well as Chronos to permit substantially higher stimulation frequencies. Furthermore, in the cortex, Chronos enabled significantly higher stimulation frequencies compared to ChR2(H134R). Our results highlight the importance of characterizing the opsin of choice in the target brain structure *in vivo* and provide guidelines for using the opsins in cortex, VTA and LC.

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Poster

092. Optogenetic Approaches to Studying Neural Circuit Function

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Topic: I.04. Physiological Methods

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Title: Optogenetic tools with varying kinetics differentially engage intrinsic network resonance *In vivo*.

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Abstract: Channelrhodopsins (ChRs) are light-gated ion channels that enable cell type-specific activation of neurons or neural circuits. Channelrhodopsin-2 has been widely used as a tool to probe circuit function in vitro and in vivo. Two additional recently developed ChR variants,

Chronos and Chrimson (Klapoetke et al 2014), are characterized by faster kinetics and a red-shifted excitation spectrum, respectively. However, because these variants have largely only been tested in vitro, little is known about their efficacy and utility in the intact brain. In addition, variations in the kinetics of optogenetic tools may regulate the degree to which they engage local network dynamics. Such variation may affect the utility and optimal use of next-generation optogenetic tools in circuit dissection. We compared ChR-evoked patterns of multi-unit (MU) activity and local field potentials (LFPs) in primary visual cortex of lightly anesthetized mice expressing Chronos, Chrimson, or ChR2 in pyramidal neurons (PNs). We assessed overall activation of PNs by measuring mean MU firing rates, as well as the temporal progression of firing rates. Previous work has found substantial increases in gamma oscillations (30-80Hz), an emergent resonant activity pattern that relies on interactions between excitatory and inhibitory neurons, upon ChR2 activation of PNs (Adesnik and Scanziani 2010). Using this evoked pattern as an assay for local network engagement, we examined the effects of ChR variant activation on the power of gamma oscillations (30-80 Hz) in the LFP. While activating all three ChR variants caused light-evoked increases in PN firing in vivo, we observed variant-dependent differences in inter-spike interval distributions and the relative power of gamma oscillations. Chronos and Chrimson did not enhance gamma oscillations, whereas ChR2 more than doubled power in the gamma band. These findings suggest that variations in kinetics of optogenetic tools can substantially affect their efficacy in neural networks in vivo, as well as the manner in which their activation engages circuit resonance.

Disclosures: **N. Jun:** None. **J.A. Cardin:** None.

Poster

092. Optogenetic Approaches to Studying Neural Circuit Function

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Topic: I.04. Physiological Methods

Support: NSF Grant 1512826

Title: Chloride indicator protein for non-invasive functional bioluminescence imaging

Authors: *K. BERGLUND[1], J. K. TUNG[2], R. E. GROSS[2];
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Abstract: Although fluorescence imaging has vastly advanced our knowledge of how the brain works, its invasive nature limits its usage: the brain has to be exposed so that excitation light, a prerequisite for fluorescence, can penetrate the brain tissue. In contrast, bioluminescence

imaging can be conducted in a non-invasive manner because the substrate required to generate biological light can be delivered to the brain systemically via the bloodstream. However, in its current form, bioluminescence does not provide any information on the brain's activity unlike commonly used fluorescent calcium indicator proteins.
We developed a functional imaging probe based on a relatively unexplored imaging modality - bioluminescence. Specifically, we rationally designed and tested novel bioluminescent proteins that change their emission spectra according to the intracellular chloride ion concentration ($[Cl^-]_i$) - an important determinant for normal and pathological neuronal activity - using blue-light emitting luciferase, GLuc, and Cl^--dependent variant of yellow florescent protein, Topaz. When expressed in an *in vitro* heterologous protein expression system, we observed expected emission changes according to manipulated $[Cl^-]_i$. As a proof-of-principle, primary neurons from rat embryos in culture were transfected with the probe and well-characterized down-regulation of $[Cl^-]_i$ during development was measured. We hope that the new probe can be utilized to detect a similar change in $[Cl^-]_i$ in the developing brain in the whole, live animals, using a small animal bioluminescence imager.
Neuronal Cl^- is involved in many normal neurophysiological activities, such as development, synaptic inhibition, pH and volume regulations. In addition, changes in neuronal Cl^- levels have been implicated in the pathological mechanisms underlying various neurological diseases (*i.e.* pain, stroke, and epilepsy). Thus, the new bioluminescence Cl^- indicator protein will be a valuable tool to non-invasively assess Cl^- fluctuations in normal and pathological neuronal processes *in vitro* and *in vivo*.

Disclosures: **K. Berglund:** None. **J.K. Tung:** None. **R.E. Gross:** None.

Poster

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Topic: I.04. Physiological Methods

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NIDA 011289 (JAK)

T32MH20068 (RS)

Sidney Frank Fellowship (RS)

Title: Bioluminescent control of optogenetics in acute brain slices

Authors: *R. ST. LAURENT[1], C. I. MOORE[1], D. LIPSCOMBE[1], B. W. CONNORS[1], U. HOCHGESCHWENDER[3,4], J. A. KAUER[1,2];
[1]Neurosci. Grad. Program, [2]Mol. Pharmacology, Physiology, and Biotech., Brown Univ., Providence, RI; [3]Neurosci. Program, [4]Col. of Med., Central Michigan Univ., Mt Pleasant, MI

Abstract: Recent expansions in the optogenetic toolbox, luminopsins, are fusion proteins of light-activated ion channels or pumps (channelrhodopsin or *Leptosphaeria maculans*) and *Gaussia* luciferase (a bioluminescent enzyme) which bypass the need for an external light source. In the presence of a corresponding luciferin, the luciferase emits cold light sufficient to open the actuator. The ability to manipulate membrane potential in excitable cells has many potential benefits for neuroscience research. For example, dopamine release is essential in processes pertaining to reward, learning, and motor control. Optogenetic studies are able to manipulate these cells on a fast time scale. However, until now we have been unable to manipulate membrane properties on a longer time scale of minutes without the use of DREADDs, which rely on endogenous signaling pathways. Here we seek to demonstrate the ability of luminopsins to alter the membrane potential and firing rate of VTA dopamine neurons using luminopsins.
We virally transduced AAV9-hSyn-LMO3 (LMO3; channelrhodopsin fused to *Gaussia* luciferase) into the VTA of C57BL/6 mice and characterized transduced cells using whole-cell patch clamp recordings in acute midbrain slices. Bath application of the luciferin, coelenterazine (CTZ), resulted in detectable bioluminescence in 100% of slices expressing luminopsins. Dopamine neurons depolarized after CTZ application (4 min after CTZ mean = 1.5 ± 2.5 mV, 8 min after CTZ mean = 8.5 ± 5.5 mV; n = 9). Bioluminescence was no longer observed at 10 min after CTZ, however, in most neurons the membrane potential did not return to baseline values. Control experiments without luminopsin viral expression showed that CTZ alone does not induce depolarization (4 min after CTZ mean = -3.5 ± 3.2 mV, 8 min after CTZ mean = -1.5 ± 4.3 mV; n = 10). Preliminary results suggest that dopamine neurons voltage clamped at -70 mV have a slow inward current (176 ± 132 pA; n = 5) that corresponds with the onset of bioluminescence and persists for tens of minutes. Although brief current injections are sufficient to increase firing rate in VTA dopamine cells, the long sustained inward current achieved through luminopsin activation did not increase firing rate (LMO3: 0.18 ± 0.10 Hz, control: 0.14 ± 0.07 Hz; n = 9-10). Our results indicate that we are able to manipulate dopamine neuron membrane potential using luminopsins.

Disclosures: R. St. Laurent: None. **C.I. Moore:** None. **D. Lipscombe:** None. **B.W. Connors:** None. **U. Hochgeschwender:** None. **J.A. Kauer:** None.

Poster

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Program#/Poster#: 92.06/KKK4

Topic: I.04. Physiological Methods

Title: Bioluminescence activated optogenetic stimulation in a rat model of spinal cord injury.

Authors: P. OTERO[1], E. D. PETERSEN[1], A. PAL[1], J. ZENCHAK[1], *U. HOCHGESCHWENDER[1,2];
[1]Neurosci., [2]Col. of Med., Central Michigan Univ., Mt Pleasant, MI

Abstract: Current methods used to treat spinal cord injury with electrical stimulation include functional electrical stimulation and intrathecal electrical stimulation. Both of these methods offer potential quality of life improvements for patients but are not entirely effective. Importantly, both approaches have the drawback of rapidly fatiguing the stimulated motor units. Optogenetics provides a method of stimulation that can be executed at the neuronal level and does not result in the same rapid fatigue as electrical stimulation. The current drawback of traditional optogenetic stimulation is its invasiveness, as this approach requires an implanted light source. Bioluminescence-driven optogenetics avoids fiber implants by providing light via a luciferase fused to the opsin (luminopsin, LMO), with the luciferase substrate applied intravenously or intrathecally. In one approach, LMO3, a fusion of a mutated version of Gaussia luciferase and Volvox channelrhodopsin 1, is expressed locally, by injection of adeno-associated virus (AAV) immediately caudal to the site of injury (T9). The reasoning is that neuronal stimulation below the lesion will make it more likely for dormant neurons to regenerate and promote formation of new connections, potentially leading to a gain in function. In another approach, LMO3 is expressed in neuronal precursor cells grafted into the damaged spinal cord. Here, stimulation of neuronal progenitor cells is expected to increase functional integration of transplanted cells into host circuitry. Both approaches have the potential to improve behavioral deficits and injury pathology and will broaden our understanding of spinal cord injury recovery and synaptic remodeling.

Disclosures: P. Otero: None. **E.D. Petersen:** None. **A. Pal:** None. **J. Zenchak:** None. **U. Hochgeschwender:** None.

Poster

092. Optogenetic Approaches to Studying Neural Circuit Function

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Topic: I.04. Physiological Methods

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CMU Graduate Student Fellowship (AP)

Title: Split Gaussia luciferase based genetically encoded calcium indicator.

Authors: *A. PAL[1], Z. ZAIDI[1], W. E. MEDENDORP[1], J. A. KAUER[3,4], B. W. CONNORS[3], C. I. MOORE[3], D. LIPSCOMBE[3], U. HOCHGESCHWENDER[1,2];
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Abstract: The detection and imaging of calcium flux in live cells provides useful insight about cellular physiology. Genetically encoded calcium indicators (GECIs) based on fluorescent proteins (GCaMPs) have been employed extensively to report calcium flux in neurons. In GCaMPs, the calmodulin-M13 protein complex is employed as a calcium sensing unit that undergoes a conformational change in the presence of calcium. However, fluorescence-based GECIs have their own set of disadvantages such as requiring external, invasive light delivery for excitation. We have employed the same calcium sensing calmodulin-M13 protein complex, but switched out the fluorescent reporter with a *Gaussia* luciferase (GLuc) enzyme reporter that is split in half. By inserting the CaM-M13 peptide between the N- and C-terminal catalytic domains of GLuc, the luciferase is inactive in the absence of calcium. Influx of calcium into the cell contracts the calmodulin-M13 complex and brings the two halves of the luciferase back together to form an active enzyme, which in the presence of its substrate (coelenterazine, CTZ) emits light. We call this split luciferase-based GECI LumiCaMPsin, or LMC. By varying locations of the split site in the GLuc molecule, sequences of the CaM-M13 peptide, length of polyG linkers between split GLuc and the CaM-M13 module, and placement of 5' and 3' targeting signals, we arrived at version LMC4. This version has low background luminescence in the absence of calcium and high light emission in its presence, making it a good candidate for a bioluminescence calcium reporter.

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Poster

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Topic: I.04. Physiological Methods

Support: Keck Foundation Grant GR529005

NIH Grant R01-NS045130

Title: Bioluminescent optogenetics (BL-OG): A systematic investigation of the neurophysiological effects of BL-OG in-vivo

Authors: ***M. GOMEZ-RAMIREZ**[1], A. I. MORE[1], B. W. CONNORS[1], J. A. KAUER[1], D. LIPSCOMBE[1], U. HOCHGESCHWENDER[2], C. I. MOORE[1];
[1]Neurosci., Brown Univ., Providence, RI; [2]Foundational Science, Col. of Med., Central Michigan Univ., Mount Pleasant, MI

Abstract: Recently, several genetic-based solutions for studying underlying neural circuits mediating perceptual processes have been developed. The most widely used tool has been optogenetics, a method that regulates neural activity of genetically-specific cell populations by controlling neurons' light-sensitive ion channels with light. The spatiotemporal precision of optogenetics and its rapidly expanding toolkit have made this technique highly useful for basic research. However, a major limitation of optogenetics is the use of invasive external devices to deliver light into tissue. Recently, our lab and others have been developing less invasive, but highly precise, approach for activating and/or deactivating genetically-specific neural circuits. Our method, termed Bioluminescent OptoGenetics (BL-OG), tethers an optogenetic component to a luciferase. The light emitted from the luciferase/luciferin interaction activates the optogenetic element, which in turn regulates neural membrane potential without the need for a physical light-emitting device. Systemic administration of a luciferin can in turn generate bioluminescence. Tethered versions of this configuration linking the bioluminescent luciferase to the optogenetic element are referred to as luminopsins (Berglund et al., 2015) and have shown efficacy in initial studies in vivo. To build on these initial demonstrations to systematically characterize the optimal use of luminopsin in vivo, we performed concomitant bioluminescent and neurophysiological recordings in anesthetized mice, with luminopsin expression in the granular and superficial layers of primary somatosensory cortex (SI). Preliminary studies show

robust bioluminescent activation with direct intracortical application. High CTZ doses caused a strong transient response followed by a slow decay. In contrast, responses to lower CTZ concentrations failed to reveal a sharp peak response and a slow decay. These preliminary data further our understanding of the BL-OG technique by providing an estimate of the temporal dynamics of bioluminescent responses as a function of CTZ dosage.

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Poster

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NIMH

NIDA

HHMI

NSF

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The U.S. Army Research Laboratory and Defense Advanced Research Projects Agency

Title: All-optical activity-guided single-cell-resolution behavioral investigation of reward in neocortical networks

Authors: *J. H. JENNINGS[1], C. KIM[1], L. YE[1], J. MARSHEL[1], M. RAFFIEE[1], C. RAMAKRISHNAN[1], A.-C. WANG[1], K. DEISSEROTH[1,2,3];
[1]Bioengineering, [2]Psychiatry & Behavioral Sci., Stanford Univ., Stanford, CA; [3]Howard Hughes Med. Inst., Stanford, CA

Abstract: Studies involving neural activity readouts in humans, primates, and rodents have highlighted the involvement of orbitofrontal cortex (OFC) in diverse reward-related tasks including feeding and social behavior. Distinct OFC neurons show different responses to various

facets of reward including anticipation, consumption, and initiation of reward seeking, but it remains unknown if these subpopulations defined by unique activity patterns functionally give rise to discrete behaviors related to reward. To dissect the individual contributions of activity-specific OFC neuronal populations to behavior, we coupled genetically encoded Ca^{2+} indicators with engineered microbial opsins to both optically monitor and manipulate the activity of OFC neurons at the single cell level with two-photon microscopy. Employing this all-optical tactic in head-fixed mice, we found that 40% of targeted OFC neurons were excited by reward-related stimuli (calorie-dense liquid), while 15% of the cells were inhibited during reward presentation and consumption (n = ~200 neurons per animal, n = 10 mice). The majority of these identified neurons also reliably responded to 6s pulse trains of single-cell resolution two-photon optogenetic stimulation, opening the door to examining whether optogenetic single-cell stimulation of these activity-specific subpopulations reproduces reward-related behaviors even in the absence of reward; these investigations are also designed to be compatible with CLARITY-based circuit tracing and multi-fiber photometry (MFP) to dissect the anatomical wiring and activity dynamics of OFC reward circuits. Together, this approach may help elucidate how individual circuit elements within neocortex can individually and collectively contribute to reward evaluation and behavior, and to what extent specific activity patterns, connectivity patterns, and/or molecular gene expression patterns help determine causal links between neural circuits and behavior.

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Poster

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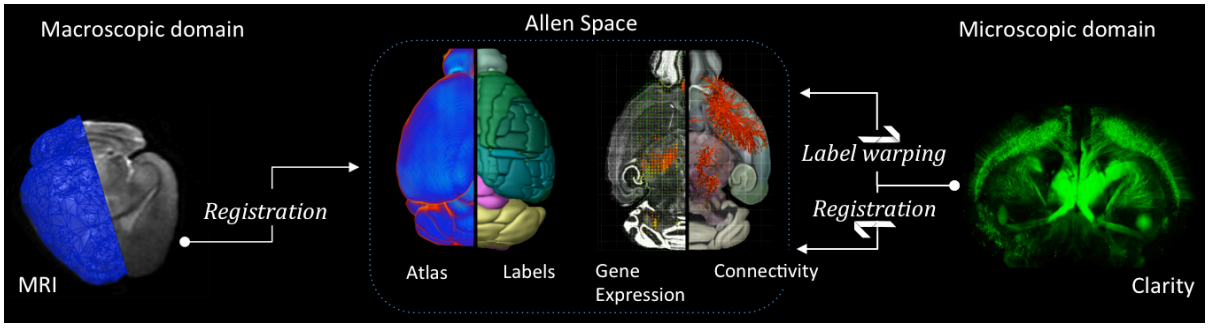
U.S. Army Research Laboratory and Defense Advanced Research Projects Agency (Cooperative Agreement Number W911NF-14-2-0013)

Title: Multimodal image registration and analysis via clarity-based light-microscopy (MIRACL)

Authors: ***M. GOUBRAN**[1], C. LEUZE[2], B. HSUEH[2], M. ASWENDT[2], L. YE[2], Q. TIAN[2], M. CHENG[2], G. STEINBERG[2], K. DEISSEROTH[2], J. MCNAB[2], M. ZEINEH[2];
[1]Dept. of Radiology, [2]Stanford Univ., Stanford, CA

Abstract: Background: MRI-histology correlation studies provide unique opportunities into understanding MRI signatures of disease state, and hence predicting pathological substrates of disease progression from *in-vivo* imaging. Clearing methods, such as CLARITY, are superior to standard histological techniques as they allow the investigation of three dimensional, intact biological systems. Combining MRI and CLARITY is a novel, powerful tool in discovering imaging correlates of pathology.
Methods: 15 Thy1-YFP mice were scanned *in-vivo* on a 7T MRI with high-resolution T1 and T2 mapping, weighted images and diffusion tensor imaging (DTI). They were then perfusion fixed with PFA, the brains were extracted and then imaged ex-vivo using contrast-enhanced CT. Afterwards, intact whole brains were cleared passively using a 1% hydrogel solution and imaged with native fluorescence using a light-sheet microscope. The brains were also cut into 3 consecutive coronal sections and imaged with a confocal microscope at a higher resolution.
Results and Discussion: We developed a pipeline for the registration, segmentation and analysis of multimodal imaging with CLARITY and the Allen mouse brain atlas. Our pipeline provides accurate registration between in-vivo, ex-vivo imaging data and CLARITY. It also warps all datasets into the Allen atlas space to make use of their extensive labels and genetic information. In addition, our tool enables unbiased brain-wide quantification of CLARITY morphological features (cell loss, size). It also enables performing structural tensor analysis and comparison with tractography from DTI. Our MIRACL pipeline can be applied in numerous neurological disorders and mouse models to uncover underlying tissue changes responsible for *in-vivo* MRI relaxometry and diffusion parameters.



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Poster

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Stanford Bioengineering Fellowship

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HHMI

NSF

Title: Crystal structure of a light gated anion channel

Authors: *Y. KIM[1,2,5], H. E. KATO[3], A. BERNDT[2,5], S. LEE[2,5], D. HILGER[3], B. KOBILKA[3], K. DEISSEROTH[2,5,4];
[2]Bioengineering, [3]Mol. and cellular physiology, [4]Psychiatry & Behavioral Sci., [1]Stanford Univ., Stanford, CA; [5]Howard Hughes Med. Inst., Stanford, CA

Abstract: Fast and reversible neuronal inhibition is an important experimental approach in optogenetics, and recent structure-guided opsin-engineering and opsin-discovery efforts have expanded the inhibition toolbox to include robust and efficient chloride-conducting channelrhodopsins. The first high-resolution structure of a cation-conducting excitatory channelrhodopsin was described in 2012, and became rapidly instrumental for developing and confirming a predictive model for light-gated pore selectivity, as well as for the initial engineering of chloride-conducting channelrhodopsins in 2014. However, a structure has not yet been obtained for any anion-conducting channelrhodopsin, which if obtained would greatly deepen insight into the operation and selectivity of the channel pore, and facilitate further design. Here we report at 3.1Å resolution the first X-ray crystallographic structure of a light-gated anion channel. This closed-state structure of the iC++ channelrhodopsin reveals a markedly distinct electrostatic surface potential lining the pore conduction pathway, compared with that of the cation-conducting C1C2, consistent with the fundamental predictions of the pore selectivity model. Although the pore constriction site revealed similar architecture compared with the cation-conducting channelrhodopsin (suggesting conserved gating properties, consistent with

electrophysiological characterization), a strikingly altered pattern of key amino acid side chain orientation was observed in the extracellular vestibule, ion pore, and cytosolic gate. Intriguing additional features included a substantially enlarged extracellular pore, which could be occupied by chloride ions and play additional roles in channel ion selectivity. This initial atomic structure of an anion-selective channelrhodopsin further illuminates the mechanism of channelrhodopsin ion selectivity and conductance, and provides a roadmap for further opsin engineering.

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Poster

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U.S. Army Research Laboratory and Defense Advanced Research Projects Agency (Cooperative Agreement Number W911NF-14-2-0013)

Title: Pathways to clinical CLARITY: Quantitative methodologies for transparent-volume analysis of irregular, soft, and heterogenous tissues in development and disease

Authors: ***B. HSUEH**[1], V. BURNS[1], P. PAUERSTEIN[1], L. YE[1], K. ENGBERG[1], A.-C. WANG[1], X. GU[1], H. CHAKRAVARTHY[1], E. ARDA[1], G. CHARVILLE[1], K. HOLZEM[2], I. EFIMOV[2,3], H. VOGEL[1], S. KIM[1], K. DEISSEROTH[1];
[1]Stanford Univ., Palo Alto, CA; [2]Washington Univ., St. Louis, MO; [3]The George Washington Univ., Washington, DC

Abstract: Volumetric tissue structural relationships are not well captured by typical thin-section histology, posing challenges for the study of tissue physiology and pathology given the natural and fundamental importance of three-dimensional structures in tissue function and dysfunction,

particularly in the nervous system. Moreover, while recent progress has been made with intact (non-sectioning-based) methods for clearing, labeling, and imaging whole organs such as the mature brain, these approaches are generally unsuitable for soft, irregular, and heterogeneous tissues that represent the vast majority of clinical samples and biopsies. Here we develop a novel two-phase hydrogel methodology with clearing speeds 25-fold greater than previously published methods ($p < 0.005$), along with a dedicated automated imaging/analysis computational platform, for high-throughput quantitative volumetric interrogation of intact soft tissue structures. We have validated and applied this platform in the examination of a variety of organs, with specific focus on the dynamics of pancreatic innervation, where automated computational analysis generated image feature datasets of several thousand mouse pancreatic islets and revealed multiphasic pruning of islet innervation through a 4-fold reduction in neural signal during the first week of life ($p < 0.005$). After characterizing islet innervation dynamics spanning pre- and post-natal development in mouse, we identified and quantified the same islet innervation measures in whole pre-natal human samples and tissue-banked juvenile pancreata (from healthy and type-I diabetic subjects), linking multiple rounds of molecular labeling using registration of the multiple labeling datastreams across entire intact samples. Finally, we develop *in silico* models and simulations to quantitatively evaluate advantages of this intact-tissue approach compared with conventional thin-section histology, revealing that traditional 2D approaches underestimate islet volumes by 20% or more ($p < 0.0005$), and have reduced statistical power due to smaller sample sizes. A human islet age classifier built using generalized linear models is sufficient to achieve >90% accuracy with as few as four 3D image features, but does not perform better than chance when using the equivalent features in 2D. Taken together, these tools and results point to a broad application domain of volumetric histology in both research and clinical settings.

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Poster

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Topic: I.04. Physiological Methods

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Simons foundation

Title: Probing large-scale network dynamics at high speed in the brain of behaving flies

Authors: *S. AIMON[1], T. KATSUKI[1], L. GROSENICK[2], M. BROXTON[2], K. DEISSEROTH[2], T. SEJNOWSKI[3], R. J. GREENSPAN[1];
[1]Kavli Inst. For Brain and Mind UCSD, La Jolla, CA; [2]Departments of Computer Sci. and Bioengineering, Stanford Univ., Stanford, CA; [3]Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: Our goal is to record and characterize fly brain activity at the scale of the whole network and close to the speed of the fastest neuronal computations, while a head-fixed fly is behaving (typically walking, grooming, or resting) and responding to various stimuli. Fast calcium or voltage sensors are expressed, either pan-neuronally, or in subsets of neurons broadly distributed in the brain (e. g. acetylcholine or dopamine neurons). We image the fly brain fluorescence using light field microscopy, which captures large volumes in a single camera exposure, albeit at reduced lateral resolution compared to the normal 2D diffraction limit of the microscope. 3D stacks are then reconstructed offline from the light field images using wave optics to model point spread functions, before applying 3D deconvolution. This technique makes it possible to image the whole brain at a frame rate of 200 Hz. We then use statistical techniques (PCA and spatial ICA) to extract maps of spatially distinct sources of activity as well as their time series. Even though PCA and ICA are mathematical algorithms that make minimal assumptions about the brain (ICA assumes spatial sparsity), most component's maps correspond to well-known anatomical structures. In some cases, neuron types can be inferred from the combination of sub-neuropile regions present in the map. The time series for each component is also extracted. With voltage sensors, the method permits the capturing of both graded potentials and spikes. The time series and the maps permit the identification of sub-neuropile areas and sometimes specific neurons involved in processing stimuli and behaviors, along with the dynamics of their activity. Patterns of spontaneous activity specific to some brain structures are also detected and help characterize the fly's internal state.

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Poster

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GE healthcare

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NIMH

Title: Methods for comparison of MRI and CLARITY data in human tissue specimen

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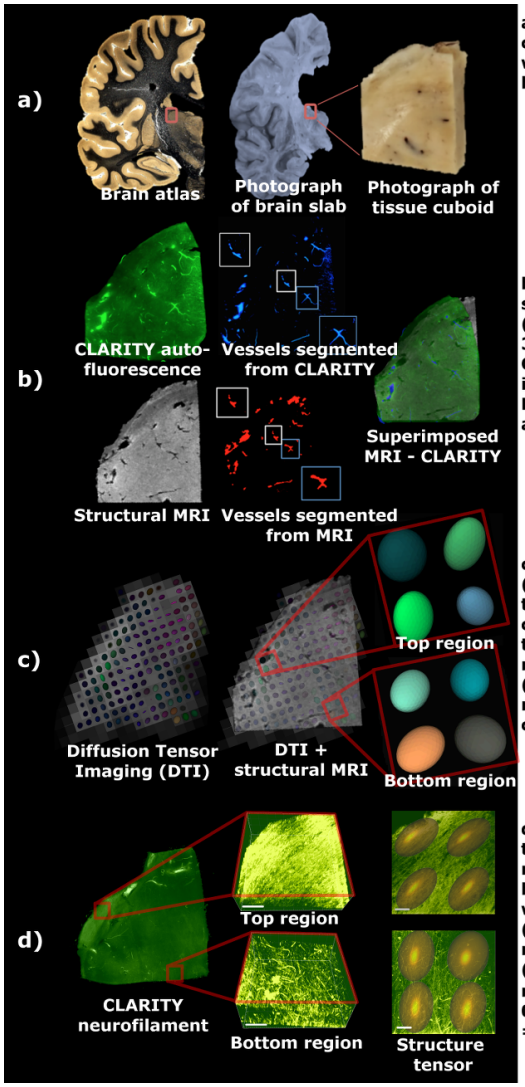
Abstract: Introduction: Relating diffusion MRI (dMRI) measurements to the underlying tissue structure through histology can help to improve the accuracy and reliability of structural connectivity measurements. In order to capture the complete nature of 3D elements such as cellular projections in both modalities, we devised a method to align MRI data with 3D histological CLARITY data and and we present a way to quantitatively compare fiber orientations within both MRI and CLARITY data.
Methods: We acquired a structural MRI scan at (FIESTA-C sequence, 350x350x300μm resolution) and a diffusion MRI scan (Diffusion-weighted EPI-sequence, $1mm^3$ resolution, $b=2000s/mm^2$, 450 diffusion directions) of a $1cm^3$ cuboid of human brain tissue containing part of the thalamus (Fig.1-a). After MRI scanning, a 200μm thin section was cut off the sample, cleared with CLARITY as described in [2] and stained for neurofilaments.
Results & Conclusion: The high resolution structural MRI scan showed a detailed visualization of the blood vessels. Since blood vessels retained their shape after tissue clearing and were well visible in the auto fluorescence image, they allowed precise alignment of MRI and CLARITY data (Fig. 1-b). For the diffusion MRI data, we measured the diffusion tensor in the aligned region (Fig.1-c), which showed an anisotropic region running along the side of the sample (Fig.1-c, top region, FA=0.30±0.10) and a less coherent region towards the bottom of the sample (Fig.1-c, bottom region, FA=0.25±0.09). Regions at corresponding locations within the the cleared, neurofilament-stained samples were analyzed by measuring the structure tensor (Fig.1-d). A similar behavior to the diffusion measurement could be observed with coherently running fiber pathways in the top region (FA=0.30±0.03) and reduced coherence in the bottom region (FA=0.21±0.05). The next steps will involve clearing of larger specimen so that the CLARITY data covers larger regions within the MRI data, which will allow for a more precise comparison

of individual voxels.
[1] Tomer, R. et al. Nat Protoc 9, 1682-1697 (2014).



a) A tissue cuboid (1cm^3) covering part of the thalamus was excised of a fixed brain human brain slab.

b) The cleared specimen and a structural MRI scan (resolution 350 x 350 x 300um) of the same region. Corresponding vessels were identified in both CLARITY and MRI dataset and used for aligment of the data.

c) Diffusion tensor imaging (resolution 1mm isotropic) of the same sample before clearing. The green tensors in the top region represent fibers running parallel to the surface (FA=0.30±0.10). The bottom region shows slightly less coherence (FA=0.25±0.09).

d) Structure tensor analysis of the neurofilament stain in that region showed a similar behaviour like the DTI scan with a higher anisotropy (FA=0.30±0.03) in the top region and reduced anisotropy (FA=0.21±0.05) in the bottom region. (CLARITY resolution 0.25 x 0.25 x 1.3 um, scale bar = 70um)

Disclosures: **C. Leuze:** None. **M. Goubran:** None. **M. Aswendt:** None. **Q. Tian:** None. **B. Hsueh:** None. **M. Zeineh:** None. **K. Deisseroth:** None. **J. McNab:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Research support provided by GE healthcare.

Poster

092. Optogenetic Approaches to Studying Neural Circuit Function

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 92.15/KKK13

Topic: I.04. Physiological Methods

Support: DARPA NeuroFAST W911NF-14-2-0013

NIH

Howard Hughes Medical Institute

Title: Stable, chronic two-photon imaging in awake, behaving rhesus macaque.

Authors: *X. SUN[1], E. TRAUTMANN[2], D. O'SHEA[2], S. RYU[1], J. MARSHEL[1], W. ALLEN[1], I. KAUVAR[1], C. RAMAKRISHNAN[1], K. DEISSEROTH[3], K. SHENOY[4];
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Abstract: Optical functional imaging, such as 2-photon (2P) calcium imaging, have become powerful tools for investigating functions of neural circuits *in vivo*. Translating these techniques to non-human primates enables recording hundreds or thousands of neurons simultaneously with single-neuron resolution, tracking the same neurons across multiple sessions, combining functional recordings with high resolution structural imaging, and identifying cell types using genetic labeling.
We have developed a platform for performing 2P imaging in awake, behaving rhesus monkeys and have demonstrated: 1) dense expression of GCaMP across PMd, M1, and S1, 2) stable imaging of neurons during a motor behavioral task, 3) imaging neurons 500 microns below the surface in highly-scattering primate cortex, and 4) tracking the same neurons across multiple days and sessions. While encouraging, the vast majority of the thousands of neurons imaged via 2P exhibited bright, filled processes and somas and did not modulate in intensity, consistent with reports of GCaMP overexpression in rodent imaging (e.g. Harvey et al., 2012). We achieved these imaging goals by addressing experimental and engineering translation challenges unique to monkey research. To screen for GCaMP constructs that express in primates, we injected 8 viral constructs at 32 sites in PMd, M1 and S1. To align with long-term monkey research timescales, the imaging chamber must maintain clarity for several months to years. We developed an imaging chamber that incorporates a transparent replaceable silicone window, which is sealed from the external environment to minimize risks of infection and opacification. Another challenge to stable imaging are the brain pulsations induced by cardiac and respiratory rhythms. To restrict brain motion, we developed a stabilization system that uses gentle pressure to restrict total XY motion to 5-10 μm for prolonged experiments. To prevent the experimental task itself from generating movement of the brain while imaging, we developed a novel rigid three-point

head restraint system. Finally, to maximize the imaging depth, we used a dichroic and light guide at the back aperture of the imaging objective to collect scattered photons and maximize the photon collection efficiency. Our results demonstrate the feasibility of two-photon imaging that can facilitate a new class of systems neuroscience experiments in behaving monkeys, complementary to electrophysiological studies.

Disclosures: **X. Sun:** None. **E. Trautmann:** None. **D. O'Shea:** None. **S. Ryu:** None. **J. Marshel:** None. **W. Allen:** None. **I. Kauvar:** None. **C. Ramakrishnan:** None. **K. Deisseroth:** None. **K. Shenoy:** None.

Poster

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NIH Grant AG038961

Title: Focused ultrasound facilitates non-invasive aav delivery for optogenetics

Authors: **S. WANG**, T. KUGELMAN, A. BUCH, S. HUSSAINI, M. HERMAN, M. KARAKATSANI, Y. HAN, K. DUFF, *E. KONOFAGOU;
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Abstract: Optogenetics is a recently developed technique that has been widely applied in the field of neuroscience. Light-sensitive protein channels such as Channelrhodopsins (ChR), are introduced into neurons either by viral transduction or transgenic manipulations. Recent advances in focused ultrasound (FUS) offer a non-invasive approach to carrying out viral transduction in the brain as shown by our group and others. In the presence of microbubbles, the blood-brain barrier (BBB) can be temporarily opened, and we have demonstrated the feasibility of non-invasive AAV delivery to the targeted brain region. Taking advantage of the FUS-mediated BBB opening, we propose a FUS-based, non-invasive adenoviral delivery scheme suitable for non-invasive optogenetic applications. The ability of FUS to facilitate AAV9-ChR-mcherry delivery was first demonstrated in wild-type mice. Caudate-Putamen and hippocampus were selected to be the targets for FUS and contrast-enhanced MRI was used to reveal the BBB opened regions. For mice receiving FUS, the following parameters were used: frequency 1.5 MHz, peak-rarefactional pressure 0.45 MPa, pulse length 10 ms, pulse repetition frequency 5 Hz,

and a duration of 120 s. Mice were allowed to survive for ten days and the ChR expression was examined with confocal imaging of the brain slides (40 μm). In addition, the region of transduction was compared with mice receiving direct infusion of the same viral particle. The bioactivity and efficiency of FUS delivered AAV was examined by performing optogenetic stimulation. Both pulsed stimulation (2s on and 2s off) and long pulse stimulation (10 s) were carried out while the animals were fully functional. Finally, the safety of the proposed technique was tested via histological analysis. Fluorescence images revealed that FUS-facilitated AAV delivery successfully transduced targeted brain regions. Furthermore, the level of transduction was comparable to that from direct infusion. When stimulated with the blue light, a higher number of spikes was observed indicating an increased level of neuronal activity. Compared to resting state, the number of spikes was significantly higher ($P<0.001$) upon stimulation. Ten-second long pulsed stimulation showed an initial increase of spike frequency followed by a gradual decrease to the baseline. These results were comparable to mice receiving direct viral infusion. Therefore, the successful non-invasive AAV delivery can provide an alternate and safer route for Channelrhodopsin neuronal transduction for optogenetic stimulation.

Disclosures: **S. Wang:** None. **T. Kugelman:** None. **A. Buch:** None. **S. Hussaini:** None. **M. Herman:** None. **M. Karakatsani:** None. **Y. Han:** None. **K. Duff:** None. **E. Konofagou:** None.

Poster

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Topic: I.04. Physiological Methods

Title: Tailoring light-delivery depth for optogenetic control of neural activity in deep-brain structures with tapered optical fibers

Authors: ***F. PISANELLO**[1], G. MANDELBAUM[2], M. PISANELLO[1,3], L. SILEO[1], A. DELLA PATRIA[1], I. A. OLDENBURG[2], T. HAYNES[2], M. S. EMARA[1,3], B. SPAGNOLO[1,3], B. L. SABATINI[2], M. DE VITTORIO[1,4];
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Abstract: In vivo optogenetic experiments in deep structures of the mouse brain are still limited by the use of flat-cleaved optical fibers, whose illumination is restricted to a small and fixed volume close to fiber facet [Neuron 86, 106 (2015); Front. Neurosci. 10:70 (2016)]. Here we exploit modal demultiplexing properties of tapered optical fibers (TOFs) to adapt light delivery

depth to the size of functional structures and to obtain spatial-resolved optogenetic control of neural activity in deep-brain regions.
A very small taper angle is designed to gradually lose the total internal reflection along the taper and therefore to deliver light along a taper segment that can be tuned from L~0.4mm to L~2mm. The same device allows for two operation modalities: (i) when light is injected into the fiber through the whole numerical aperture, light is delivered homogeneously along the whole segment; (ii) when light is coupled with an angle into the fiber, the user can instead select a well-defined sub-portion of L that emits light. The approach was tested in motor cortex and in striatum, in both head-restrained and free-moving animals.
For the motor cortex experiment we used the *VGAT-ChR2* mouse line (ChR2 in all inhibitory interneurons). Experiments with modality (i) show that the light emission geometry of TOFs permits sustained inhibition of neural activity at delivered powers 5-times smaller than the threshold recorded for standard flat-cleaved fibers. The experiments in striatum were instead carried out in mice expressing ChR2 in the indirect spiny projection neurons of the striatum (*Ador2a-Cre; Ai32*). Immunohistochemistry for c-fos (protein product of an immediate early gene whose expression is regulated by neuronal activity) after TOF-based light delivery (in modality (i)) revealed a more uniform c-fos induction across ~2 mm of the dorsal ventral axis of the striatum, with respect to the ~0.5mm obtained with standard fibers, for the same delivered light power.
In both motor cortex and striatum, coupling light at different angles into the fiber (modality (ii)) [Neuron 82, 1245 (2014); Biom. Opt. Exp. 6 4014 (2015)] allows for obtaining site-selective light delivery in three independently addressable subregions of these structures by using a single implant. This property can also be used with multiple lasers at multiple wavelengths and different input angles to obtain multicolor light delivery at different depth.
The simplicity of this technique, together with its versatility, reduced invasiveness and compatibility with both laser and LED sources, indicate this approach can greatly complement the set of existing methods for light delivery in optogenetic experiments.

Disclosures: **F. Pisanello:** None. **G. Mandelbaum:** None. **M. Pisanello:** None. **L. Sileo:** None. **A. Della Patria:** None. **I.A. Oldenburg:** None. **T. Haynes:** None. **M.S. Emara:** None. **B. Spagnolo:** None. **B.L. Sabatini:** None. **M. De Vittorio:** None.

Poster

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Topic: I.04. Physiological Methods

Support: ICTSI and the Indiana University Health – Indiana University School of Medicine Strategic Research Initiative

Title: Comparison of network effects of optogenetic and electrical stimulation on the synchronized oscillations in a computational model of parkinsonian basal ganglia

Authors: S. RATNADURAI-GIRIDHARAN[1], C. C. CHEUNG[1], *L. L. RUBCHINSKY[2,1]; [1]Indiana Univ. Purdue Univ. Indianapolis, Indianapolis, IN; [2]Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Deep brain stimulation (DBS) is used as a therapeutic procedure to treat symptoms of several neurological and neuropsychiatric disorders by controlling electrical activity of neural circuits. In particular it is used to treat motor symptoms of Parkinson's disease (PD), associated with excessive oscillatory synchronized activity in the beta frequency band. An alternative way to stimulate neural circuits is an emerging technology of optogenetics. It is not clear if optogenetics will eventually be possible to implement in clinical practice. However it is emerging as a widely used experimental tool to control brain networks. Thus the goal of his study is to explore how effective an optogenetic stimulation in comparison with electrical stimulation in their network effects on elevated synchronized oscillatory activity. We use a computational model of subthalamic and pallidal circuits, which was developed to reproduce experimentally observed beta-band activity patterns. We model electrical stimulation as well as optogenetic stimulation of two types (excitatory via channelrhodopsin and inhibitory via halorodopsin). All three modes of stimulation can decrease beta synchrony. The actions of different stimulation types on the beta activity differ from each other. Electrical DBS and optogenetic excitation have somewhat similar effects on the network. They both cause desynchronization and suppression of the beta-band bursting. As intensity of stimulation is growing, they synchronize the network at higher (non-beta) frequencies in almost tonic dynamics. Optogenetic inhibition effectively reduces spiking and bursting activity of the targeted neurons. We compare the stimulation modes in terms of the minimal effective current delivered to basal ganglia neurons in order to suppress beta activity below a threshold. Optogenetic inhibition usually requires less effective current than electrical DBS to achieve beta suppression. Optogenetic excitation, while as not efficacious as optogenetic inhibition, still usually requires less effective current than electrical DBS to suppress beta activity. Our results suggest that optogenetic stimulation may introduce smaller effective currents than conventional electrical DBS, but still achieve sufficient beta activity suppression. Thus optogenetic stimulation may be more effective than electrical stimulation in control of synchronized oscillatory neural activity.

Disclosures: S. Ratnadurai-Giridharan: None. **C.C. Cheung:** None. **L.L. Rubchinsky:** None.

Poster

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Topic: I.04. Physiological Methods

Support: AIHS postdoctoral fellowship

CIHR postdoctoral fellowship

Title: Opto-panx1: engineering a new, optically-controlled pannexin 1 channel

Authors: *A. W. LOHMAN[1], W. ZHANG[2], R. E. CAMPBELL[2], R. J. THOMPSON[1];
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Abstract: Pannexin 1 (Panx1) forms a large pore ion channel that regulates several cellular and (patho)physiological processes including ATP release, phagocyte chemotaxis, inflammasome assembly and neuronal excitotoxicity. Characterization of Panx1 has expanded over the past decade, but pharmacological and genetic manipulations of the channel have been enigmatic due to overlapping targets for antagonists and the potential for compensation by other Panx isoforms. As a result, ascribing specific contributions of Panx1 signalling in health and disease has proved difficult. Here, we have developed a new optogenetic tool for the isolation and study of Panx1 signalling, called opto-Panx1. Opto-Panx1 consists of a two-protein system. We first engineered an optically-activated Hepatitis C Virus protease (HCVp) by inserting a photocleavable protein (PhoCl) between HCVp and an inhibitory peptide which blocks the protease active site. Next, we mutagenized the caspase cleavage site in the Panx1 C-tail to a consensus HCVp motif ($Panx1^{HCV}$). Proteolytic truncation of the Panx1 C-tail opens the channel pore. We assessed the functional status of opto-Panx1 by TO-PRO-3 dye uptake and electrophysiology. Photostimulation of opto-Panx1 with 380nm light promoted dye uptake and increased whole cell currents in transfected HEK293T cells, but not cells expressing a non-cleavable mMaple-HCVp variant. Chronic photoactivation induced cell blebbing, consistent with reported Panx1 activities in apoptotic cells and ischemic neurons. These responses were not observed in cells solely expressing PhoCl-HCVp or $Panx1^{HCV}$ or cells co-expressing PhoCl-HCVp and $Panx1^{WT}$, indicating specificity of the system. For *in vivo* applications, we generated a bi-cistronic AAV vector for dual expression of PhoCl-HCVp and $Panx1^{HCV}$ with a proximal floxed DsRed-STOP codon element allowing Cre recombinase-driven expression. Transfection of this construct in HEK293T cells along with Cre recombinase revealed a loss of DsRed fluorescence and induction of opto-Panx1 expression, as evidenced by increased green fluorescence (from PhoCl). This new optogenetic tool provides sensitive spatio-temporal control over Panx1 activity for examining cell type specific contributions of these channels across a broad range of scientific disciplines.

Disclosures: **A.W. Lohman:** None. **W. Zhang:** None. **R.E. Campbell:** None. **R.J. Thompson:** None.

Poster

092. Optogenetic Approaches to Studying Neural Circuit Function

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Topic: I.04. Physiological Methods

Title: Comparison of optogenetic and electrical intracranial self-stimulation of the mouse VTA

Authors: ***T. C. WEIDNER**, D. VINCENZ, M. J. BROCKA, J. TEGTMEIER, A. KOLODZIEJ, K. TAKAGAKI, F. W. OHL, J. GOLDSCHMIDT, M. T. LIPPERT;
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Abstract: Optogenetic methods have become a mainstay of systems neurophysiology and have replaced electrical stimulation in many fields of basic research. However, there are numerous experimental contexts where optogenetic methods cannot be used yet. For example, optogenetic stimulation is currently not feasible in human patients. In addition, one must also relate new optogenetic findings to the large body of neurophysiological research that has been performed before the invention of optogenetics. However, it is often difficult to relate findings using optogenetics to findings from electrical stimulation. In this study, we compare electrical and optogenetic stimulation of the ventral tegmental area (VTA) in mice. The VTA and its dopaminergic circuitry are both important from a clinical point of view as well as an area of high interest in optogenetic research. While indirect comparisons of optogenetic and electrical VTA stimulation have been done previously, matching of stimulation intensity between the electrical and optical modality has not been reported yet. Here, we matched the intensity of electrical and optogenetic stimulation by the strength of self-stimulation behavior evoked by different current strengths and light levels. We used two different transgenic mouse lines which are typically used in optogenetic VTA studies, TH::Cre and DAT::Cre mice. In both groups, we transduced 10 male animals with AAV-EF1a-DIO-hChR2(H134R)-eYFP-WPRE-pA and implanted a custom-made optrode into the left VTA. Following a three week expression period, the mice were tested in a lever-pressing task for optogenetic and electrical intracranial self-stimulation (ICSS) at various intensities. The resulting responses were fitted with a Gompertz-model and two behaviorally equivalent intensities were chosen for imaging with electrical and optogenetic stimulation. The mice were passively stimulated at these intensities and the spatial patterns of neural activity measured using SPECT-imaging of regional cerebral blood flow. Our results show that the application of behaviorally equivalent optogenetic and electrical stimuli resulted in

surprisingly similar activity patterns in the Nucleus accumbens (Nacc), medial prefrontal cortex (mPFC), Raphe nuclei (DRN) and dorsal striatum. Previously described differences in brain activity between electrical and optogenetic stimulation of the VTA may therefore be related to unequal intensities of stimulation rather than to a fundamental difference in the stimulation modalities.

Disclosures: T.C. Weidner: None. **D. Vincenz:** None. **M.J. Brocka:** None. **J. Tegtmeier:** None. **A. Kolodziej:** None. **K. Takagaki:** None. **F.W. Ohl:** None. **J. Goldschmidt:** None. **M.T. Lippert:** None.

Poster

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Program#/Poster#: 92.21/KKK19

Topic: I.04. Physiological Methods

Title: Coupling optogenetic stimulation and microdialysis *In vivo* to investigate how distinct stimulation patterns regulate dopamine levels

Authors: *M. SKIRZEWSKI, I. KARAVANOVA, A. BUONANNO;
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Abstract: Optogenetics has been widely used to investigate electrophysiological synaptic function and circuits *in vitro* and *in vivo*, and to correlate them to behaviors. However, relatively little is known about how distinct optogenetic stimulation paradigms regulate the release and accumulation of neurotransmitters and neuromodulators in the brain. These parameters are important because, as has been reported previously at corticostriatal synapses, the release of low concentrations of dopamine during pacemaker or irregular firing leads to long-term depression, whereas the release of high concentrations of dopamine during bursting induces long-term potentiation (Williams et al, Neuroscience 39:1-16, 1990; Reynolds et al, Nature 413:67-70, 2001). Importantly, these dopamine-dependent forms of synaptic plasticity provide the basis for context-dependent changes underlying reward-related learning in primates and rodents. Because presently it is not clear how different protocols of optogenetic manipulations (tonic vs. phasic) widely used to modulate dopamine-dependent behaviors affect dopamine levels, particularly in brain areas such as prefrontal cortex or hippocampus where microdialysis is the only suitable method sensitive enough to detect extracellular dopamine levels (0.5 - 1.5 nM), we have set-out to combine these two experimental approaches. Preliminary findings indicate that tonic vs. phasic optogenetic stimulation on dopaminergic processes in dorsal striatum leads to different locomotor activity behaviors in mice, supporting the idea that distinct optogenetic stimulation

paradigms differentially regulate the accumulation of dopamine. Here, we provide direct evidence of how *in vivo* extracellular dopamine levels in prefrontal cortex and dorsal striatum are regulated during tonic vs. phasic optogenetic stimulation of midbrain dopaminergic neurons. The combined optogenetic-microdialysis approach is allowing us to investigate the properties of dopamine accumulation, and provide a complementary functional study in support for electrophysiological and behavioral analysis to determine the direct relevance of extracellular dopamine levels to behaviors. *This work was supported by the Eunice Kennedy Shriver NICHD Intramural Research Program, NIH.*

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Poster

092. Optogenetic Approaches to Studying Neural Circuit Function

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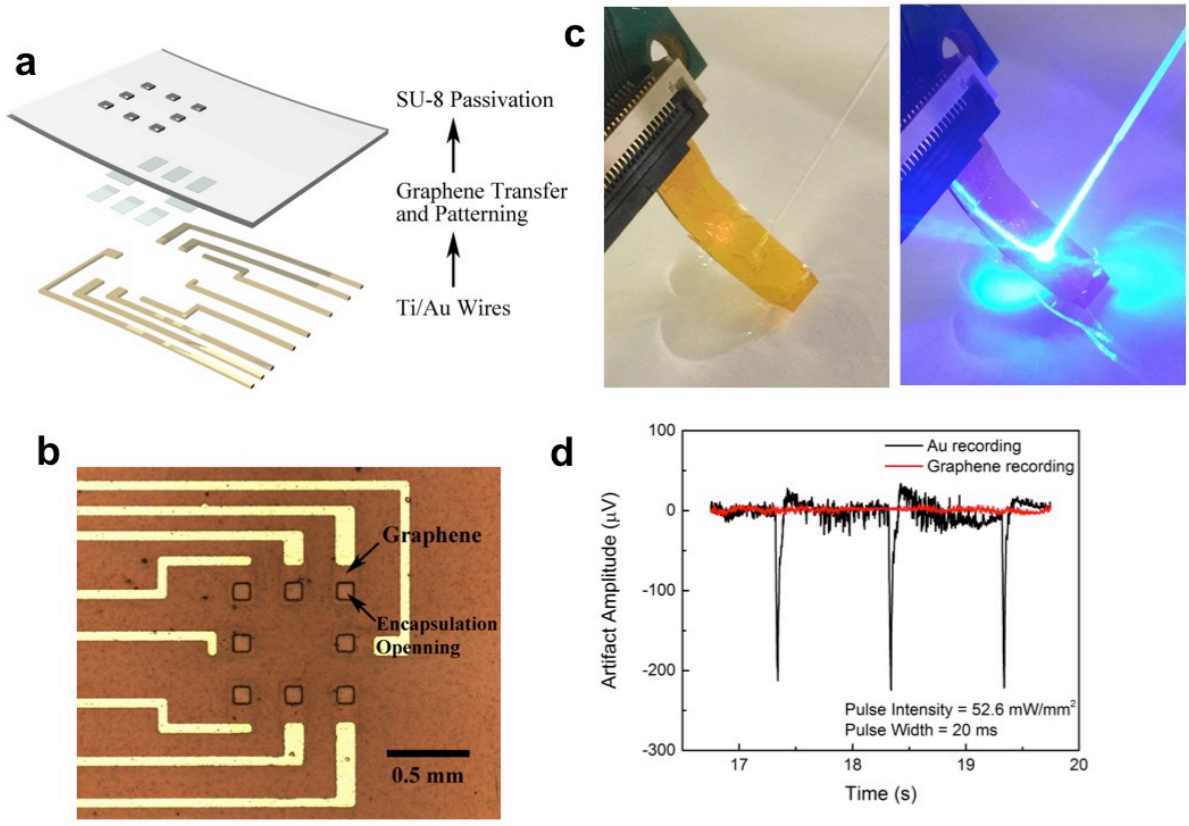
Topic: I.04. Physiological Methods

Title: Transparent microelectrodes eliminating light-induced artifacts for simultaneous optogenetics and electrophysiology.

Authors: H. LYU[1], X. LIU[2], *D. KUZUM[2];
[1]Univ. of California San Diego, San Diego, CA; [2]Univ. of California San Diego, La Jolla, CA

Abstract: Emerging light and optic-based methods, such as optogenetics and multiphoton microscopy, have revolutionized neuroscience research. However, light-induced artifacts in metal-based microelectrodes significantly limit simultaneous use of these tools. Due to Becquerel and photo-thermal effects, these artifacts appear as transients in recordings and can interfere with local field potentials or spike recordings, depending on the frequency and duration of the light stimulus. In this work, light-induced artifacts in Au and transparent graphene electrodes fabricated in the same batch are systematically investigated. Graphene and Au electrodes were fabricated on transparent polyimide (Fig. 1a,b). Monolayer graphene was transferred onto patterned metal contacts. Standard optical fiber with 200 μm diameter was used to apply light pulses on the electrode to study light-induced artifacts during optogenetic stimulation (Fig 1c). The recordings by the Au electrode exhibit severe light-induced artifacts, while no light-related artifacts were observed for the graphene electrode. Rhythmic square pulse stimulation with 20 ms pulse duration at 10 Hz and was applied to both graphene and Au electrodes (Fig 1d). Power spectrum density analysis on the recording of the graphene electrode did not reveal any features at 10 Hz and its harmonic frequency components. However, Au electrode recordings showed fundamental frequency component, and harmonic peaks at 20 Hz,

30 Hz and 40 Hz. In summary, we have demonstrated that transparent graphene electrodes can enable artifact free optogenetics and electrophysiology while conventional metal electrodes drastically suffer from light-induced artifacts. With its other superior properties, such as high mechanical strength, good conductivity, transparency and biocompatibility, graphene electrodes are extremely promising for studies involving electrical recordings and optical stimulation or imaging.



Disclosures: **H. Lyu:** None. **X. Liu:** None. **D. Kuzum:** None.

Poster

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Research to Prevent Blindness

Beckman-Argyros Award from the Arnold and Mabel Beckman Foundation

Title: Vision restoration - neuronal imaging of red-shifted channelrhodopsin responses in the living eye

Authors: *S. K. CHEONG[1], J. M. STRAZZERI[1,2], D. R. WILLIAMS[3,1], W. H. MERIGAN[2,1]; [1]Ctr. for Visual Sci., [2]Flaum Eye Inst., [3]Inst. of Optics, Univ. of Rochester, Rochester, NY

Abstract: ***Purpose:*** The rate of progress in developing new approaches to vision restoration is limited by the inability to troubleshoot each approach at the level of individual neurons in the living eye. Here we describe the application of an *in vivo* method to record and track the response of retinal neurons in a study of optogenetic vision restoration in blind mice. We used a red-shifted channelrhodopsin (ChrimsonR) and stimulated the eye using red light, outside the activation spectra of mouse photoreceptors and melanopsin. We recorded ChrimsonR mediated light response directly from retinal neurons using *in vivo* adaptive optics imaging of a genetically encoded calcium indicator (GCaMP6s) in a mouse model of photoreceptor degeneration (*rd10*). ***Methods:*** Transgenes GCaMP6s and/or ChrimsonR-tdTomato were transfected into retinal neurons using adeno-associated viral vectors. *In vivo* functional calcium imaging of GCaMP6s, excited by 488 nm over a 5 x 6.7° field of view, was performed using a custom built adaptive optics scanning light ophthalmoscope to measure cell responses to light stimulation. Uniform field, 8.6° diameter, 0.2 Hz square wave stimuli were presented in Maxwellian view using two LEDs: 365 nm to drive S-opsin, and 620 nm to drive ChrimsonR. Response amplitude and phase were computed at the stimulus frequency (f1). Responses were also recorded from wild-type (C57BL/6J) mice transfected with only GCaMP6s. ***Results:*** Robust coexpression of GCaMP6s and ChrimsonR-tdTomato was observed in many neurons following serial injections. In *rd10* mice without ChrimsonR, no cells showed light evoked activity to 620 nm or 365 nm stimulation. In rd10 mice with ChrimsonR, cells showed robust responses to 620 nm stimulation (that activates ChrimsonR) but not to 365 nm stimulation (that activates S-opsin); responses to 620 nm were recorded in mice up to 119 days of age, 90 days post injection. In wild-type mice without ChrimsonR, many cells showed light evoked activity to 365 nm but none responded to 620 nm stimulation. ***Conclusions:*** ChrimsonR allows study of optogenetic vision restoration using stimulating light outside the activation spectra of mouse photoreceptors. *In vivo* calcium imaging of retinal neurons allows recording of restored visual responses directly at the site of therapy in the eye and may be applied to multiple methods of vision restoration.

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Poster

092. Optogenetic Approaches to Studying Neural Circuit Function

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 92.24/KKK22

Topic: I.04. Physiological Methods

Support: National Institute of Neurological Disorders and Stroke Grant 5R01NS086804

NSF CAREER Award CBET-1253890

CMSE Grant DMR-1419807

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McGovern Institute for Brain Research at MIT

Samsung Scholarship

Title: Flexible multifunctional polymer fibers for integrated optogenetics

Authors: *S. PARK[1], Y. GUO[5], X. JIA[6], H. CHOE[2], B. GRENA[3], J. KANG[4], H. YOON[4], G. B. CHOI[2], Y. FINK[3], P. ANIKEEVA[3];
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Abstract: Multifunctional devices for optogenetic stimulation and neural recording may offer benefits to the basic study of the nervous system. Since optogenetic experiments typically rely on viral delivery of opsin genes and require visible light, an invasive two-step surgery is often employed. As the majority of optogenetic studies still rely on silica fibers outfitted with metallic electrodes, the modulus mismatch between these devices and the brain tissue lead to profound foreign-body response posing a barrier to long-term opto-electrophysiology. Consequently, there remains a need for flexible and multifunctional neural probes that combine viral delivery with optogenetic stimulation and electrophysiological recording in freely moving rodents. Here we introduce an all-polymer probe that integrates an optical waveguide, 6 electrodes, and 2 microfluidic channels. This device produced via a thermal drawing process has a cross sectional diameter of 200 μm, and connectorized with optical, electrical and microfluidic interfaces weighs <0.5g. The choice of materials enabled low-loss optical transmission, and the development of a custom conductive polyethylene (CPE) composite with graphite (5% by weight) yielded electrodes whose impedance is comparable to that of metallic microwires (100s kΩ) enabling electrophysiological recordings of isolated action potentials with high signal to noise ratio

(SNR). The utility of our devices was confirmed by recording the optically-induced neural activity 2 weeks following the delivery of the adeno-associated virus carrying a gene for channelrhodopsin 2 (AAV5-CaMKIIα::ChR2-EYFP) into medial prefrontal cortex (mPFC) of wild type mice. Optical stimulation in the premotor area resulted in increase of locomotor activity consistent with ChR2-facilitated excitation. Multiple implantations were also performed to allow optogenetic studies of projections from the basolateral amygdala to the mPFC or the ventral hippocampus (vHPC). These circuits exhibited distinctly different latencies of optically evoked signals, and furthermore the activity was correlated to the behavioral response. Consistent with prior studies, stimulation of the BLA inputs into the vHPC resulted in a decreased time spent in the center during a standard open field test. Finally, the flexibility of our probes was manifested in their enhanced biocompatibility as corroborated by reduced glial response and blood-brain barrier breach following up to 90 days of implantation. As our device allowed for minimally-invasive optogenetics in freely moving mice with a one-step surgery, we anticipate its future applications in systems neuroscience studies.

Disclosures: **S. Park:** None. **Y. Guo:** None. **X. Jia:** None. **H. Choe:** None. **B. Grena:** None. **J. Kang:** None. **H. Yoon:** None. **G.B. Choi:** None. **Y. Fink:** None. **P. Anikeeva:** None.

Poster

092. Optogenetic Approaches to Studying Neural Circuit Function

Location: Halls B-H

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Program#/Poster#: 92.25/KKK23

Topic: I.04. Physiological Methods

Support: R21EY024362

P51OD010425

R01EY023277

R01EY019258

Title: Selective optogenetic control of Purkinje cells in monkey cerebellum

Authors: ***Y. EL-SHAMAYLEH**[1,2], Y. KOJIMA[1,2], R. SOETEDJO[1,2], G. D. HORWITZ[1,2]; [1]Physiol. & Biophysics, Univ. of Washington, Seattle, WA; [2]Washington Natl. Primate Res. Ctr., Seattle, WA

Abstract: A central goal of systems neuroscience is to understand how distinct cell types contribute to brain function. The ability to manipulate gene expression in specific cell types in

small animals (e.g. flies and mice) has already revealed key principles of neural coding and the neural basis of behavior, but these animals are poor models for higher brain function. Primates are a superior model in this respect, but achieving cell type-specific manipulations in primates (e.g. through optogenetics) have been challenging. Here, we report robust Purkinje cell-specific expression of channelrhodopsin-2 in the primate cerebellum. We injected the cerebellar cortex of three rhesus monkeys with recombinant AAV vectors. Vector constructs contained a 1 kb L7 promoter fragment which targets Purkinje cells in rats and mice but, to our knowledge, has not been tested in monkeys. Physiological recordings confirmed potent optogenetic activation of transduced cerebellar neurons (N = 3 monkeys). Optical stimulation of the oculomotor vermis increased the probability of both simple and complex spikes, and was sufficient to perturb saccade trajectories with a latency of ~13 ms. Sustained optical stimulation caused horizontal saccade hypometria in one direction and hypermetria in the opposite direction. Histological examination of cerebellar sections via immunohistochemistry and fluorescence microscopy confirmed the specificity of viral vector-mediated opsin expression (N = 3 monkeys). In contrast, control injections of a vector containing the ubiquitous CMV promotor transduced a broad range of cerebellar cell types (N = 1 monkey). Thus, AAV vectors containing a 1 kb L7 promotor fragment provide an effective tool for the causal interrogation of Purkinje cell contributions to cerebellar function in primates. More broadly, this study highlights the promise of cell type-specific promoters in conjunction with viral vector-mediated gene delivery to manipulate the activity of targeted neural populations in macaques.

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Poster

092. Optogenetic Approaches to Studying Neural Circuit Function

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Brain Sciences Project of CNSI, NINS grant no. BS271006

JSPS Research Fellowships for Young Scientists (265926)

Title: Efficient ArchT-mediated optogenetic inhibition by red-shifted off-peak 594-nm light *In vivo*

Authors: *R. SETSUIE[1], K. TAMURA[1], M. TAKEDA[1,2], K. MIYAMOTO[1], Y. MIYASHITA[1,2];
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Abstract: Manipulation of a large number of neurons is a prime requirement for optogenetic intervention to behavior in mammals with large brain sizes, such as primates. The spatial volume effectively illuminated by a single optical fiber is restricted by attenuation of the emitted light due to distance as well as scattering and absorption by brain tissue. In many *in vivo* studies, a proton pump ArchT has been driven by 532-nm light according to *in vitro* cellular studies that reported the excitation peak in the 530~550-nm range. The 532-nm light, however, can be strongly absorbed by hemoglobin, and may not be the most effective for large brain volume inhibition. One of the alternative approaches is to use a longer wavelength to avoid the tissue absorption. Thus we examined the efficacy of red-shifted 594-nm light in ArchT-mediated large-volume inhibition of neuronal activity in comparison with 532-nm light.
ArchT was expressed in the primary somatosensory cortex of Wistar rats by injecting a virus vector (AAV5-CaMKIIa-ArchT-GFP; 2 μl; titer, 0.5~2.0×10^13 GC/ml). To measure the efficacy of photoinhibition at different distances from the optical fiber, we designed an optical device with one tungsten microelectrode surrounded by four non-overlapping side-emitting optic fibers at horizontal distances of 250, 500, 700, and 1000 μm from the electrode. Light-responsive single-/multi-unit activities were recorded under urethane anesthesia (1.2 g/kg) as described previously (Tsubota et al, 2015). The decreases in the spontaneous firing rate during illumination were measured with 532-nm or 594-nm DPSS lasers. In each unit, the light power, matched between 532-nm and 594-nm, was optimized for different optic fiber distances so that changes in the firing rate could be measured accurately without floor or ceiling effects (within the linear range) by considering the inverse square attenuation of light with distance. We found that the off-peak 594-nm and on-peak 532nm light suppressed the firing with a comparable efficacy at 250 μm (e.g., 60% at 0.6mW). At each distance, 594-nm light showed a suppression efficacy consistent with the prediction of inverse square attenuation. On the other hand, with distance, suppression by 532-nm light became lower than the inverse square attenuation. Correspondingly, at 1000 μm, its suppression was approximately half that of 594-nm light.
Thus our results suggest that 594-nm light is more effective than commonly used 532-nm light for ArchT-mediated large volume silencing in mammalian cortices *in vivo*. We propose that red-shifted off-peak 594-nm light is beneficial for behavioral intervention in primates.

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Poster

092. Optogenetic Approaches to Studying Neural Circuit Function

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Topic: I.04. Physiological Methods

Support: NSERC CFC 212519

Heart and Stroke Foundation 000328

Title: *In vivo* all-optical electrophysiology of neuronal structures via two-photon microscopy of genetically-encoded calcium indicators and optogenetics

Authors: *J. R. MESTER[1,2], P. BAZZIGALUPPI[4,3], P. L. CARLEN[4,3], J. G. SLED[5,2], B. STEFANOVIC[1,2];
[1]Physical Sci., Sunnybrook Hlth. Sci. Ctr., Toronto, ON, Canada; [2]Med. Biophysics, [3]Univ. of Toronto, Toronto, ON, Canada; [4]Toronto Western Hosp., Toronto, ON, Canada; [5]Hosp. for Sick Children, Toronto, ON, Canada

Abstract: Simultaneous optogenetic stimulation of individual neurons and recording of fluorescent calcium indicators emission in vivo has been made possible by the development of red-shifted opsins such as C1V1, Chrimson, and ReaChR. As reported recently, C1V1 and GCaMP variants can be excited simultaneously with minimal cross-excitation. This is advantageous for in situ two-photon fluorescence microscopy (2PFM) for the purpose of all-optical electrophysiology. In this work, we used a transgenic mouse line expressing GCaMP6f in cortical pyramidal neurons (Jackson Labs) transfected with an adeno-associated virus (AAV) driving expression of C1V1(t/t) in these neurons (AAV9.CamKIIa.C1V1(E122T/E162T).TS.eYFP.WPRE.hGH, University of Pennsylvania vector core, AAV-eSYN-C1V1(E122T/E162T)-TS-mCherry, Vector biolabs). AAV injections were performed 2-3 weeks prior to 2PFM imaging. Prior to imaging, mice were anesthetized under isoflurane (5% induction, 1% maintenance) and implanted with a cranial window. 2PFM was done using a twin FV1000MPE microscope (Olympus). C1V1 and GCaMP6f were simultaneously illuminated with 1040nm and 900nm pulsed light using two Ti:Sapphire lasers (Mai Tai DeepSee, Newport) so as to photostimulate C1V1 while exciting GCaMP6f, respectively. The dual wavelength 2P illumination was enabled by beam combining via a custom 950nm low-pass dichroic filter (Chroma Technologies). GUI-based galvanometer control (Fluoview, Olympus) was used to raster scan or to follow a spiral trajectory with 1040-nm illumination. We imaged GCaMP6f and C1V1 reporter (YFP/mCherry) to localize co-expressing cells. By investigating soma, dendrites, axons, and axonal boutons, we found that the primary cellular expression of C1V1 with our transfection protocol was non-somatic. Interrogation of these cellular compartments resulted in increased frequency of GCaMP6f spiking events (spike

sizes 25-100% dF/F) in a broader spatial region than that spanned by photostimulation trajectory during stimulus paradigm. Furthermore, the spikes were not always time-locked with the stimulus paradigm, suggesting either delayed responses, postsynaptic events, or unrelated spontaneous activity. However, with current anesthesia protocol, the baseline level of spontaneous activity is very low when no stimulus is delivered, so it is unlikely that these spikes are unrelated to the stimulus. Further work on this methodology is warranted to enable its use as a viable alternative to invasive electrophysiology methods.

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Poster

092. Optogenetic Approaches to Studying Neural Circuit Function

Location: Halls B-H

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Topic: I.04. Physiological Methods

Support: NIH NINDS K12 Grant NS049453

Burroughs Wellcome Fund Career Award for Medical Scientists

Title: Simultaneous two-photon calcium imaging and optogenetics using GCaMP6 and ChrimsonR

Authors: *E. M. GOLDBERG;
Neurol., Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Two-photon microscopy of calcium-sensitive probes facilitates optical imaging of the activity of many neurons at high speed and with cellular-level resolution, including relatively deep in the light-scattering environment of brain tissue. Optogenetics utilizes light-sensitive molecules to manipulate neuronal activity, also with cellular-level resolution, and with millisecond temporal precision. Recent and rapid advances in the ability to combine these techniques has expanded the tools available to investigate mechanisms of neural circuit function. Here, we demonstrate the simultaneous combination of two-photon calcium imaging of the genetically-encoded indicator GCaMP6f with one-photon photostimulation of the red-shifted channelrhodopsin variant ChrimsonR in cultured neurons and in acute brain slices *in vitro*. This was accomplished with simple modifications to a commercial two-photon microscope system. Photostimulation with 660 nm light of primary rat hippocampal neurons in culture, as well as granule cells and parvalbumin-positive interneurons in the hippocampal dentate gyrus in acute

brain slices, expressing GCaMP6f and ChrimsonR.tdTomato, produced robust GCaMP6f-mediated signals. Imaging:photostimulation did not require use of a shutter (and hence avoids sequential imaging:photostimulation) or post-hoc correction of photostimulation artifact. Severe light scattering and lack of single neuron precision will limit the broad applicability of this approach, but it may nevertheless have narrow applications for specific research questions *in vitro* and perhaps *in vivo*.

Disclosures: E.M. Goldberg: None.

Poster

092. Optogenetic Approaches to Studying Neural Circuit Function

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Topic: I.04. Physiological Methods

Support: NIH Grant R44AG046030

Title: Integration of optogenetic stimulation with neuronal and neurotransmitter recordings

Authors: *D. A. JOHNSON, E. NAYLOR, S. GABBERT, D. V. AILLON, D. A. JOHNSON; Pinnacle Technology, Inc, Lawrence, KS

Abstract: The integration of optogenetics and *in vivo* brain responses requires researchers to coordinate disparate tools and timing issues. Standardized techniques and turn-key systems will allow researchers to focus on the experiment and not system design. Pinnacle has developed an LED-based light delivery method small enough to reside on a rodent's head yet powerful enough to deliver sufficient light for opsin activation (> 100 mW/mm^2 for most wavelengths) and combine this with biopotential and neurotransmitter detection. Pinnacle's system includes LED fiber probes, precise timing, TTL I/O, various recording headstages (EEG/EMG, sleep, seizure, biosensors, etc.) and synchronized video. No optical commutator is required. In validation experiments, wild-type control mice (C57BL6/J) and transgenic mice expressing channelrhodopsin in the hippocampus and cortical regions [(Thy1-COP4/EYFP)9Gfng - Jackson Laboratory] were stimulated in the hippocampus for 20 s with blue (445 nm) or deep red (660 nm) light at 20 Hz, 10% duty cycle, (intensity > 100 mW/mm^2) while measuring EEG via a depth electrode in the hippocampus and a cortical electrode in each hemisphere. The same experiment was also performed with the addition of a glutamate biosensor implanted in the prefrontal cortex (40 s stimulation). All surgical procedures were approved by the University of Kansas IACUC. Control mice (C57BL6/J) showed no response to optical stimulation including no Becquerel effect. In response to blue light stimulation, all transgenic mice expressing

channelrhodopsin had EEG frequency changes in the hippocampus corresponding to the timing of the stimulation pulse. In 70% of the trials, the response propagated to both hemispheres resulting in large-amplitude, cortical seizure-like, activity lasting 20 – 30 s after stimulation. Glutamate in the prefrontal cortex consistently declined (0.17 +/- 0.02 uM) during periods of blue light stimulation. As an additional control, stimulation at 660 nm (deep red) did not result in any measurable response in either EEG or extracellular glutamate concentration.
This research was supported by NIH grant #R44AG046030.

Disclosures: **D.A. Johnson:** A. Employment/Salary (full or part-time): Pinnacle Technology, Inc. **E. Naylor:** A. Employment/Salary (full or part-time): Pinnacle Technology, Inc. **S. Gabbert:** A. Employment/Salary (full or part-time): Pinnacle Technology, Inc. **D.V. Aillon:** A. Employment/Salary (full or part-time): Pinnacle Technology, Inc. **D.A. Johnson:** A. Employment/Salary (full or part-time): Pinnacle Technology, Inc..

Poster

092. Optogenetic Approaches to Studying Neural Circuit Function

Location: Halls B-H

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MIT Media Lab

Title: Two-photon holographic control of single neurons expressing somatic opsins

Authors: ***O. A. SHEMESH**[1], D. TANESE[2], V. ZAMPINI[2], C. LINGHU[1], E. PAPAGIAKOUMOU[2], E. RONZITTI[2], K. D. PIATKEVICH[1], V. EMILIANI[2], E. S. BOYDEN[1];

[1]MIT Media Lab. and McGovern Inst. for Brain Res., MIT, Cambridge, MA; [2]Neurophotonics Laboratory, CNRS UMR8250 Paris Descartes Univ., Paris, France

Abstract: Optogenetics, the use of light-driven microbial opsins to mediate optical control of neuronal activity, is in widespread use for the activation and silencing of populations of neurons. Natural neural codes, however, vary from neuron to neuron even within a single class of cells, with the firing of even single neurons capable of altering behavior or brain state. Thus, an intriguing question is whether it is possible to drive individual neurons. In recent years, two-photon (2P) photostimulation of individual neurons expressing optogenetic proteins has begun to be explored. 2P activation is essential for selective targeting of single neurons because it excites opsins precisely where the light is aimed, unlike single photon stimulation wherein the stray light would activate opsin-bearing neurons away from the targeted cell. Of the different 2P stimulation approaches, computer generated holography (CGH) stands out since it enables precise sculpting of the illumination volume and simultaneous illumination of an entire cell or even multiple cells at the same time. Holography also enables millisecond temporal resolution and even sub-millisecond temporal jitter of action potential generation, meaning that CGH could support single cell, sub-millisecond optogenetic control. Despite recent advances in 2P stimulation, there remains a major problem. In a neuronal network, cell bodies are densely surrounded by neurites of neighboring cells. Thus, if neurons within a region are bearing densely expressed opsins, even 2P stimulation of a single neuron's cell body may excite opsins on dendrites or axons that are passing by, causing artifactual activation of nearby neurons. To avoid this crosstalk, we designed a soma-targeted opsin, which is selectively expressed in the cell body. We screened for soma targeting proteins and found a protein fragment that could limit GFP expression to the cell body of neurons. We termed this fragment 'SomaTag', and fused to it the powerful channelrhodopsin CoChR. We found SomaTag-CoChR to localize to the cell body of neurons in the mammalian cortex, and in combination with holographic 2P stimulation, could support optogenetic stimulation of single cells in mammalian brain slices with millisecond temporal resolution. This new SomaTag-CoChR, in conjunction with optimized 2P optics, may enable a diverse set of neural codes and computations to be probed in a causal fashion in systems and circuit neuroscience.

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Poster

093. Genomics, Proteomics, and Systems Biology

Location: Halls B-H

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Topic: I.02. Systems Biology and Bioinformatics

Support: NRF fund 2015M3C7A1029113

KRIBB fund KGM5201611

Title: Quantitative analysis of phosphoproteome in hippocampal neurons from mouse model of developmental delay

Authors: S.-H. LIM, S. CHOI, J. MOON, *J.-R. LEE;
KRIBB, Daejeon, Korea, Republic of

Abstract: Lack of synaptic formation is one of the common phenomena emerging in neurons of developmental delay. Protein kinases and protein phosphatases regulate many neuronal functions, so the balance between the activities of protein kinases and protein phosphatases is very important in the neuron. Although many synaptic proteins have been known to be phosphorylated or dephosphorylated by synaptic functions, a systematic analysis of phosphoproteins has not been performed about neuronal development. Here we carried out the extensive analysis of phosphopeptides in the hippocampal neurons from a mouse model of developmental delay using Orbitrap MS/MS system. Synaptic proteins were extracted from hippocampal neurons with anionic detergent and the filter-aided sample preparation (FASP) was adopted to remove the residual anionic detergent for full activity of trypsin. After enrichment of phosphopeptides was carried out, labeling with iTRAQ isobaric tag was employed for the quantitative analysis of phosphopeptides. Among phosphopeptides of hippocampal neurons more than 30,000, phosphopeptides more than 600 were significantly increased and decreased in hippocampal neurons of developmental delay compared to wild type. With western blotting the phosphorylation of synaptic proteins were confirmed, and the effects of their phosphorylation and dephosphorylation on synaptic formation were examined. Thus, through the extensive analysis of neuronal phosphopeptides, new signal pathways and unique functions of protein kinases and phosphatases could be elucidated for neuronal development and synaptic formation.

Disclosures: **S. Lim:** None. **S. Choi:** None. **J. Moon:** None. **J. Lee:** None.

Poster

093. Genomics, Proteomics, and Systems Biology

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Topic: I.02. Systems Biology and Bioinformatics

Support: The Israeli Ministry of Science, Space and Technology

Allen and Jewel Prince Center for Neurodegenerative Processes of the Brain

Title: Multi-dimensional characterization of the brain's immune populations

Authors: *B. KORIN, T. BEN-SHANNAN, T. DUBOVIK, A. ROLLS;
Technion - Israel Inst. of Technol., Haifa, Israel

Abstract: Immune cells play a crucial role in brain regulation and malfunction. Yet, we are still limited in our understanding of the complexity and variability of the brain's immune populations and the vast majority of research has been focused on resident microglia and infiltrating monocytes/macrophages. To cope with this gap we adapted mass cytometry (CyTOF), an emerging technology that revolutionizes immunology research, to analyze multiple cell populations in the brain, providing a bird's eye view of the system. CyTOF is a variation of flow cytometry in which antibodies are labeled with heavy metals, rather than fluorophores, and analyzed by their time of flight in a mass spectrometer. This overcomes the overlap in emission curves evident in fluorescent labeling, enabling the use of tens of markers to analyze simultaneously multiple cell populations. Using this strategy, we detected a wide range of immune cell subsets present in the naïve brain, including two subsets of resident microglia, several subsets of infiltrating monocytes/macrophages, granulocytes, dendritic cells (DCs), B cells, natural killer (NK) cells, CD4 and CD8 T cells. By isolating the meninges and choroid plexus from the parenchyma, we determined the relative distribution of those cells between these compartments. Analysis of the expression of cytokine receptors (e.g. IL-1R, IL-2R, IL-4R, IL-6R, TNF-αR and IFN-γR) on various immune populations in the brain revealed the differential expression of receptors across the resident and infiltrating immune cells. This characterization is especially relevant for understanding the effects of cytokines on brain activity. Finally, we examined the changes in the brain following peripheral bacterial infection (with *E. coli*), demonstrating the differential outcomes in the brain and the blood as a result of the peripheral manipulation. Taken together, such multidimensional characterization of the brain's vibrant immune environment provides a new perspective and a valuable tool for our understanding of brain-immune communication.

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Poster

093. Genomics, Proteomics, and Systems Biology

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Topic: I.02. Systems Biology and Bioinformatics

Support: MOST 103-2311-B-002-026-MY3

Title: Explore the exocytotic proteins that interact with Synapsin Ia in a phosphorylation-dependent manner

Authors: *H.-J. YANG[1], C.-T. WANG[2,3,4,1];
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Abstract: Synapsins (Syns), a family of evolutionarily conserved phosphoproteins, are widespread in the nervous system and localize to synaptic vesicles (SVs). Syns are encoded by three distinct genes (*syn I*, *syn II*, and *syn III*) and consist of ten homologous proteins by alternative splicing, i.e., Syn Ia-b, IIa-b, and IIIa-f. Among all homologous proteins, Syn Ia is the best studied in regulating the dynamics of SVs by controlling their storage and mobilization in a phosphorylation-dependent manner. These physiological functions of Syn Ia are mainly mediated by interaction with certain presynaptic proteins (such as cytoskeleton) through phosphorylation of Syn Ia. To date, only a few of exocytotic proteins are found to directly interact with Syn Ia in a phosphorylation-dependent manner. In this study, we explore new exocytotic proteins that interact with Syn Ia through phosphorylation of Syn Ia. To begin with, we performed Bioinformatics approaches. First, we conducted systematic database search to find the proteins that can interact with Syn Ia. Second, we performed the sequence-based protein-protein interaction (PPI) prediction with the software MirrorTree. Based on the results from database search and PPI prediction, we selected the candidate proteins that are exocytotic proteins and likely affect SV release. Furthermore, we performed endogenous co-immunoprecipitation for these candidate proteins. We found that the interaction between Syn Ia and Synaptophysin (Syp) was changed after overexpression of the Syn Ia phosphodeficient mutant, suggesting that the Syn Ia-Syp interaction may be regulated by phosphorylation of Syn Ia. Our results suggest that Syp may potentially involve in Syn Ia's regulation of SV exocytosis in a Syn Ia phosphorylation-dependent manner.

Disclosures: H. Yang: None. **C. Wang:** None.

Poster

093. Genomics, Proteomics, and Systems Biology

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Topic: I.02. Systems Biology and Bioinformatics

Support: NIH/NCCIH Grant P50AT006273

Title: Effects of aged garlic extract and fruarg on lipopolysaccharide induced gene profiling and signaling pathways in microglial cells

Authors: *H. SONG[1,7,2], Y. LU[8,3,2], Z. QU[1,7,2], V. V. MOSSINE[3], J. HOU[2,4], J. CUI[1,2], B. PECULIS[3], T. P. MAWHINNEY[5], J. CHENG[2,4], M. C. GREENLIEF[5,2], K. FRITSCHE[2,6], F. J. SCHMIDT[3], R. B. WALTER[8], D. B. LUBAHN[2,3], G. Y. SUN[1,3,2,7], Z. GU[7,1,2];
[1]Pathology and Anatom. sciences, [2]Ctr. for Botanical Interaction Studies, [3]Biochem., [4]Computer Sci., [5]Chem., [6]Animal Sci., Univ. of Missouri, Columbia, Columbia, MO; [7]Ctr. for Translational Neurosci., Univ. of Missouri Sch. of Med., Columbia, MO; [8]Xiphophorus Genet. Stock Ctr., Texas State Univ., San Marcos, TX

Abstract: Aged garlic extract (AGE) is widely used as a dietary supplement on account of its protective effects against oxidative stress and inflammation. But less is known about specific molecular targets of AGE and its bioactive components, including *N*-α-(1-deoxy-D-fructos-1-yl)-L-arginine (FruArg). Our recent study showed that both AGE and FruArg significantly attenuate lipopolysaccharide (LPS)-induced neuroinflammatory responses in BV-2 microglial cells. This study aims to unveil effects of AGE and FruArg on gene expression profiles due to LPS-stimulated neuroinflammatory responses by conducting a genome-wide search. In the present study, RNA-Seq analysis by Illumina HiSeq 2500 was used to assess the global gene expression in the mouse immortalized microglial BV-2 cells to investigate the protective effects of AGE and FruArg on the transcriptional responses in LPS-induced cells. Results showed that LPS treatment significantly altered 2563 genes. AGE repressed 67% of the transcriptome alteration induced by LPS, whereas FruArg accounted for the protective effect by reducing expression of 54.8% of total genes altered by LPS. Key pro-inflammatory canonical pathways induced by the LPS stimulation included toll-like receptor, IL-6 signaling, and Nrf2-mediated oxidative stress pathway, along with elevated expression levels of genes, such as *Il6, Cd14, Casp3, Nfkb1, Hmox1,* and *Tnf*. These genes could be modulated by both AGE and FruArg treatment. These findings suggest that AGE and FruArg are capable of alleviating oxidative and neuroinflammatory responses stimulated by LPS in BV-2 cells by modulating gene expression in the key pro-inflammatory canonical pathways. Inhibition of the expression of multiple immune- and inflammation-related genes suggested that AGE and FruArg could serve as candidates for the prevention of inflammation-mediated neurodegenerative diseases.

Disclosures: H. Song: None. **Y. Lu:** None. **Z. Qu:** None. **V.V. Mossine:** None. **J. Hou:** None. **J. Cui:** None. **B. Peculis:** None. **T.P. Mawhinney:** None. **J. Cheng:** None. **M.C. Greenlief:** None. **K. Fritsche:** None. **F.J. Schmidt:** None. **R.B. Walter:** None. **D.B. Lubahn:** None. **G.Y. Sun:** None. **Z. Gu:** None.

Poster

093. Genomics, Proteomics, and Systems Biology

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 93.05/KKK33

Topic: I.02. Systems Biology and Bioinformatics

Support: NIMH grant MH094670

Title: Dynamic DNA methylation landscape of neurons from hippocampus and frontal cortex during postnatal development

Authors: *Y. HE[1,2], E. A. MUKAMEL[3], M. HARIHARAN[1], C. LUO[1,5], J. LUCERO[6], R. CASTANON[1], J. R. NERY[1], T. J. SEJNOWSKI[6,4,5], J. R. ECKER[1,5], M. M. BEHRENS[6]; [1]Genomic Analysis Lab., The Salk Inst. For Biol. Studies, La Jolla, CA; [2]Bioinformatics and Systems Biol. program, [3]Dept. of Cognitive Sci., [4]Div. of Biol. Sci., UCSD, La Jolla, CA; [5]Howard Hughes Med. Inst., [6]Computat. Neurobio. Lab., The Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: The growing number of studies focusing on dissecting cell-type specific transcriptional patterns in brain highlights the community effort to profile the molecular signatures of brain cells. However, research on epigenetic signatures, which are closely related to gene regulation and more directly impacted by disease-associated genetic variants, has only recently started. Especially, the temporal and spatial epigenetic changes in brain cells remain far under-explored.

In this study we have characterized the genome-wide DNA methylation (DNAm) patterns of neurons from two distinct brain regions during postnatal maturation. NeuN+ nuclei were isolated from frontal cortex and hippocampus of mice at postnatal weeks 1, 4 and 10 and their DNA methylation and transcription landscape were profiled using MethlC-seq and RNA-seq. NeuN+ nuclei from both brain regions showed dramatic global accumulation of non-CG methylation and a slight increase in genome-wide CG methylation as the brain matured, which is consistent with previous findings in whole frontal cortex in both mouse and human. Despite the similar global DNAm dynamics, hundreds of thousands CG differentially methylated regions (CG DMRs) were identified, marking regulatory elements responsible for the distinct transcriptional patterns of neurons from different brain regions. Interestingly, a subset of CG DMRs was clustered forming thousands of hypomethylated regions stretching more than 2 kilobases. These regions strongly overlapped with genes that are region specific transcriptional markers, implying DNAm as a unique signature for spatial distribution of cells in brain. In addition, the large block of hypomethylation correlated with gene up-regulation, as well as lower level of non-CG methylation. Consistent with this, hundreds of genes showed cell-type specific intragenic non-CG methlyation. To expand our scope, we generated methylome data for pyramidal neurons and

the underrepresented inhibitory Parvalbumin (PV) interneurons and further dissect the methlyation patterns observed in these cell types. Lastly, the data generated in this study provides a valuable resource for the community to understand the epigenetic differences between neurons, as well as regions, and its functional implications, especially in cell identity determination. This work is supported by NIMH grant MH094670.

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Poster

093. Genomics, Proteomics, and Systems Biology

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Topic: I.02. Systems Biology and Bioinformatics

Support: NRF Grant 2014K1B1A1073720

Title: A novel electrochemical detection method for aptamer biosensors of thrombin

Authors: ***H. YOO**[1,2], W. SUN[2], S. YANG[3], J. PARK[2], C.-H. JI[2], S. JUN[2];
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Abstract: Aptamers are single-stranded DNA or RNA molecules which can bind to specific target analytes. Aptamers are known to be able to detect a variety of analytes with high stability and high sensitivity. However, there are still several challenges for the realization of aptamer-based biosensors. One of the challenges to overcome is how to transduce the chemical binding between aptamers and target analytes. An aptamer-based detection of analytes was analyzed using electrochemical impedance spectroscopy (EIS). After thrombin-specific aptamers were coated on the gold electrode, EIS data showed that the binding between the aptamers and thrombin increased the impedance magnitude. The EIS data was also used to model the electrode-electrolyte interface to precisely examine the effect of thrombin-aptamer binding. As a result, it was shown that resistance is the most feasible component for aptamer-based thrombin detection. Based on this, this study has proposed a new simple aptamer detection method using a current source device. This approach is to obtain the resistance value by observing the voltage values to be measured between the two electrodes in particular the current pulse caused to put the voltage pulse as a function generator to a current source device. As a result, the value of voltage applied to the both end electrodes was increased, as the thrombin concentration increases. It

showed the potential for use of aptamer-functionalized gold electrodes in biosensors for in-field detection of proteins.

Disclosures: **H. Yoo:** None. **W. Sun:** None. **S. Yang:** None. **J. Park:** None. **C. Ji:** None. **S. Jun:** None.

Poster

093. Genomics, Proteomics, and Systems Biology

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Topic: I.02. Systems Biology and Bioinformatics

Support: NIH Grant U42 OD010918

Title: Complex microbiota targeted rederivation: a method to study effects of gut microbiota on model phenotypes

Authors: ***M. L. HART**[1], A. C. ERICSSON[2], C. L. FRANKLIN[3];
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Abstract: Recent studies indicate that the gut microbiota (GM) can significantly impact both local and systemic host physiologic processes. With the rising concern to optimize experimental reproducibility and translatability, it is essential to consider the GM in study design. To assess the role of microbiota on model phenotypes, complex microbiota targeted rederivation (CMTR) can be used. With CMTR, mice of the desired model are rederived using surgical embryo transfer into surrogate dams with one or more desired GM profiles. Unfortunately, differing GM are often present in inbred strains of mice which can complicate the use of CMTR as these strains frequently have poor reproductive indices and variations in maternal care. To overcome these limitations, we exploited the benefits of outbred mice as surrogates by establishing colonies of CD1 mice with differing GM profiles. CD1 embryos were transferred into CD1 or C57BL/6 surrogate dams that varied by GM composition and complexity to establish three separate colonies. Using targeted 16S rRNA amplicon sequencing, female offspring were found to have similar GM profiles to surrogate dams. Furthermore, breeding colonies of CD1 mice with distinct GM profiles were maintained for four generations, demonstrating stability of GM profiles within these colonies. We then compared changes in the phenotype of B6 IL-10$^{-/-}$ mice rederived by CMTR using either CD1 colonies or the inbred strains from which the colonies were derived. Cecal and colonic histologic lesion scores differed significantly between groups, but no differences were seen when surrogate source of GM (CD1 vs inbred strain) was

compared. These findings underscore that CMTR using outbred CD1 colonies is an invaluable experimental resource in the assessment of GM effects on model phenotype with applicability to virtually any model. With the emerging interest in the gut brain axis, the effect of the GM on systemic disease models such as neuroscience models are a priority for further investigation at the MU MMRRC.

Disclosures: **M.L. Hart:** None. **A.C. Ericsson:** None. **C.L. Franklin:** None.

Poster

093. Genomics, Proteomics, and Systems Biology

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Program#/Poster#: 93.08/KKK36

Topic: I.02. Systems Biology and Bioinformatics

Support: UCSF Program for Breakthrough Biomedical Research

Title: Transcriptional identities of major cell types in the human brain

Authors: *K. W. KELLEY[1], M. C. OLDHAM[2];
[1]Neurology, The Eli and Edythe Broad Ctr. of Regeneration Med., [2]Dept. of Neurolog. Surgery, Univ. of California San Francisco, San Francisco, CA

Abstract: Understanding the molecular basis of cellular identity in the human brain is essential for ascertaining the functions of different cell types and their susceptibilities to pathologies that underlie neurological diseases. However, acquiring this information has been difficult due to pragmatic and technical challenges. By analyzing gene coexpression relationships in 62 datasets representing over 7000 non-pathological adult human brain samples spanning all major neuroanatomical regions and technology platforms, we identified consensus transcriptional signatures of astrocytes, oligodendrocytes, microglia, and neurons. We developed a new metric to describe cellular identity called gene expression 'fidelity', which ranks genes according to their sensitivity and specificity of expression for a given cell type and provides a mathematical bridge between the analysis of gene expression data from bulk tissue samples and single cells. Predictive gene expression modeling using high-fidelity genes revealed that nearly half of the variation in gene expression levels measured from bulk tissue samples can be explained by variation in the abundance of major cell types. Furthermore, we show how predictive modeling can be used to identify cell type-specific transcriptional differences in disease, among brain regions, and between species. Our work provides a broad foundation for understanding the molecular basis of cellular identity in the human brain and offers a generalizable strategy for defining cell types as vectors of genes ranked by expression fidelity.

Disclosures: **K.W. Kelley:** None. **M.C. Oldham:** None.

Poster

093. Genomics, Proteomics, and Systems Biology

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Program#/Poster#: 93.09/KKK37

Topic: I.02. Systems Biology and Bioinformatics

Title: A customized comparative genomic hybridization array for the analysis of copy number variations and exon-dosage anomalies in neurological disorders.

Authors: *V. LA COGNATA[1,2], G. MORELLO[1], G. GENTILE[1], V. D'AGATA[2], S. CAVALLARO[1];
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Abstract: Neurological disorders are a highly heterogeneous group of pathological conditions characterized by multifactorial etiology relying on genetics, epigenetics and/or environmental contributions. The very complex nature of this group of diseases makes the investigation of their genetic basis rather difficult, mostly if performed gene by gene through traditional methodological approaches. For this reason, high-throughput genotyping technologies are increasingly replacing the classical detection methods, providing higher resolution and a complete window of candidate genomic anomalies. Among the advanced biotechnologies, microarray-based comparative genomic hybridization (aCGH) represents a powerful molecular tool to explore the pathogenetic role of unbalanced structural rearrangements, also in a diagnostic setting. Here, we report the design strategy, development, validation, and implementation of *NeuroArray*, an exon-centric customized aCGH tailored to detect single/multi-exon deletions and duplications in a large panel of multi- and monogenic neurological disorders. This targeted design allows a focused evaluation of structural imbalances in clinically relevant genes at exon-level resolution. An increasing use of the *NeuroArray* platform may offer new insights in investigating potential overlapping gene signatures among neurological conditions and defining genotype-phenotype relationships.

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Poster

093. Genomics, Proteomics, and Systems Biology

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Program#/Poster#: 93.10/KKK38

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH Grant 0072705

Title: Profiling serotonergic neurons from donors at different states of satiation with single-neuron transcriptomics.

Authors: *E. C. DABE[1,2], A. B. KOHN[2], R. GILLETTE[3], L. L. MOROZ[2];
[1]Univ. of Florida Whitney Lab., Saint Augustine, FL; [2]Neurosci., Univ. of Florida McKnight Brain Inst., Gainesville, FL; [3]Mol. and Integrative Physiol., Univ. of Illinois Urbana-Champaign, Urbana, IL

Abstract: The giant serotonergic metacerebral cells MCCs of *Pleurobranchaea californica* are intrinsic neuromodulators of the well-studied feeding network in *P. californica* and are conserved across opistobranchs. Serotonin (5HT) is a critical factor in both behavioral arousal and appetite. Analysis of indole metabolites has previously shown that satiated animals have reduced levels of serotonin (5-HT) and its metabolite 5-HT-SO_4 (but not of its precursor tryptophan or the metabolite 5-hydroxyindole acetic acid) 24 hrs post-feeding, compared to unfed controls. This suggests reduction in synthesis and possibly in release of serotonin in satiated animals. To evaluate these changes and their regulation at the genomic level, we isolated MCC neurons from satiated and unfed *P.californica* 24 hours post-feeding and processed them for single-neuron RNA-seq (min. n=6). Other groups of 5-HT neurons (min. n=3), neurons of other neurotransmitter phenotypes (min. n=3) and whole CNS ganglia or specific regions were also sequenced on Illumina Next-Seq to form the first *P. californica* hybrid reference transcriptome. Normalized RNA expression levels in transcripts per million (TPM), and differential transcript expression were calculated using RSEM and DESEQ2. Overall, we observed a significant decrease in transcript expression of tryptophan hydroxylase and an increase in expression of serotonin transporter (SERT) in satiated animals. Changes in other 5-HT pathways genes were not detected. This finding matches with the observed decrease in 5-HT levels and further suggests that there is a potential increased re-uptake of 5-HT during satiation. We also analyzed homologous MCC transcriptomes from four opistobranch species and discerned several potential transcription factors that could be regulating the 5-HT gene cohort in molluscs.

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

Poster

093. Genomics, Proteomics, and Systems Biology

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Program#/Poster#: 93.11/KKK39

Topic: I.02. Systems Biology and Bioinformatics

Support: IHDCYH #126790

IHDCYH #136908

CHUSJ

Title: Perinatal Inflammatory imprinting in a neonatal animal model of white matter injury: a DNA methylation study

Authors: *S. MCGRAW[1,2], W. C. PIERRE[1,2], L.-M. LEGAULT[1], V. BERTRAND-LEHOUILLIER[1], I. LONDONO[1], G. A. LODYGENSKY[1,2];
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Abstract: **Introduction:** Preterm infants are vulnerable to inflammation-induced white matter injury (WMI) which is associated with neurocognitive impairment and neuro-psychiatric manifestation in adulthood. Epigenetic mechanisms play a role in normal development and pathological adaptation in response to environmental challenges. DNA methylation is considered the most stable and well-characterized epigenetic modification. We hypothesize that perturbations in normal profiles following inflammation-induced WMI in the neonatal period could be implicated in the processes leading to known neuropsychiatric diseases in adulthood. **Objective:** The aim of this study was to assess DNA methylation profile in the brain of neonatal rats subjected to WMI compared to controls at 24h (P4) and 21 days (P24) post-injury. **Methods :** We injected LPS (1mg/kg) or sterile saline in the left side of the corpus callosum of P3 rat pups. Brains were collected at P4 (n=8) and P24 (n=8). We extracted genomic DNA from ipsilateral brain hemisphere and produced multiplex libraries of reduced representation bisulfite sequencing to establish genome-wide quantitative DNA methylation profiles. Genomic regions (100bp, 2 CpG min, 15x sequencing) with a ±≥10% average CpG methylation differences between groups of replicates (Ctrl vs LPS) were designated as differentially methylated tiles (DMTs). Functional enrichment analyses of genic associated DMTs were generated using Metascape (http://metascape.org). **Results:** A total of 1590 and 1838 DMTs were observed at P4 and P24 respectively. At P4 and P24, inflammation induced hypermethylation in genes related to nervous system development and developmental growth, respectively and hypomethylation in genes associated with inflammatory pathways. (see table). **Conclusion:** Neonatal WMI resulted in perturbed brain DNA methylation profiles of genes promoting inflammatory pathway at P4

that remained at P24. It also altered brain DNA methylation profiles inhibiting pathways related to normal development of nervous system (P4) and developmental growth and morphogenesis (P24). Further analysis of target genes through qPCR will be performed to determine if alterations in DNA methylation are associated with aberrant gene expression regulation.

Table 1:Pathways affected by changes in DNA methylation profile following neonatal WMI

Conditions	Pathways	Gene names	Methylation Difference %	Annotation
P4 hypermethylated	Nervous system development	RELN	13.06	Intron
P4 hypomethylated	Inflammation	TNF	-19.21	5' UTR
			-10.48	3' UTR
P24 hypermethyled	Developmental growth	TGFBR2	10.33	Intron
P24 hypomethyled	Inflammation	PIK3R1	-10.14	Promoter-TSS
		C3	-11.68	Intron

Disclosures: **S. McGraw:** None. **W.C. Pierre:** None. **L. Legault:** None. **V. Bertrand-Lehouillier:** None. **I. Londono:** None. **G.A. Lodygensky:** None.

Poster

093. Genomics, Proteomics, and Systems Biology

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Program#/Poster#: 93.12/KKK40

Topic: I.02. Systems Biology and Bioinformatics

Support: Beckman Young Investigator Grant, Arnold and Mabel Beckman Foundation

Title: Discovery proteomics for small neuronal populations using mass spectrometry: toward single cell proteomics

Authors: *P. NEMES[1], S. B. CHOI[1], M. ZAMARBIDE[2], M. MANZINI[2];
[1]Dept. of Chem., [2]Dept. of Pharmacol. and Physiol., George Washington Univ., Washington, DC

Abstract: Characterization of gene expression is central to understanding how molecular processes orchestrate development and function of the nervous system. To address vast cell-to-cell differences (heterogeneity) in the brain, new instruments are needed, particularly those capable of single-cell analysis. High-resolution electrospray ionization mass spectrometry (HRMS) is able to characterize the proteome, but requires averaging hundreds of thousands to millions of cells at the expense of potentially losing information specific to small populations of neurons or individual neurons. Here we present an ultrahigh-sensitivity HRMS approach that enables the identification of a substantial number of proteins in limited populations of neurons. The HRMS instrument integrates a custom-built microanalytical capillary electrophoresis platform and HRMS to achieve trace-level detection capability. For example, angiotensin II was detected at a ~350-zeptomol lower limit of detection. Next, this platform was used analyze ~500 picogram protein digest from neurons that were isolated from the mouse hippocampus. With 10,000-to-100,000-times higher volumetric sensitivity than feasible by traditional HRMS technology, microanalytical HRMS allowed us to simultaneously characterize the expression of 350+ non-redundant protein groups without knowing their neuronal presence ahead of time. The identified proteins included many gene products that have been ascribed to neurons, such as synaptic proteins (e.g., synapsin 1 and 2) and the neural cell adhesion molecule (NCAM). Trace-level detection of the neuron proteome by microanalytical HRMS opens new avenues to study gene expression in small populations of neurons in the brain. Additionally, refinement of this technique will open the road to mapping gene expression also in single neurons, so to help better understand basic molecular processes underlying normal brain development and function.

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Poster

093. Genomics, Proteomics, and Systems Biology

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Topic: I.02. Systems Biology and Bioinformatics

Support: NIH Grant U01 MH098977

NIH Training Grant 5T32AG000216-24

Title: Single-cell RNAseq of human cerebral cortical neurons reveals transcriptional heterogeneity

Authors: G. E. KAESER[1], *J. J. CHUN[2], Y. C. YUNG[1], B. B. LAKE[3], R. AI[3], N. S. SALATHIA[4], A. CHEN[1], X. SHENG[1], J.-B. FAN[4], W. WANG[3], K. ZHANG[3];
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Abstract: The well-known diversity of human brain neurons underlies myriad aspects of neural function. Does the transcriptional landscape within single neurons reflect this diversity as well? Here we report efforts from a NIMH U01 supported team to develop a scalable pipeline to sequence and quantify the transcriptome of over 3,200 single neurons from post-mortem human brain, analyzing six Brodmann Areas (BAs) of the cerebral cortex. Single neuronal nuclei were sorted and processed for total RNA sequencing, followed by use of an iterative, unbiased clustering and classification method that identified 16 neuronal subtypes. These subtypes were further annotated using known gene markers and cortical cytoarchitecture. Neuronal clusters were identified based upon differentially expressed genes (DEGs) that first divided excitatory and inhibitory neurons expressing classical neurotransmitter and transporter genes. Further analyses split neurons according to cortical layer, developmental origin, and BA. These analyses demonstrated a robust and scalable method for identifying and categorizing single neuron transcriptomes, while identifying expressed genes sufficient to distinguish novel and orthologous neuronal subtypes as well as transcriptionally defined BAs within the human brain. The remarkable heterogeneity identified in single neuronal transcriptomes may further reflect activities of complex neuronal networks that vary with time and function. In addition, previous studies from our group have identified increased genomic mosaicism in normal and Alzheimer's disease cortical neurons; the transcriptomic heterogeneity is consistent with normally occurring genomic mosaicism, which may be altered in diseased conditions.

Disclosures: G.E. Kaeser: None. **J.J. Chun:** None. **Y.C. Yung:** None. **B.B. Lake:** None. **R. Ai:** None. **N.S. Salathia:** None. **A. Chen:** None. **X. Sheng:** None. **J. Fan:** None. **W. Wang:** None. **K. Zhang:** None.

Poster

093. Genomics, Proteomics, and Systems Biology

Location: Halls B-H

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Program#/Poster#: 93.14/KKK42

Topic: I.02. Systems Biology and Bioinformatics

Title: The endogenous disrupted in schizophrenia 1 (DISC1) interactome in human neural progenitor cells

Authors: *B. J. WILKINSON[1,3], M. P. COBA[2,3];
[2]Dept. of Psychiatry and Behavior, [1]USC, Los Angeles, CA; [3]Zilkha Neurogenetic Inst., Los Angeles, CA

Abstract: Disrupted in Schizophrenia 1 (DISC1) was originally identified as contributing to schizophrenia and bipolar disorder through a balanced translocation in a Scottish family. Since its discovery, the role of DISC1 in psychiatric disease along with many of its biological functions, including its proposed function as a scaffolding molecule, have been explained through its protein interactions. Although multiple binding partners have been described, these have been identified through yeast-two hybrid screens or protein-over expression systems using non-stoichiometrical protein ratios. Moreover, there has been controversy about the specificity of DISC1 commercial antibodies, therefore making it difficult to validate and interpret the context of DISC1 interactomes. Here, we show human *in-vivo* protein-protein interactions of endogenous DISC1 in human neural progenitor cells for the first time. We introduced a 3X-FLAG tag coding sequence at the C-termini of the endogenous DISC1 coding sequence using the CRISPR-Cas9 genome engineering system in human pluripotent stem cells. Following differentiation to neural progenitors, we isolated DISC1 protein interactions via immunoprecipation followed by tandem mass spectrometry, enabling the identification of the endogenous human DISC1-interactome. We then expanded the DISC1 interactome by immunoisolation of DISC1 protein interactors including AKAP9, PDE4DIP, and TNIK and determined their respective interactomes. This expanded network allowed for the mapping of cellular localization and the clustering of psychiatric disease risk candidates in common signaling pathways.

Disclosures: B.J. Wilkinson: None. **M.P. Coba:** None.

Poster

093. Genomics, Proteomics, and Systems Biology

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Program#/Poster#: 93.15/KKK43

Topic: I.02. Systems Biology and Bioinformatics

Support: NIH Grant 5U01MH105985

Title: Single neuronal methylomes reveal epigenomic diversity in the mammalian brain.

Authors: *C. LUO[1], E. A. MUKAMEL[2], R. CASTANON[4], J. LUCERO[4], J. R. NERY[4], C. L. KEOWN[3], Y. HE[4], L. KURIHARA[5], C. SCHUMACHER[6], T. HARKINS[5], M. BEHRENS[4], J. R. ECKER[4];
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Abstract: Epigenomic marks such as cytosine DNA methylation (mC) have highly diverse patterns across brain neuronal and non-neuronal cell types. Human and mouse brains accumulate high levels of non-CG methylation (mCH) at locations throughout the genome that are inversely correlated with gene expression. In addition, more than 200,000 regions showing differential CG methylation were identified between three cortical excitatory and inhibitory neuron types. Approximately 16% of the mouse genome contains differential mC signatures that allows neuronal cell types to be distinguished by low coverage single cell WGBS (whole-genome bisulfite sequencing). We are developing single cell methylome techniques for the classification of mammalian brain cells types using mC patterns, and to explore the epigenomic heterogeneity of neurons with single-cell resolution. To meet the need of large-scale single cell methylome profiling of thousands of cells, we developed a new method for the preparation of single cell methylome libraries with improved complexity using the Adaptase™ technology. Single cell methylomes are generated from single neuronal nuclei isolated from mouse frontal cortex using FACS coupled to a mouse INTACT (isolation of nuclei tagged in specific cell types) strategy. We demonstrated robust neuron type classification using single cell methylomes, readily separating excitatory and inhibitory populations and also identifying distinct inhibitory cells that are similar to PV+ or VIP+ inhibitory cells. The data further allowed an accurate classification of pyramidal neurons in superficial versus inner layers of mouse frontal cortex. The single cell methylome method will enable unbiased characterizations of brain epigenomic diversities without the need of isolating specific cell populations.

Disclosures: C. Luo: None. **E.A. Mukamel:** None. **R. Castanon:** None. **J. Lucero:** None. **J.R. Nery:** None. **C.L. Keown:** None. **Y. He:** None. **L. Kurihara:** None. **C. Schumacher:** None. **T. Harkins:** None. **M. Behrens:** None. **J.R. Ecker:** None.

Poster

094. Light Microscopy: Advances in Technologies, Software, and Analysis

Location: Halls B-H

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Topic: I.03. Anatomical Methods

Support: FWF Grant P 23102-N22

Title: Fast recording of whole mouse brains with aspheric light sheet microscopy

Authors: *H.-U. DODT[1,2], S. SAGHAFI[1], C. HAHN[1,2], K. BECKER[1,2], M. PENDE[1,2], I. SABDYUSHEVA-LITSCHAUER[2], M. WANIS[1];
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Abstract: Visualization of complete neuronal networks in the brain is an important goal of neuroscience. To this end chemical clearing and recording of mouse brains with light sheet microscopy has become increasingly popular in recent years. Several microscopic setups have been developed for light sheet microscopy but often it is difficult to view preparations like whole mouse brains with different magnifications and to exchange easily objectives.
We developed therefore a setup where microscope objectives are dipped from above in the clearing solution and can be exchanged in less than a minute. As no low magnification objectives for clearing solutions are available on the market we developed correction devices for existing air objectives (2X, 4X, 20X). These devices correct for spherical aberration and allow dipping of objectives into all clearing solutions described in the literature.
Gaussian light sheets for large fields of view have to be rather thick throughout their extension. If made thin in the center they deviate strongly in axial direction towards the edges of the field of view. We thus developed a completely new light sheet generator using aspherical optical elements. It generates a very thin long light sheet with a vastly extended field of view. This light sheet generator allowed us to image whole mouse brains using a single stack of images with a corrected 2X objective and additional demagnification showing clearly neuronal dendrites and axons. For imaging with higher resolution we could easily switch to higher power objectives in our setup. As clearing we used sDISCO, a stabilized version of our 3DISCO clearing, which allows the storage and imaging of GFP labelled preparations for long times. Apart from brains we also imaged adult GFP labelled drosophilae and cm large pieces of human cancers in autofluorescence.
We believe that the development of a light sheet generator for very long thin light sheets is a crucial step towards the imaging of very large samples with microscopic resolution.
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Ertürk A, Becker K, Jährling N, Mauch CP, Hojer CD, Egen JG, Hellal F, Bradke F, Sheng M, Dodt HU (2012) Three-dimensional imaging of solvent-cleared organs using 3DISCO, *Nat. Prot.* **7**: 1993-95
Saghafi S, Becker K, Hahn C, Dodt HU (2014) 3D-ultramicroscopy utilizing aspheric optics, *J. Biophoton.* **7**: 117-25

Disclosures: **H. Dodt:** None. **S. Saghafi:** None. **C. Hahn:** None. **K. Becker:** None. **M. Pende:** None. **I. Sabdyusheva-Litschauer:** None. **M. Wanis:** None.

Poster

094. Light Microscopy: Advances in Technologies, Software, and Analysis

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 94.02/KKK45

Topic: I.03. Anatomical Methods

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EC Grant 284464 (LASERLAB-EUROPE)

Italian Ministry for Education, University and Research, Flagship Project NanoMAX

Private Foundation "Ente Cassa di Risparmio di Firenze"

Title: Adaptive correction of defocus in light sheet microscopy of cleared mouse brains

Authors: *L. SILVESTRI[1,2], M. MÜLLENBROICH[1], A. DI GIOVANNA[1], L. SACCONI[1,2], F. PAVONE[1,3,2];
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Abstract: Light sheet microscopy, coupled with chemical clearing of the sample, is raising as a major tool to reconstruct brain anatomy across large areas - even entire mouse brains - with micron-scale resolution. Indeed, the selective illumination of the sample in the focal plane of the detection objective allows obtaining three-dimensional resolution with a wide-field detection scheme. On the one hand, the uncoupling between illumination and detection speeds up image acquisition by several orders of magnitude with respect to point-scanning techniques like confocal or two-photon microscopy. On the other hand, separation of illumination and detection optical paths renders the system sensitive to low-order optical aberrations, like defocus, which are self-corrected in confocal microscopy. Thus, albeit in theory light sheet microscopy can achieve high resolution imaging in whole cleared mouse brains, in practice specimen-induced defocus degrades image quality in different regions of the sample.
Here, we describe an optical method to measure defocus in a light sheet microscope in real time, allowing fast defocus correction via closed-loop motion of detection objective. With the proposed autofocus system image contrast and resolution are kept high across the entire sample, allowing reliable tracing of small structures, like small capillaries.

Disclosures: **L. Silvestri:** None. **M. Müllenbroich:** None. **A. Di Giovanna:** None. **L. Sacconi:** None. **F. Pavone:** None.

Poster

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Italian Ministry for Education, University and Research, Flagship Project NanoMAX

Private Foundation "Ente Cassa di Risparmio di Firenze"

Title: Integrated dual approach for 3D reconstruction of myelinated fibers orientation: combination of polarized light imaging and two-photon fluorescence microscopy

Authors: I. COSTANTINI[1], L. SILVESTRI[1], M. AXER[2], M. MENZEL[2], D. GRÄßEL[2], K. AMUNTS[2,3], *F. S. PAVONE[4,5,6];
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Abstract: Connectomics aims at defining the interactions existing among brain regions through the reconstruction of the neuronal fiber network. 3D-Polarized light imaging (3D-PLI) allows to assess fiber orientation and inclination angles in histological brain sections thanks to the birefringent properties of the myelin sheaths. This optical method enables a fast analysis of fixed mouse and human brains without any exogenous labeling. A single 3D (fiber) orientation vector is obtained for each voxel and reflects the net effect of all comprised fibers. In this work, we employ an integrated dual approach that combines 3D-PLI with two-photon fluorescence microscopy (TPFM) to study the mixture of various fiber orientations within the sample (and voxel) of interest. We exploit the higher axial and radial resolution of TPFM optical sectioning in combination with myelin autofluorescence to perform the 3D reconstruction of fiber orientation within each brain section. We demonstrate that the correlation between the two methods permits to reconstruct areas of the brain with the possibility of characterizing a specific sector of the sample at high resolution, below micrometer level. Our study shows that the integration of different techniques allows an improved analysis of brain connectomics, providing a novel tool for an implemented 3D reconstruction of nerve fiber orientations in fixed samples.

Disclosures: I. Costantini: None. **L. Silvestri:** None. **M. Axer:** None. **M. Menzel:** None. **D. Gräßel:** None. **K. Amunts:** None. **F.S. Pavone:** None.

Poster

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Topic: I.03. Anatomical Methods

Support: Allen Institute for Brain Science

Title: 3D reconstruction of mouse white matter tracts in a Common Coordinate Framework with a combined approach

Authors: *S.-L. DING, J. ROYALL, P. LESNAR, Y. LI, B. FACER, Q. WANG, N. DEE, A. BERNARD, J. PHILLIPS, C. KOCH, S. SUNKIN, H. ZENG, J. A. HARRIS, L. NG;
Data analysis and Annotation, Allen Inst. For Brain Sci., Seattle, WA

Abstract: 3-D reconstruction of white matter (WM) tracts allows more accurate targeting of individual fiber tracts for lesion, stimulation and fiber tracing studies. We aimed to reconstruct about 90 WM tracts in the mouse brain, including some left undefined in commonly used brain atlases using our average 3D template derived from serial two-photon tomography of 1675 mice brains. The delineation of the WM tracts was made with a 3D drawing tool, ITK-SNAP, on the basis of inherent contrast features in the anatomical common template in combination with myelin basic protein, neurofilaments (SMI-32 and NF-160), parvalbumin (PV), and calbindin reference stains. Although WM tracts generally exhibited lower signal intensity (darker) than gray matter structures (brighter) in the anatomical template, these features were not necessarily homogenous between different WM tracts or along individual WM tracts themselves. In the case of isolated and solid WM bundles such as the anterior commissure, fornix, fasciculus retroflexus, and mammilothalamic tract, contours and trajectories were easily defined without the need of additional data. In most other cases, however, WM tracts adjoined, merged (mix) or intersected other bundles and/or portions of gray matter structures at particular locations, leading to complex signal intensities along their paths, thus necessitating the correlation of template signal intensity with reference data for accurate delineation of boundaries and trajectories. For example, the medial lemniscus travels through the medulla, pons, and midbrain on its way to the thalamus, exhibiting significant changes in shape, size, location, topography, and signal intensity throughout. The trajectory and contour of the medial lemniscus was confirmed by analysis of sequential PV-stained sections, which revealed strong staining and a distinct fiber orientation pattern. In certain cases, other stains were used, as were tracer experiments from the Allen Mouse Brain Connectivity Atlas. This data set was especially useful when an anterograde tracer (rAAV) was injected in a desired anatomic structure restrictively (e.g. red nucleus). In this scenario, trajectories of a fiber bundle (e.g. rubrospinal tract originated from the red nucleus) were confidently traced with modest adjustments according to contours exhibited in other

reference data. Finally, the lateral, third, and fourth ventricles as well as the cerebral aqueduct and central canal were also delineated based on low signal intensity (dark), with the exception of the regions occupied by the choroid plexus, which display higher signal intensity (less dark) and were included in the corresponding ventricles.

Disclosures: **S. Ding:** None. **J. Royall:** None. **P. Lesnar:** None. **Y. Li:** None. **B. Facer:** None. **Q. Wang:** None. **N. Dee:** None. **A. Bernard:** None. **J. Phillips:** None. **C. Koch:** None. **S. Sunkin:** None. **H. Zeng:** None. **J.A. Harris:** None. **L. Ng:** None.

Poster

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Program#/Poster#: 94.05/KKK48

Topic: I.03. Anatomical Methods

Title: Ribbon imaging: high-speed scanning of specialized 3d rois fitted to neuronal structures using an electrical tunable lens

Authors: *M. S. SMIRNOV, L. YAN, R. YASUDA;
Max Planck Florida Inst., Jupiter, FL

Abstract: During high-resolution imaging of subcellular live neuronal activity, rapid image acquisition is often critical to capture the structural and biochemical changes which occur in sub-second timescales. However, the process of raster scanning is poorly adapted to the complex morphological structure of neuronal processes, resulting in a large amount of scanned 'dead space'. We introduce Ribbon Imaging: a low-cost method to selectively scan only the labeled regions in fluorescent biological tissue during live, two-photon excitation without relying on a motorized stage. By manually selecting custom, non-uniform regions of interest, or ribbons, the galvanometer scanning path is reshaped, significantly reducing acquisition time by ignoring non-fluorescent dead space. With the use of an electrically tunable lens in the excitation path, we are able to further increase our scanning efficiency by rapidly and precisely altering focal distance during the acquisition of each ribbon, allowing us to image structures spanning multiple Z planes in a single frame. The ability to quickly image *in vivo* along the Z axis allows for high time-resolution experiments which would otherwise be prohibitively time-consuming due to the vertical orientation of cortical neurons. By combining ribbon imaging with FRET-FLIM, the lifetimes of molecular sensors can be imaged for the first time in 3D over long distances and without the need to collect Z stacks. I will present data showing rapid changes in subcellular molecular activity measured using FRET-FLIM spanning along curved dendrites in hippocampal slices. Furthermore, I will show structural changes in dendritic spines imaged along neurons *in*

vivo at speeds over 20x faster than allowed by standard two-photon microscopy. All hardware and pixel mapping is controlled through a user-friendly interface and accessible MATLAB code built into Scanimage.

Disclosures: **M.S. Smirnov:** None. **L. Yan:** None. **R. Yasuda:** None.

Poster

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Topic: I.03. Anatomical Methods

Support: NSF EBICS 0939511

Title: Helmholtz phase tomography for label-free imaging of neuronal intracellular transport

Authors: M. E. KANDEL[1], H. SHAKIR[2], C. A. BEST[2], *G. POPESCU[3];
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Abstract: Neuron growth and development is guided by the transport of intracellular cargo, providing a quantifiable measurement of growth and development. It is nevertheless cumbersome to simultaneously assay the intracellular traffic along with the complete 3D geometry of the extension. Where confocal imaging systems are used, strong optical sectioning is achieved at the expense of photobleaching and phototoxicity. Recent improvements in light sheet microscopy have provided decreased exposure compared to point scanning methods, but such systems are expensive and still suffer from problems associated with fluorescent tags. Often the assay of intracellular traffic is not performed simultaneously with the structure of the neuron, and instead intracellular traffic is measured using a widefield fluoresce technique, while the structure is acquired using a confocal system after fixation.
In order to overcome these obstacles, we show that conventional widefield microscopes can be augmented with phase sensitive modules to provide sub-nanometer sensitivity. The resulting phase map is a measurement of the intrinsic structural information which enables the direct application of the physical equations governing mass transport. In this work we propose and validate a new method for tomographic reconstruction that relies on inverting the 3-dimensional deterministic signal associated with the object's structural informational, namely we directly invert the Helmholtz equation. Crucially, owing to the sectioning capabilities of Spatial Light Interference Microscopy (Wang et al, Opt Express, 2011), we find that our approach does not require the use of a point-spread function, avoiding numerical and physical artifacts typical of

nonlinear structures.
To validate our new found approach, we acquire time-lapse tomograms and apply our method to calculate surfaces areas over which we track mass flow rates, yielding a flux through the neuron's processes. We believe this new analysis scheme is widely applicable to quantifying neuron transport and is particularly well suited to imaging fine structures such as synapses.

Disclosures: **M.E. Kandel:** None. **H. Shakir:** None. **C.A. Best:** None. **G. Popescu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Phi Optics.

Poster

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Topic: I.03. Anatomical Methods

Support: NIH Grant MH085802

NIH Grant NS090473

Simon Center for the Social Brain/SFARI

Picower Institute for Engineering Colloboration

Title: Third harmonic generation imaging of intact human cerebral organoids to assess key components of early neurogenesis in Rett Syndrome

Authors: ***M. YILDIRIM**[1,2], D. FELDMAN[1], T. WANG[4], D. OUZOUNOV[4], S. CHOU[1], J. M. SWANEY[1], K. CHUNG[1], C. XU[4], P. SO[2], M. SUR[1,3];
[1]Picower Inst. for Learning and Memory, [2]Dept. of Biol. Engin., [3]Simon Ctr. for Social Brain, MIT, Cambridge, MA; [4]Sch. of Applied and Engin. Physics, Cornell Univ., Ithaca, NY

Abstract: Rett Syndrome (RTT) is a pervasive, X-linked neurodevelopmental disorder that predominantly affects girls. It is mostly caused by a sporadic mutation in the gene encoding methyl CpG-binding protein 2 (MeCP2).The clinical features of RTT are most commonly reported to emerge between the ages of 6-18 months and implicating RTT as a disorder of postnatal development. However, a variety of recent evidence from our lab and others demonstrates that RTT phenotypes are present at the earliest stages of brain development including neurogenesis, migration, and patterning in addition to stages of synaptic and circuit development and plasticity. We have used RTT patient-derived induced pluripotent stem cells to

generate 3D human cerebral organoids that can serve as a model for human neurogenesis *in vitro*. We aim to expand on our existing findings in order to determine aberrancies at individual stages of neurogenesis by performing structural and immunocytochemical staining in isogenic control and MeCP2-deficient organoids. In addition, we aim to use Third Harmonic Generation (THG) microscopy as a label-free, nondestructive 3D tissue visualization method in order to gain a complete understanding of the structural complexity that underlies human neurogenesis.
As a proof of concept, we have performed THG imaging in healthy intact human cerebral organoids cleared with SWITCH. We acquired an intrinsic THG signal with the following laser configurations: 400 kHz repetition rate, 65 fs pulse width laser at 1350 nm wavelength. In these THG images, nuclei are clearly delineated and cross sections demonstrate the depth penetration capacity (> 1mm) that extends throughout the organoid. Imaging control and MeCP2-deficient human cerebral organoids in 2D sections reveals structural and protein expression-based alterations that we expect will be clearly elucidated via both THG and three-photon fluorescence microscopy.

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Poster

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Topic: I.03. Anatomical Methods

Support: BBSRC Grant No. BB/M020231/1

Title: Algorithmic optimization of DIC microscopy images for electrophysiology and neuronal-tissue reconstruction

Authors: ***B. COLLYER**[1], M. G. THOMAS[2], M. J. WALL[2], P. BANIUKIEWICZ[1], T. BRETSCHNEIDER[1], A. SHMYGOL[3], M. J. E. RICHARDSON[1];
[1]Warwick Systems Biol. Ctr., [2]Sch. of Life Sci., [3]Warwick Med. Sch., Univ. of Warwick, Coventry, United Kingdom

Abstract: Differential interference contrast (DIC) microscopy is a standard technique for imaging transparent biological specimens that are problematic to observe under traditional bright-field illumination. DIC microscopes use the interference of coherent parallel light-beams with slightly differing optical paths to form sharply defined images of the specimen that show an artificial light-shadow effect. Though this shadowing facilitates visual identification of tissue

components, it complicates tissue reconstruction as it is phase and not intensity related. During electrophysiological experiments DIC microscopy video is used to find and identify cells; however, the continuous feed of images is rarely retained or exploited algorithmically to aid the experimentalist in cell identification or reconstruction of the local tissue environment. Here we present preliminary results from a study aimed at extracting useful experimental information from this under-utilized resource. We automated the acquisition of large data sets comprised of multiple DIC images at dense submicrometre intervals in the xyz-planes from neocortex, hippocampus and uterine endometrium. Using deconvolution methods and phase-to-intensity conversion algorithms, the extent to which these data can be used to generate 3D models of tissue was investigated.

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Poster

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Topic: I.03. Anatomical Methods

Support: HumanBrainProject Grant 604102

LASERLAB-EUROPE Grant 284464

Title: High-throughput whole mouse brain vasculature imaging with micrometric resolution using light sheet microscopy

Authors: ***A. P. DI GIOVANNA**[1], A. TIBO[2], L. SILVESTRI[1,4], M. C. MÜLLENBROICH[1], L. SACCONI[1,4], P. FRASCONI[2], F. S. PAVONE[1,4,3];
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Abstract: Cerebral blood vessels are charged with the fundamental task of insuring and regulating blood supply depending on neuronal demand, which is known as “neurovascular coupling”. However, in spite of its essential role, we still miss a complete map of brain vasculature network on a brain wide scale, which it is referred to as “angiome”. Moreover, mainstream functional imaging methods like BOLD-fMRI rely on the level of blood oxygenation for indirect measure of neuronal metabolism. A greater understanding of vascular organization

would then also provide a better interpretation of these methodologies. Methods based on micro-optical sectioning tomography (MOST) have shown the possibility to label and image the entire mouse brain vascular system with high resolution and contrast. On the other hand, these approaches still provide a moderate throughput (about 1 week to image a single sample) and use a staining protocol designed for transmission imaging rather than fluorescence, preventing simultaneous imaging of blood vessels and fluorescently-labeled neurons. We describe an approach enabling high throughput *ex vivo* whole mouse brain vasculature imaging using light sheet microscopy in combination with CLARITY technique and a specialized blood vessels labeling. The procedure preserve endogenous fluorescence, allowing for simultaneous imaging of vessels and neurons in transgenic animals. Contrary to serial sectioning techniques, the samples is preserved during imaging acquisition, allowing for successive analysis. Noteworthy, the fast whole brain image acquisition of the method proposed foster a proper characterization of morphological variability both in physiological and pathological conditions. We speculate that the high-contrast provided by our staining and imaging protocol will also be beneficial for the development of segmentation algorithms, which should be fully automatic and capable of dealing with datasets exceeding one TeraByte.

Disclosures: **A.P. Di Giovanna:** None. **A. Tibo:** None. **L. Silvestri:** None. **M.C. Müllenbroich:** None. **L. Sacconi:** None. **P. Frasconi:** None. **F.S. Pavone:** None.

Poster

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NIH NS25304

Brightfocus Foundation

NSF MRI 1337573

NIH HL68702

Title: C-DSLM: cleared tissue digital scanned light-sheet microscopy

Authors: ***D. SHEPHERD**[1,2], D. RYAN[6], E. GOULD[3], J. SINGH[2], G. SEEDORF[2], P. PARSA[4], O. MASHIZADEH[5], S. ABMAN[2], S. VIJAYARAGHAVAN[4], W. MACKLIN[3], D. RESTREPO[3];
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Abstract: Optical tissue clearing has revolutionized researchers' ability to perform fluorescent measurement of individual cells, protein patterning, and structural features within intact tissue samples and organs. However, one complication shared by all optical clearing methods is a spatially inconsistent refractive index, leading to light scattering and focal plane shifts that limit the application of high-resolution fluorescence microscopy to ideal samples, specialized microscope designs, or limited imaging depths. To overcome these technical limitations and lack of flexibility in current light-sheet fluorescence microscope designs, we implemented C-DSLM (cleared tissue digital scanned light-sheet microscopy), a method that utilizes electrotunable lenses (ETL) inserted in both the excitation and detection arms to generate volumetric images without mechanical manipulation of the sample while simultaneously correcting for shifts in focal plane due to spatial heterogeneity of refractive index. C-DSLM has sub-micron in-plane and micron axial resolution, compensates for inhomogeneous refractive index, reduces background using HiLo background subtraction, allows imaging deep within large samples without sectioning, eliminates mechanical drift as well as physical distortion for small deformable samples, and is compatible with any clearing protocol. These advancements enable high-resolution fluorescent imaging without complicated refractive index matching, custom microscope objectives, computationally expensive deconvolution, or destructive sample sectioning. In addition, we have developed a computational analysis pipeline to reconstruct and filter volumetric images to aid researchers in identification of key features within these extremely large imaging datasets. We demonstrate the flexibility and power of our methodology by quantifying features in tissue from multiple animal models, including two unique measurements: 1) quantification of individual oligodendrocyte cells and myelin networks within intact optically cleared mouse brain and spinal cords; and 2) quantification of changes in the vascular and neuronal networks due to experimental retinopathy of prematurity within intact optically clear albino rat eyes.

Disclosures: **D. Shepherd:** None. **D. Ryan:** None. **E. Gould:** None. **J. Singh:** None. **G. Seedorf:** None. **P. Parsa:** None. **O. Mashizadeh:** None. **S. Abman:** None. **S. Vijayaraghavan:** None. **W. Macklin:** None. **D. Restrepo:** None.

Poster

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Dr. Gernot Pomrenke of the Department of Defense: Air Force Office of Scientific Research FA9550-12-1-0261

Title: An integrated photonic probe for light sheet microscopy

Authors: ***F. YE**[1], B. AVANTS[1], J. ROBINSON[1,2,3];
[1]ELECTRICAL AND COMPUTER ENGINEERING, Rice Univ., Houston, TX;
[2]Bioengineering, RICE UNIVERSITY, Houston, TX; [3]Neurosci., Baylor Col. of Med., Houston, TX

Abstract: The ability to record the neural activity from many individual cells in three dimensions in the living brain is a major technological challenge. Optical techniques that measure changes in calcium or voltage provide a promising route toward this goal, but delivering light to specific regions deep within the brain is limited by the scattering of neural tissue. By focusing light to a single illumination plane, researchers can perform high-speed volumetric imaging or optically stimulate cells within specific layers of tissue. Typically, selective plane illumination relies on macroscopic cylindrical lenses that are not compatible with integrated microdevices which can be implanted into the body or arrayed on a chip. To overcome this limitation, we use planar metallic lenses combined with integrated photonic devices to create a microscale probe capable of producing planar illumination. Our results show that a single metallic slit is an efficient nanophotonic element for light sheet illumination. Further, we have integrated the microlens onto the AlN resonant waveguide gratings that radiate vertically from a planar chip. To scan this plane without moving the probe, we implemented three gratings with resonant ring filters tuned to unique resonant wavelengths within the absorption band of GCaMP. We can thus illuminate individual planes at specific depths within the tissue by tuning the excitation wavelength. This combination of radiating photonic elements and planar metallic lenses enables us to achieve light sheet illumination that can be used for deep brain imaging with an integrated probe that does not require a large-footprint pulsed laser needed for multiphoton microscopy.

Disclosures: **F. Ye:** None. **B. Avants:** None. **J. Robinson:** None.

Poster

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NSF EAGER to GAA

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Title: Design and implementation of multi-signal, time-lapse digital reconstructions of neuronal morphology

Authors: *S. NANDA[1], H. CHEN[2], R. DAS[3], H. PENG[4], D. N. COX[3], G. A. ASCOLI[1]; [1]Krasnow Inst. For Advanced Studies, Fairfax, VA; [2]Dept. of Computer Sci. and Bioimaging Res. Ctr., The Univ. of Georgia, Cortical Architecture Imaging and Discovery Lab., Athens, GA; [3]Neurosci. Inst., Georgia State Univ., Atlanta, GA; [4]Allen Inst. for Brain Sci., Seattle, WA

Abstract: Mature neuronal arbors develop from growth processes regulated by complex molecular interactions. The convergence of extracellular, intracellular, and activity-dependent molecular events on the cytoskeletal effectors, primarily actin filaments and microtubules, facilitates neural development as well as maintenance of mature morphology. Several effective techniques to digitally reconstruct and analyze neuronal morphologies exist, but the quantification of their structural dynamics remains challenging. Current descriptions of neuron morphology are static and do not contain precise representations of intracellular components. Recent advances in tissue labeling and imaging techniques necessitate the co-evolution of the standard SWC format for representing digital arbor tracings. Generation of time-varying reconstructions is required, co-registering subcellular information with neuronal morphology. Additionally, large numbers of augmented reconstructions are required to develop data-driven mechanistic models of neuronal development and of structural plasticity.
Here we present the definition of a new multichannel file structure and a new Vaa3D plug-in to handle this new type of data. We also introduce a design to tag dynamic structural changes in a time-coded manner. Next, we illustrate ongoing progress in using the multichannel/time-lapse system on developing neurons in the *Drosophila* larva. Time-varying images of overall neuronal morphology along with fluorescently labeled subcellular cytoskeletal components are digitally traced into the aforementioned file structures. These new reconstructions enable complete statistical analysis of the structural changes and the underlying molecular processes. Lastly, we will demonstrate how stochastic computational simulations of neuronal growth, statistically constrained by and validated against these novel reconstructions, can help select the most

experimentally promising genetic alterations to gain additional biological insight.
The designed data structure and research approach are also broadly applicable to other types of multichannel/time-lapse neuronal imaging, such as quantification of voltage changes or tracking the arbor-wide movement of any subcellular component of interest.

Disclosures: S. Nanda: None. **H. Chen:** None. **R. Das:** None. **H. Peng:** None. **D.N. Cox:** None. **G.A. Ascoli:** None.

Poster

094. Light Microscopy: Advances in Technologies, Software, and Analysis

Location: Halls B-H

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Topic: I.03. Anatomical Methods

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Wellcome Trust 090946/Z/09/Z

Title: Automated 3D analysis of confocal microscopy images by 3D-ACE reveals widespread molecular and cellular adaptions in the spinal nociceptive circuitry of monoacylglycerol lipase knockout mice

Authors: *C. I. PONGOR[1], S. G. WOODHAMS[1], B. BENJAMIN[1], B. DUDOK[2], L. BARNA[2], M. KANO[3], K. SAKIMURA[4], M. WATANABE[5], I. KATONA[2];
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Abstract: Immunofluorescence staining and confocal microscopy are widely used to study specific molecular alterations underlying physiological or pathophysiological processes. However, changes in fluorescence intensity levels can either directly reflect up-or downregulation of the target protein or be indirectly caused by the appearance or disappearance of subcellular profiles harbouring the target protein. Due to the limited resolution and sensitivity of confocal microscopy, reliably distinguishing between these two possibilities in a high yield manner is often difficult and laborious. To circumvent this obstacle, we developed 3D-ACE; an open-source, easy-to-use analysis tool with a graphical user interface.
The 3D-ACE plug-in for Fiji enables unbiased, object-based 3D analysis of co-expression of

target proteins on large batches of confocal microscopy images, thereby facilitating automatized analysis of large sample sizes. We chose to write 3D-ACE in Python, a language widely in the scientific community, and utilized the plugin "3D Objects counter" for the image processing platform Fiji and ImageJ. In short, the plug-in first segments the images, then finds objects in the two channels and determines which objects overlap. A database of properties of objects, such as their volume, intensity, and overlapping ratio is created. Quantitative data can be attained by setting filters for this database.
As a proof of principle, we applied 3D-ACE to reveal the molecular and cellular adaptations following deletion of monoacylglycerol lipase (MGL), the predominant degrading enzyme of the endocannabinoid messenger 2-AG. Unexpectedly, our data uncovered a remarkable cell-type-specific reorganization of the local excitatory, but not inhibitory, circuits in the superficial laminae of the spinal dorsal horn. These extensive molecular and cellular adaptation mechanisms may explain the lack of the expected analgesic phenotype in MGL knockout mice, and therefore the failure of chronic complete blockade of MGL, a theoretically promising treatment for chronic pain via elevation of 2-AG signalling at CB_1 receptors. . Finally, to test the validity of the workflow, results from the automated analysis were compared to a manual method for two of the sample sets. Importantly, both methods yielded similar results, but the automated analysis was far more efficient. 3D-ACE analyzed an order of magnitude more structures in a fraction of the time (200x faster), producing stronger statistical significance and removing selection bias when compared to the manual analysis.

Disclosures: **C.I. Pongor:** None. **S.G. Woodhams:** None. **B. Benjamin:** None. **B. Dudok:** None. **L. Barna:** None. **M. Kano:** None. **K. Sakimura:** None. **M. Watanabe:** None. **I. Katona:** None.

Poster

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Support: Wellcome Trust: 090946/Z/09/Z

Momentum II. Program of the Hungarian Academy of Sciences: LP2013-54/2015

National Institutes of Heath: NS089575

Title: VividSTORM: a novel open-source software for super-resolution and confocal microscopy images

Authors: *V. MICZÁN[1,2], L. BARNA[1], B. DUDOK[1,3], A. HORVÁTH[2], J. R. GLAVINICS[1,2], Z. I. LÁSZLÓ[1,3], I. KATONA[1];
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Abstract: Super-resolution light microscopy techniques can visualize the nanoscale molecular composition of brain circuits with similar localization precision of target proteins such as immunogold electron microscopy. Moreover, the possibility to simultaneously visualize multiple proteins, together with the faster tissue processing protocols and the higher immunolabeling density represents potential major advantages over electron microscopy in several neuroscience applications. Indeed, we have recently shown that combining Stochastic Optical Reconstruction Microscopy (STORM) with confocal microscopy allows rapid and efficient nanoscale molecular imaging within morphologically and neurochemically defined cellular and subcellular context in intact brain circuits (Dudok et al., 2015, Nature Neuroscience, 18:75-86). To further facilitate the correlated visualization and data analysis of super-resolution and confocal images, we have developed the open-source software VividSTORM, and recently implemented multiple new features not yet available in any other microscopy software packages. VividSTORM offers the simultaneous, correlated visualization and analysis of both pixel-intensity-based (such as widefield, TIRF, confocal, STED or SIM) and coordinate-based (such as STORM or PALM) microscopy data. By using the graphical user interface of VividSTORM, the key steps of the correlated analysis of the two different imaging modalities are: i) to overlay the pixelated image and the single-molecule localization coordinates; ii) to outline the labelled target cell or subcellular structure on the conventional microscopy image as region-of-interest (ROI); iii) to filter those specific single molecule localization points which belong to the identified target profile; iv) to perform coordinate-based analysis of the super-resolution imaging data. We implemented several novel tools in VividSTORM in order to facilitate this process including: manual and automatic, fiducial-marker-based or image-based alignment methods; an unbiased ROI selection feature by automated 2D or 3D segmentation of the pixelated image using a modified Morphological Active Contours Without Edges (MACWE) algorithm; and the analysis of molecular clustering by using multi-scale Bayesian or DBSCAN methods. The batch analysis of pre-selected ROIs is also supported to further facilitate high-throughput correlated confocal and super-resolution data analysis and functional interpretation of quantitative molecular observations within identified cellular and subcellular structures.

Disclosures: **V. Miczán:** None. **L. Barna:** None. **B. Dudok:** None. **A. Horváth:** None. **J.R. Glavinics:** None. **Z.I. László:** None. **I. Katona:** None.

Poster

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Topic: I.03. Anatomical Methods

Support: NIH Grant U01MH105971

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Title: HeadLight: plug & play tool for visualization andanatomical analysis of whole brain datasets

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Abstract: Today whole-brain imaging in multiple modalities enables neuroscientists to acquire brain datasets of different animal models at various developmental stages. Here we present a toolchain called “HeadLight” (a cross-platform GUI application built using CMake, C++, ITK, VTK and matlab) that can be used to process and analyze large image-based datasets. Using this plug & play tool, the neuroscience community can register whole-brain datasets to reference brain volumes linked to available atlases, as well as build their own atlases for different types organisms based on data acquired by different imaging modalities. As an example, we demonstrate the use of Headlight for co-registration of two mouse brain atlases, one generated by magnetic resonance imaging (MRI) and the second created by serial two photon (STP) tomography that is linked to the Allen mouse brain atlas. We also use the reference STP (RSTP) framework to register brains imaged by light-sheet fluorescent microscopy (LSFM), demonstrating a cross imaging-platform versatility. The datasets registered in Headlight can be browsed alongside a view of hierarchical anatomical structures. In addition, a built-in cell-counting module, based on Graphical Processing units (GPU), allows for fast detection of fluorescently labeled cells. The cell count-based data can be used to generate unbiased cell density maps across the entire mouse brain, as well as to create 2D “flat” maps of cortical cell distribution in different layers. Headlight thus provides a versatile whole-brain registration and viewing platform that can be easily used by many neuroscience laboratories.

Disclosures: K.U. Venkataraju: None. **P. Osten:** None.

Poster

094. Light Microscopy: Advances in Technologies, Software, and Analysis

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Topic: I.03. Anatomical Methods

Title: Smartscope 2: automated imaging for morphological reconstruction of fluorescently-labeled neurons

Authors: *B. R. LONG[1], Z. ZHOU[2], X. LIU[2], J. TING[2], E. LEIN[2], M. HAWRYLYCZ[2], H. PENG[2];
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Abstract: Fast and accurate quantification of morphology is a critical challenge in determining neuronal cell types in the brain. To address this challenge, we introduce SmartScope2, the first open source, automated neuron reconstruction machine that integrates automated bioimage analysis and rapid multiphoton imaging. The system integrates a commercially-available microscope with open-source software control for rapid 3D visualization and analysis. We show that SmartScope2 can automatically image and digitally reconstruct neuronal morphology with reduced imaging, storage and analysis loads by leveraging on-line data analysis and sparse neuronal geometry.

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Poster

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Topic: I.03. Anatomical Methods

Title: The structural alteration of medial prefrontal cortex of a genetic rat model of depression subjected to maternal separation

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Abstract: Background: Superimposed effect of early life adversity, namely maternal separation is known as one of the possible risk factors for the development of the neuropsychiatric disorders. Quantitative investigation of the structure of cerebral cortex in preclinical studies could help to increase our knowledge about the neurostructural underpinnings of the early life stress. Therefore, in the present study, we aimed to investigate the superimposed effect of maternal separation as an environmental factor in a genetic rat model of depression (Flinders Line rats) on the neurostructural components of medial prefrontal cortex (mPFC).
Methods: The degree of neuronal minicolumnarity was tested by a newly developed cylinder *K*-function, in layer III of medial prefrontal cortex of rats. The acquisition of 3D coordinates of neurons was performed by optical disector in a well-defined region using optical microscopy. Alteration in shape, orientation and volume of neurons in layer III of mPFC between three groups of rat models: Flinders Sensitive Line (FSL) as depressed rats, FSL with maternal separation (FSL-MS) and Flinders Resistant Line (FRL) rats as a control group were tested by a newly developed estimator of volume tensors.
Result: Findings of this study related to the degree of neuronal columnarity in three animal groups demonstrated a subtle anisotropy in parallel to mPFC layers in the FSL-MS but not significant difference. Volume tensor estimation showed no significant alteration from sphere shape in neurons obtained from the FSL-MS ($p>0.05$) while the obtained results of other groups indicated a prolate shape ($p<0.05$).
Conclusion: Our results suggest that the interaction between the environmental factor (maternal separation as an early life adversity) and genetic factor (genetic animal model of depression) is reflected in a quantifiable structural alteration of mPFC including neuronal shape factor.

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Poster

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Topic: I.03. Anatomical Methods

Title: A high volume throughput of 3D morphometrics of the CNS

Authors: *M. C. WU[1], J. HO[2], D. D. QUACH[1];
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Abstract: 3D visualization is essential to disclose both cytoarchitecture and the morphology of the CNS. With the advanced microscopy, the immunofluorescence method can further examine biochemical property of individual neurons. Nevertheless, using this protocol is unable to achieve the goal of a high volume, high throughput imaging and morphometrics at multiple ROIs (Regions of Interest) of the brain, mainly due to signal degradation. Thus, this method is only able to characterizie morphological features of a small population of neurons within one or limited number of ROIs of the brain. We have developed a high-throughput protocol added to the current imaging platform that combines Golgi-Cox/IHC and Nikon Eclipse microscopy to produce 3D profiles of multiple neuron populations at multiple ROIs of the brain. In brief, a series of Golgi-stained sections (200 um thick) of the brain will be digitized using pre-set imaging parameters to register x, y, z coordinates of the ROIs, then screened through internal morphological features of the neuron, such as 1) apical or basal dendrites of pyramidal cells in the cortex or hippocampus, and 2) the dendrites of medium spiny neurons in the striatum, etc. With precisely registered 3D coordinates of sampled neurons of the entire brain, the rigorous high magnification digitization is proceeded under 100x (oil immersion) lens using the customized high-powered computing desktop system. Thus, we are able to digitize 5 to 7 neurons per day, which generates thousands of Gigabytes storage. This throughput will be accelerated by improved computing powered system. With the 3D automatic image analysis software, we are able to generate a large-scale 3D morphometric data bank eventually that can be complementary to current leading brain bank, i.e., Allen Brain Atlas, Human Connectome and Blue Brain projects.

Disclosures: **M.C. Wu:** None. **J. Ho:** None. **D.D. Quach:** None.

Poster

095. New Approaches for Neuromodulation

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Topic: I.04. Physiological Methods

Support: NIH/NINDS Grant R01NS079288

NIH/NINDS Grant R01NS072171

Title: Wireless control of cellular function by activation of EPG, a novel gene responsive to electromagnetic fields

Authors: *J. BANERJEE[1,2], S. PARK[1,2], M. SORRELL[1,2], J. PEVSNER[1], W. GUGGINO[3], A. GILAD[2], G. PELLED[1,2];
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Abstract: Major advances in molecular and synthetic biology have revolutionized the capability to control cell excitability in living organisms. Yet, the majority of the technologies that manipulate cellular function in a cell-spatio-temporal-specific manner demand the use of optics, drugs, radio-wave or ultrasound. Identification of genes responsible for controlling cellular function by non-invasive electromagnetic fields (EMF) is still in its infancy. In the present study we investigated the potential of an alternative and novel method to wirelessly control cellular function through the transmission of non-invasive EMF.
Various aquatic species use electromagnetic fields for orientation and navigation, but the cellular mechanism by which this is accomplished remains unknown. One of these species is the *Kryptopterus bicirrhis* (glass catfish). Using expression cloning in *Xenopus laevis* oocytes, we identified a single gene from *K. bicirrhis* that, once expressed in the oocytes, produces changes in the oocyte's membrane current when activated by EMF. We termed this gene the *electromagnetic perceptive gene (EPG).* Bioinformatic analysis of EPG showed that this previously uncharacterized gene encoded a putative membrane associated protein. The encoded protein possesses a highly conserved UPAR_LY6 domain that may interact with the beta subunits of voltage gated sodium channels. We expressed EPG in HEK293T mammalian cells and confirmed its expression in the membrane. To examine whether remote activation of EPG can modulate neuronal function, neuronal cultures were transduced using adeno-associated virus expressing EPG under the excitatory neuron specific promoter, Ca^{2+}/calmodulin-dependent protein kinase II (pAAV2-CaMKIIprom::-EPG-IRES-hrGFP). The cultured neurons were loaded with the calcium indicator Fura 2-AM. 10 s long EMF pulses (250 mT) induced an average of 21 ± 9% increase in intracellular calcium in EPG-expressing neurons (n=9; $p<0.0005$), but no responses in control or non-infected neurons. Thus, the data demonstrates that wireless activation of EPG in neuronal culture results in significant changes in neuronal activity. Furthermore, stereotaxic injections of AAV expressing EPG in the rat hippocampus have resulted in location specific EPG expression suggesting that EPG can be expressed in a specific cellular population and in a specific location in the rodent brain.
We anticipate that this discovery has the potential to transform the neuroscience field, by providing a tool that offers non-invasive, cellular-specific, temporal-specific and region-specific stimulation.

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Poster

095. New Approaches for Neuromodulation

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Topic: I.04. Physiological Methods

Support: NIH/NINDS Grant R01NS079288

NIH/NINDS Grant R01NS072171

Title: Neuromodulation of hippocampus function by EPG, a novel gene responsive to electromagnetic fields, decreases seizure activity in the kainic acid rat model of epilepsy

Authors: *B. E. THEISEN[1,2], G. BAR-KLEIN[3,4], H. DIETZ[3,4], A. FATEMI[1,2], A. GILAD[5], G. PELLED[1,5];
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Abstract: Epilepsy is a common chronic neurological disorder effecting 0.5-1% of the population. Evidence from patients and animal studies suggest that neuromodulation can reduce seizure occurrences. For example, studies have shown that deep brain stimulation can inhibit seizure development and optogenetics approaches have shown to modify seizure-onset in epilepsy. However, these invasive technologies could lead to unpredictable and undesirable side effects.
We have recently discovered and cloned a new gene from the *Kryptopterus bicirrhis* (glass catfish) that is sensitive to electromagnetic fields. We term this gene electromagnetic perceptive gene (EPG). Once expressed in cultured neurons, wireless induction of electromagnetic fields leads to increases in intracellular calcium concentrations. Furthermore, immunohistochemistry confirmed that the EPG can be expressed under cell-specific promoters in specific brain location. Here, we tested whether the EPG technology could be used as a neuromodulation approach in the kainic acid (KA) rat model of epilepsy.
Six adult rats were treated with KA (i.p.) to induce spontaneous recurrent seizures. Two to three months after KA administration, telemetric ECoG electrodes were implanted in the cortex allowing continuous recordings. Two weeks following electrode implantations, the baseline number of seizures was determined for each KA rat as the average number of seizures per day over a six days period. Then, bilateral stereotaxic injections of virus containing the EPG (pAAV2-CMVprom::-EPG-IRES-hrGFP) into the rats' hippocampus were performed. A viral vector containing only GFP reporter was used as control. Two weeks following stereotaxic viral injections, the average number of seizures was reevaluated. KA rats that have been injected with virus containing the EPG showed significant reduction in the number of seizures (17.12±4.3 initially to 9.12±3.8 seizures per day, 46.86±9.7% decrease; $p<0.05$; $n=4$). KA rats that were

injected with the virus containing only GFP (control) did not show significant reduction in the number of seizures (12.67±2.6 to 10.25±4.6, 15.96±4.8% decrease; n=2). Four weeks following initial viral injections, electromagnetic activation of the EPG in the KA rats was performed, which led to further decreases in seizure activity (3.6±1.23 seizures per day, 80.35±4.2% decrease).
This preliminary data suggests that wireless activation of EPG by external electromagnetic fields, or possibly, by electromagnetic changes induced by the seizure themselves, leads to suppression of seizures. Further investigation could lead to a new, non-invasive alternative to current clinical standards.

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Poster

095. New Approaches for Neuromodulation

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 95.03/KKK63

Topic: I.04. Physiological Methods

Title: Wireless ultrasonically-powered neurostimulators with bioimpedance measurement capacity

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Abstract: Despite the immense improvement in the quality of life achieved using the state-of-the-art neurostimulation devices, existing neurostimulators suffer from important limitations of size, battery life, and tissue damage due to lead placement and implantation. To address these limitations, we have been developing a platform for tetherless sub-millimeter size neuroelectric devices that rely on ultrasonic energy harvesting. Our present work focuses on passive neuroelectronic devices that in addition to neurostimulation, report tissue impedance to provide a sensitive indicator of blood flow for the conditions involving changes in tissue perfusion, such as a peripheral vascular disease. We demonstrate an improved microscale implantable impedance measuring system that employs the transit time nature of ultrasound to achieve much higher dynamic range and improved tissue impedance characterization. Experiments using tissue models allowed us to characterize device impedance measurements in the range of physiological tissue conductivities to an accuracy of 10%. Changes in tissue blood flow can translate to the changes of tissue impedance as high as hundred percent and thus this suggests a high sensitivity.

We discuss our results in terms of their potential to serve as a completely integrated feedback control system that would allow both neurostimulation control of a physiologic response such as vasodilation, and then immediately and locally monitor tissue impedance as an indicator of the achieved result and the efficacy of treatment. The ability to wirelessly stimulate and record its effect from the same tissue using scalable passive implantable devices will enable dynamic control over treatment protocols and paves a way towards adaptable, personalized neuroelectric medicine.

Disclosures: D. Celinskis: None. **B.C. Towe:** None.

Poster

095. New Approaches for Neuromodulation

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Topic: I.04. Physiological Methods

Support: NIH R24braincircuits R24-MH106107

Title: Studying the effects of focal 3mhz ultrasound on neural activity in transgenic mice

Authors: *T. SATO, D. WU, M. FLANNERY, M. SHAPIRO, D. TSAO;
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Abstract: Transcranial focused ultrasound (tFUS) is a highly promising tool for non-invasive neuromodulation. Studies have shown the potential for modulating and inducing sensations in the human somatosensory cortex (Legon et al. 2014, Lee et al. 2015). However, the mechanisms for how ultrasound affects neurons and what parameters are critical are unknown. Human studies have shown large variability within subjects, and it is very difficult to optimize parameters in humans. A refinement of our understanding is needed to apply ultrasonic neuromodulation in humans in a safe, systematic manner. Many of the studies so far *in vivo* and *ex vivo* in animal models have utilized electrophysiological means or coarse imaging methods (BOLD, PET) requiring intensive labor with invasive probes that could affect how ultrasonic energy interacts with neurons or offering poor spatial and temporal time scales. In transgenic mice with widespread cortical GCaMP6 expression, calcium imaging of neural activity provides a non-invasive means of studying large populations of neural activity with high temporal and spatial resolution. The long wavelength of low-frequency (500kHz or lower) ultrasound that is normally used in neuromodulation studies, however, precludes the use of simple means to deliver ultrasound to small target regions of the mouse brain with a high dynamic range in power. In this study, we apply 3MHz ultrasound, with a focal size of <800um, while observing responses to

both baseline signals and responses to visual stimulation. In addition, we use a fiber-optic hydrophone to directly measure *in vivo* the pressure and temperature changes in this preparation to account for the complexities of reflections and interference patterns, instead of simply using measurements of the ultrasound field in water or using computational methods to estimate the ultrasound field in a preparation.

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Poster

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Brain Canada

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CIHR

Title: High-frequency deep brain stimulation of the fornix improves memory consolidation and causes network-level neuroanatomical remodelling in an Alzheimer's mouse model

Authors: *D. R. GALLINO[1], G. A. DEVENYI[3], J. GERMANN[2], S. FREY[4], A. P. MATHIEU[2], M. CHAKRAVARTY[5];
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Abstract: Deep brain stimulation (DBS) involves the targeted delivery of high-frequency electrical stimulation to brain regions affected by neuropsychiatric disorders using a surgically implanted electrode. Recently a clinical trial has been initiated to determine the utility of DBS of the fornix for the treatment of Alzheimer’s disease. Here we study the longitudinal effects of

DBS on brain structure and behaviour using a mouse model of Alzheimer's disease. We overcome MRI-compatibility issues of traditional metal electrodes using carbon fibre-based electrodes for delivery of DBS.
10 week-old male and female 3xTg mice received bilateral electrode implantion +/- 0.75 mm bilaterally, perpendicular to the skull plane at bregma, and at a depth of 3.25 mm to target the body of the fornix. Sham (n = 2M/6F) or monophasic stimulation (n = 3M/4F) was delivered for 1 hour at settings homologous to those used in humans: 100 Hz, 100 μA, ~1.5 V with pulse width of 100 μs. Memory and cognitive flexibility was assessed weekly in water maze with changing platform positions. MRI image acquisition occurred 3 days before stimulation, 3 days and 6 weeks post stimulation. Animals were imaged in a 7T Bruker Biospec 70/30 USR using a T2 weighted 3D FLASH acquisition with 100 μm isotropic voxels.
Latency and pathlength in the water maze did not significantly differ between sham and stimulated animals. However, during weeks 3-4 post stimulation, animals showed a significant (P=0.013) bias to return to the previous week's platform position (fig.1). Deformation-based morphometric analysis revealed significant (FDR 5%) relative growth of the anterior thalamus after 6 weeks in stimulated animals (fig.1).
Our findings suggest that acute DBS of the fornix enhances memory consolidation and makes memories harder to extinguish 3-4 weeks post stimulation. This may be mediated by remodelling of the anterior thalamus and hippocampus. The innovative use of carbon fiber electrodes allowed animals to be imaged with minimal invasiveness due to MRI acquisition artifacts and lend themselves well to preclinical longitudinal designs.

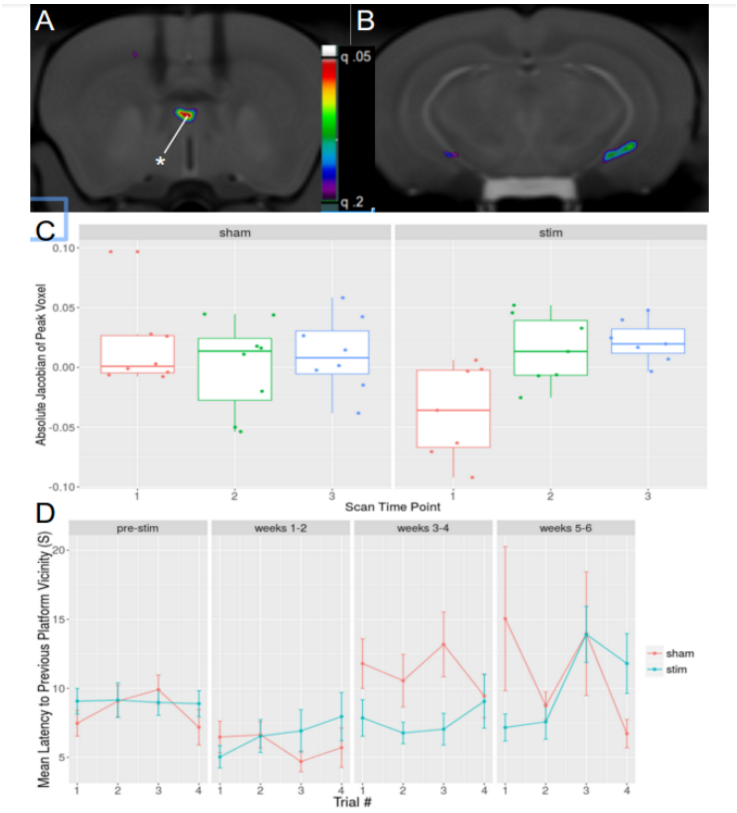

Figure 1. A&B. T statistic maps of relative deformation in stimulated animals when compared to sham with respect to time. Anterior thalamus and ventral hippocampus shown. FDR from 20% to 5% displayed in color. Underlays are coronal views of final registration. C. Absolute deformation values from peak voxel (denoted in A by asterisk) in anterior thalamus plotted by scanning time point (3 days prior, 3 days and 6 weeks post-stimulation). D. Latency to the previous week's platform position in water maze, displayed as mean +/- SE. During weeks 3 and 4, stimulated mice return in significantly less time.

Disclosures: D.R. Gallino: None. **G.A. Devenyi:** None. **J. Germann:** None. **S. Frey:** None. **A.P. Mathieu:** None. **M. Chakravarty:** None.

Poster

095. New Approaches for Neuromodulation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 95.06/KKK66

Topic: D.01. Sensory Disorders

Title: A new paradigm for neural restoration: low level random electrical stimulation can enhance vibration perception in diabetic neuropathy

Authors: *E. GIOKAS[1], K. MIGDAL[2], J. MICHALIK[2], L. REYES[2], P. BREEN[3], J. M. SERRADOR[4,2];

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Abstract: Peripheral sensory loss is a common problem in patients with diabetes. Loss of feeling in the foot can lead to chronic foot problems and amputations. Reversing this sensory loss could greatly improve quality of life and prognosis. A novel paradigm that has been shown to improve sensory perception is the use of stochastic noise applied at the sensory receptor. Our hypothesis was that applying stochastic noise to the conduit nerve, rather than the sensory receptor, would improve neural traffic and vibration perception threshold.
Twelve healthy controls (1 Female, Age: 50±13 yrs; Height: 173±9 cm; Weight: 83.8±12.9 kg; BMI: 28.2±5.2) and five participants with Diabetes (Age: 59±9 yrs; Height: 174±1 cm; Weight: 94.7±15.6 kg; BMI: 30.8±5.1; HbA1c: 7.7±0.9) volunteered for the study. Electrodes were placed proximally to the medial and lateral malleoli such that the tibial nerve was stimulated. We evaluated the vibration perception threshold (VPT) of the plantar side of the hallux (big toe) using a Neurothesiometer. Stimulation was randomly applied, with two control conditions (no electrical stimulation) and four stimulation conditions (0±15μA, 0±30μA, 0±45μA, 0±60μA, 0±75μA, 0±90μA, 0±105μA). Participants were unable to perceive stimulation. Both participants and experimenter performing the VPT measures were blinded to stimulation condition. Assessment of sensory function in the big toe of participants demonstrated that healthy controls had significantly lower VPTs (Mean: 12.2; Range 2.5-21 V) than participants with diabetes (Mean: 28.5; Range 6-50 V, P=0.011). Application of the low levels of subsensory random stochastic electrical noise resulted in significant improvement in VPT. Effects of stimulation on VPT was different for each individual with overall 60.3% of the trials resulting in improvements in VPT (Range 14.3-100% for each participant) while 23.8% resulted in impairment of VPT. There was no difference between baseline and sham (0 μA) conditions. Stimulation resulted in significant improvements in VPT ($P<0.001$) in both controls (Baseline: 12.2±6.5 vs Optimal Level: 9.5±5.5 V) and diabetics (Baseline: 28.5±17.5 vs Optimal Level: 23.1±15.8 V) with greater absolute improvement in diabetics. However, examining percent improvement, effects were similar between controls (+25.8±19.4%) and diabetics (+19.9±11.7%).
These findings represent a novel method to treat sensory loss in diabetic neuropathy. Since participants cannot feel the stimulation, it could provide an effective treatment with no side effects. This work was supported by the War Related Illness and Injury Study Center within the Department of Veteran Affairs.

Disclosures: **E. Giokas:** None. **K. Migdal:** None. **J. Michalik:** None. **L. Reyes:** None. **P. Breen:** None. **J.M. Serrador:** None.

Poster

095. New Approaches for Neuromodulation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 95.07/KKK67

Topic: D.07. Vestibular System

Title: Modulation of body mass composition using galvanic vestibular stimulation.

Authors: *J. MCKEOWN, P. D. MCGEOCH, H. PETERSON, V. S. RAMACHANDRAN; Ctr. for Brain & Cognition, UC San Diego, LA Jolla, CA

Abstract: In a series of experiments participants consumed high calorie diets for prolonged periods, in some cases up to 10,000 calories a day, while at the same time refraining from physical exercise. In most cases the subjects gained a surprisingly modest amount of weight. This observation supports the concept that there is a "set-point" for body mass composition as modulated by the hypothalamus. Deviation too far in either direction from this set-point is restricted by ill-understood mechanisms.

It has been known for some time that animals living in a state of chronic centrifugation show a marked change in their body mass composition. In particular, they go through a process of "de-fatting", with their total body fat dropping from in excess of 20% to around 5%. Experiments using a type of mutant mouse that is missing the otolith organs from its inner ear have shown that, rather than being a non-specific effect of hypergravity, this change in body mass composition appears to be mediated by a specific vestibulo-hypothalamic pathway.

We attempted to replicate its effect by stimulating the otolith organs using galvanic vestibular stimulation (GVS). This technology involves passing a small electric current into the skin overlying the mastoid process, and has been shown at currents less than 3mA to preferentially activate the otolith organs.

In a pilot study we recruited 10 overweight (BMI 25-30) or obese (BMI >30) subjects. Three were randomly selected as blinded control subjects and 7 were given between 20 and 40 hours of binaural GVS in a 0.5 Hz sinusoid as tolerated up to 2mA. All the participants had their body mass composition measured at the start and end of the study period using dual energy X-ray absorptiometry. We found that in contrast to the control group, the GVS group had up to a 16% reduction in their total body fat. This observation was supported by an analysis of secretion of leptin, the "satiety hormone", in the saliva of subjects undergoing GVS.

We postulate that prolonged vestibular stimulation most likely acts via the parabrachial nucleus in the pons which is known to integrate vestibular, sympathetic and parasympathetic afferent activity. Via this pathway GVS modulates the function of the dorsomedial hypothalamus. We suggest that repeated GVS acts to alter the set-point for body mass to lower the proportion of body fat.

Disclosures: **J. McKeown:** None. **P.D. McGeoch:** None. **H. Peterson:** None. **V.S. Ramachandran:** None.

Poster

095. New Approaches for Neuromodulation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 95.08/KKK68

Topic: D.07. Vestibular System

Support: NIH/NIA Grant AG049917

Title: Blood pressure and heart rate responses to vestibular stimulation in aged and young-adult male and female rats.

Authors: ***G. P. MARTINELLI**[1], G. R. HOLSTEIN[2];
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Abstract: Acute changes in blood pressure (BP) are regulated by the baroreflex, which conveys baroreceptor signals initially to the solitary nucleus and ultimately to presympathetic neurons in the rostral ventrolateral medulla (RVLM). This pathway maintains homeostasis through negative feedback. In contrast, the vestibular system can modulate BP through a more direct and feed-forward mechanism, the vestibulo-sympathetic reflex (VSR), which is activated during movement and changes in posture re:gravity (e.g. when rising from a seated or lying position). The VSR assures adequate cerebral perfusion during these postural adjustments, allowing humans to stand up without fainting due to blood pooling in the lower body. VSR dysfunction has been postulated to underlie neurogenic orthostatic hypotension in some individuals, and orthostatic hypotension is significantly more prevalent and severe in the elderly.
The present study compared BP and heart rate responses to tilt and sinusoidal galvanic vestibular stimulation (sGVS) in isoflurane-anesthetized young adult (4-6 mo) and aged (24-26 mo) male and female F344 rats obtained from the NIA Aged Rodent Colonies. At least one week prior to stimulation, animals were implanted with an intra-aortic telemetric device (DSI Technologies) that transmitted physiological data including HR, BP (systolic, diastolic and mean arterial pressure), HR variability, body temperature and other relevant parameters. These data were analyzed offline using proprietary Ponemah software (DSI). For tilt stimulation, anesthetized rats were placed prone on a servo-controlled tilt table, and raised in the pitch plane from horizontal to 70° nose-up (0.91g). Animals remained in that position for 5 min before being returned to the prone position for 10 min. This sequence was repeated 5 times per session per rat. For sGVS, single 3 mA and 0.05 Hz sinusoids were presented 10 times at 4 min intervals. Testing was

repeated 3 times in each session with a 45 min rest period between each stimulus sequence. Each animal was tested at least twice with each stimulus.
To identify the vestibular neurons activated by these stimuli, rats were perfused with mixed aldehydes 90 min after the stimulation and the brainstems were sectioned serially. One set of tissue sections was stained for c-Fos protein to map the locations of the vestibular neurons activated by pIRs of the individual end organs. Other sets of sections were used for immunofluorescence detection of glutamate, GABA and PACAP in activated vestibular neurons. We found marked differences in the physiological responses of aged vs young adult animals, and corresponding alterations in the activated VN neurons.

Disclosures: G.P. Martinelli: None. **G.R. Holstein:** None.

Poster

095. New Approaches for Neuromodulation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 95.09/KKK69

Topic: D.07. Vestibular System

Support: NIH/NIDCD grants DC008846 (GRH)

DC006685 (RDR)

DC013798 (SMR)

Title: Vestibular nuclear neurons of the vestibulo-sympathetic reflex (VSR) activated by pulsed infrared stimulation of vestibular end organs.

Authors: *G. R. HOLSTEIN[1], G. P. MARTINELLI[2], R. D. RABBITT[3], S. M. RAJGURU[4]; [1]Depts Neurol, Neurosci, Anat/Cell Bio, [2]Dept. Neurol., Icahn Sch. of Med. at Mount Sinai, New York, NY; [3]Univ. of Utah, Salt Lake City, UT; [4]Univ. of Miami, Miami, FL

Abstract: Studies in humans and experimental animals show that vestibular system activation can modulate blood pressure (BP). In humans, otolith stimulation using linear acceleration causes BP changes that are attenuated in patients with bilateral vestibular dysfunction. Off-vertical-axis rotation, a specific otolith stimulus, 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. Linear acceleration, head/body-up tilt and electrical stimulation of the vestibular nerve increase SNA and/or raise BP in experimental animals as well. These responses are mediated by a VSR pathway from the vestibular end organs to the vestibular nuclei (VN), which in turn project to presympathetic neurons in the rostral

ventrolateral medulla (RVLM). We previously demonstrated in rats that the VSR pathway can be activated by tilt and sinusoidal galvanic vestibular stimulation and that activated VSR neurons in VN and RVLM accumulate c-Fos protein. Maps of cFos-positive activated vestibular neurons that were also backfilled following a retrograde tracer injection in RVLM showed that VN neurons of the VSR are located in the caudal half of the medial and spinal VN. More recently, we found that pulsed infrared stimulation (pIRs) can also be used to activate the VSR.
In the present study, pIRs (1863nm, 100-250pps, 250μs) was directed through the round window toward the vestibular end organs in rats using customized optical fibers. Changes in BP and heart rate were recorded in response to pIRs of semicircular canal cristae and otolith maculae. Eye movements evoked by vestibular stimulation were measured using a video-based eye tracking system (ISCAN Inc, Woburn, MA) and a custom MATLAB program was used for analysis. These experiments documented the contributions of individual end organs in modulating BP through the VSR pathway. To identify the vestibular neurons activated by these stimuli, rats were perfused with mixed aldehydes 90 min after the pIRs and the brainstems were sectioned serially. One set of tissue sections was stained for c-Fos protein to map the locations of VN neurons activated by pIRs of individual end organs. Other sets of sections were used for immunofluorescence detection of glutamate, GABA and PACAP in pIRs-activated VN neurons. Taking advantage of the high spatial and temporal specificity of the stimulus, this study demonstrated the locations of central vestibular neurons specifically activated by pIRs of individual vestibular end organs.

Disclosures: G.R. Holstein: None. **G.P. Martinelli:** None. **R.D. Rabbitt:** None. **S.M. Rajguru:** None.

Poster

095. New Approaches for Neuromodulation

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Program#/Poster#: 95.10/KKK70

Topic: D.07. Vestibular System

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NIH Grant R01DC008846 (GRH)

NIH Grant RO1DC011481 (RDR)

Title: Pulsed infrared stimulation of the vestibular system evokes vestibulo-ocular and vestibulo-sympathetic reflex responses.

Authors: *S. RAJGURU[1], W. JIANG[2], G. R. HOLSTEIN[3], G. P. MARTINELLI[3], C.-P. RICHTER[4], R. D. RABBITT[5];
[1]Biomed. Engin. and Otolaryngology, [2]Biomed. Engin., Univ. of Miami, Coral Gables, FL; [3]Icahn Sch. of Med. at Mount Sinai, New York, NY; [4]Northwestern Univ., Chicago, IL; [5]Univ. of Utah, Salt Lake City, UT

Abstract: Bilateral vestibulopathy in patients leads to postural instability and chronic disequilibrium. Infrared neural stimulation (INS) has been investigated as an alternative neurostimulation modality that does not require direct contact with neural tissue and has been shown to produce significant post-synaptic afferent responses in the vestibular system. In the present study, we have investigated the physiological effects of pulsed infrared stimulation of the vestibular system in a rat model and carried out histopathological analysis. The animals were placed in a custom-designed modified stereotaxic frame that allows delivery of head rotations in pitch, roll, and yaw planes tracked using rotary potentiometers. A head post was cemented to the skull to secure the animal's head during stimulation and recording procedures. Pulsed infrared radiation (IR: 1863nm, 100-250pps, 250μs) was directed through the round window toward the vestibular end organs. Evoked vestibulo-ocular and vestibulo-sympathetic reflex responses were measured. Eye movements were measured using a video-based, two-channel eye tracking system (ISCAN Inc, Woburn, MA) and analyzed in MATLAB. We observed significant in-torsion followed by sinusoidal movements of the ipsilateral eye by frequency modulated infrared pulses. Depending upon the modulation frequency of IR, amplitude of oscillating eye movement ranged from 1 to 7º. At lower modulation frequencies (0.05-0.25Hz), an increase in the stimulation frequency was matched by a corresponding increase in the magnitude of the eye movement. The eye movements continued in response to IR after 30+ minutes of continuous stimulation. Simultaneously, a drop in blood pressure (BP) and heart rate (HR) was recorded during pulsed infrared stimulation of the vestibular system. The results compared well with previous observations of HR and BP following galvanic stimulation of the vestibular system. To identify the vestibular endorgans activated by infrared stimuli, histology and micro-computed tomography were used. Following the experiments, the radiation energy was increased to a level that would induce tissue damage. The cochlea and vestibular end organs were harvested to quantify the damage and determine the beam path. Alternatively, high-resolution microCT was used to image the structures as well as the location of the optical fiber fixed in place following experimentation. Overall, the results demonstrate selective activation of vestibular endorgans by pulsed IR and suggest its potential applications in inner ear research.

Disclosures: S. Rajguru: None. **W. Jiang:** None. **G.R. Holstein:** None. **G.P. Martinelli:** None. **C. Richter:** None. **R.D. Rabbitt:** None.

Poster

096. Automation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 96.01/LLL1

Topic: I.06. Computation, Modeling, and Simulation

Support: NSFC Grant 91432105

NSFC Grant 91432116

NSFC Grant 91232000

Title: An automatic method for segmenting brain nucleus boundaries on cell-resolution cytoarchitectural image sequence

Authors: ***Z. FENG**, A. LI, H. GONG, Q. LUO;
Britton Chance Ctr. for Biomed. Photonics, Wuhan Natl. Lab. For Optoelectronics, Hubei, China

Abstract: It has always been a manual way to delineate the brain region and nuclei of brain tissue on cell-resolution 3D image dataset, which was time-consuming as well as error-prone. The key problem of automation of nucleus segmentation on cytoarchitectural image is to recognize the blurry boundaries formed by isolated neurons. We propose a new method that could extract the blurry boundaries of brain nuclei on cell-resolution dataset automatically. This method parameterizes the boundaries of nuclei with closed cubic spline and guides the evolution of parameterized boundaries using the cytoarchitectural information around the nodes of boundary. It takes the nucleus boundary on the first image of the image sequence as the initial value, and calculates the boundaries of nucleus on the remaining images of the sequence. We applied this method to the cerebellum area and olfactory area, segmented the mitral layer of Main Olfactory Bulb, granular layer of Lobule II and the molecular layer of Lobule II on consecutive 100 coronal sections automatically and precisely.

Disclosures: **Z. Feng:** None. **A. Li:** None. **H. Gong:** None. **Q. Luo:** None.

Poster

096. Automation

Location: Halls B-H

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Program#/Poster#: 96.02/LLL2

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant R01DC008343

NIMH Grant P50MH100024

Title: Unbiased automated phenotyping of rodent behavior in nonsocial and social contexts

Authors: *Y. KWON, G. K. ADAMS, G. J. BERMAN, R. C. LIU;
Dept. of Biol., Emory Univ., Atlanta, GA

Abstract: Animal behaviors are complex, yet our methods for quantifying them have been relatively simplistic. This has limited how well we can meaningfully phenotype animals to establish behavioral differences whose neural mechanisms may be of interest. Traditional methods to assess behaviors generally look only at the timing or quantity of specific events that have been predefined for an observer or apparatus to identify. However, for a given animal model, it may not always be known ahead of time what behaviors are most important to track to reveal functional distinctions. Hence, it is essential to develop unbiased methods to phenotype behavior. Such tools have begun to be used in model systems such as Drosophila and C. elegans, but less has been done to analyze rodent behavior. Pursuing such methods for rodents could help elucidate behaviors that have thus far escaped careful quantification, such as natural social behaviors, which are of increasing interest from a neuropsychiatric perspective. We exploit algorithms developed to identify behaviors based on their degree of stereotypy (Berman et al, 2014) to create a behavioral state map.Behavioral mapping employs unsupervised machine learning algorithms to learn behaviors from video recordings. Starting with the underlying structure of postural movement data, animal images undergo postural decomposition via PCA. A spectogram for postural modes are created from wavelet transforms, and then via t_SNE, video time points are mapped into a two-dimensional plane. Here, for the first time, we apply these mapping methods to identify stereotyped behavioral states in individual rodents in both social and nonsocial contexts. Our preliminary data suggests that the rodent behavioral repertoire can coarsely be clustered into 20 different behavioral states. Animals traverse these states differently depending on the social context. This proof-of-principle demonstration now lays the groundwork to begin quantifying subtleties in natural social interactions between individuals, which is often difficult to define a priori. Our methods could have application for phenotyping rodent models of autism spectrum disorder (ASD). ASD is characterized by deficits in social communication/interaction and by restricted, repetitive patterns of behavior. Current mouse models of ASD largely focus on phenotypes such as social interaction, ultrasonic vocalization

production, and repetitive self-grooming (Crawley, 2012), but these behaviors represent only a small subset of ASD characteristics. Unbiased behavioral phenotyping may be useful to delineate more robust representations of ASD phenotypes.

Disclosures: **Y. Kwon:** None. **G.K. Adams:** None. **G.J. Berman:** None. **R.C. Liu:** None.

Poster

096. Automation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 96.03/LLL3

Topic: I.06. Computation, Modeling, and Simulation

Support: U01CA198932-01

Title: Template-based interactive registration, segmentation and quantification workflows for light microscopy images

Authors: ***I. BOWMAN**[1], K. COTTER[1], M. BAY[2], M. ZHU[1], M. Y. SONG[1], N. N. FOSTER[1], M. S. BIENKOWSKI[1], S. YAMASHITA[1], A. W. TOGA[1], H. HINTIRYAN[1], H. DONG[1]; [1]USC Stevens Neuroimaging and Informatics Inst., Los Angeles, CA; [2]Broad CIRM Ctr. and Dept. of Stem Cell Biol. and Regenerative Medicine, USC Keck Sch. of Med., Los Angeles, CA

Abstract: Within the Mouse Connectome Project (MCP) group, we developed a new image processing workflow to aid in the collection and analysis of long-range neural network data. A typical MCP study involves dozens of C57Bl/6J mouse brains. Each animal (case) is injected with two anterograde (PHA-L & BDA) and two retrograde (CTb & FG) chemical tracers to respectively establish the input and output connections of each injection site. Animals survive a few weeks following the injections to allow tracer transport time before euthanization, fixation, and brain excision. Individual brains are cut into 50 μm coronal sections, which undergo fixation and immunostaining before being mounted onto slides for imaging under 10x objective magnification. Epifluorescent scans of a single section comprise five channels (one channel for each tracer plus one channel for cytoarchitectural background), each stored at up to 2 GB. Opening, viewing, and editing a single 2 GB scan is non-trivial even on state of the art equipment -- manually annotating the connectivity of hundreds of scans is intractable and can take a team of researchers months. We created our new image processing workflow to overcome the annotation obstacles. Workflow interaction is performed via a novel interface for image registration: the user observes correspondence point templates to evaluate and provide feedback to the image warping algorithm, and evaluate output of subsequent processing. After warping, the user sets and observes threshold values using an interactive segmentation interface. The

values are applied to the image scans to extract labeled axons, terminals (output connections), and cells (input connections) from tissue background. The segmented images provide graphical reconstructions of labeling registered to a common spatial frame. A final annotation stage employs an overlap indexing method to the segmented images. This comprehensive annotation output of our registration workflow spans multiple cases, and quantifies connectivity from numerous injection sites to their corresponding upstream or downstream regions of interest. Analyzing this data allows the user to identify clusters, hubs, motifs and other graph analysis metrics as well as visualize connectivity information. Applying our registration workflow to the MCP brain data has significantly expedited our analysis of brain connectivity, as well as furthered our ability to present these findings to the neuroscientific community.

Disclosures: I. Bowman: None. **K. Cotter:** None. **M. Bay:** None. **M. Zhu:** None. **M.Y. Song:** None. **N.N. Foster:** None. **M.S. Bienkowski:** None. **S. Yamashita:** None. **A.W. Toga:** None. **H. Hintiryan:** None. **H. Dong:** None.

Poster

096. Automation

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Program#/Poster#: 96.04/LLL4

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH R01 NS39600 (BISTI)

NIH R01 NS086082 (CRCNS)

NSF BRAIN DBI 1546335 (EAGER)

Title: Circuitry profiling in the *Drosophila* brain through machine learning

Authors: *R. ARMANANZAS, S. NANDA, G. A. ASCOLI;
Krasnow Inst. For Advanced Study, George Mason Univ., Fairfax, VA

Abstract: Recent and accelerating advances in experimental neuroscience, bioengineering, and information technology are rapidly pushing Cajal's "neuron doctrine" to the verge of empirical and computational testability. Prominent research efforts are unveiling how circuits constitute the basic functional units of nervous systems. Among them, *Drosophila* is currently the species with more promising results in mapping brain-wide connections at the individual neuron level. The pioneering FlyCircuit Database has already traced and co-registered the neurite wiring corresponding to approximately 10% of the cells in female *Drosophila* brain.
Here, we first assigned each of the 16,206 FlyCircuit v1.0 neurons to one of 58 brain regions by

determining the geometrical memberships of the somata within 3D meshes of the neuropil structures. A multi-label classification method (ML-KNN) allowed us to establish regions for those cells located outside of the meshes' boundaries due to imperfect registration. Next, using axonal and dendritic terminal data from the morphological reconstructions, we algebraically computed potential pre- and post- synaptic connectivity based on spatial co-occurrences. This resulted in a largely sparse matrix of connectivity likelihoods that was subsequently filtered to discard isolated cells.
For the detection of distinct circuits, we applied the Large Average Submatrix (LAS) co-clustering method. LAS was able to identify a total of 20 separate biclusters, i.e. sets of pre- and post- synaptic neurons connected through differentiated patterns. Each bicluster connectivity signature was profiled using an outlier detection algorithm based on Kolmogorov-Smirnov statistic and bootstrapping. Finally, to reveal the hub neurons in each profile, we fitted the individual connectivity scores to different logistic distributions. The core cells and connections forming the circuitry motif were those whose inverse cumulative distribution function was equal to or greater than 0.90.
The continuously increasing comprehension of the functional importance of circuitry brings the challenge to develop and select the most suitable, powerful, and practical approaches for quantitative analytics. Applying machine learning techniques to the elucidation of canonical circuits may yield important connectivity patterns that can help explain at least some of the observed behavioral mechanisms.

Disclosures: **R. Armananzas:** None. **S. Nanda:** None. **G.A. Ascoli:** None.

Poster

096. Automation

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Program#/Poster#: 96.05/LLL5

Topic: I.06. Computation, Modeling, and Simulation

Support: NSFC Grant 91432105

NSFC Grant 91432116

NSFC Grant 91232000

Title: A fine-grained parallel method for reformatting terabyte-scale volumetric images into hierarchical data

Authors: *Y. LI, A. LI, H. GONG, Q. LUO;
Wuhan Natl. Lab. for Optoelectronics, Huazhong Univ. of Sci. and Technol., Hubei, China

Abstract: Techniques for three-dimensional imaging of whole mammalian brains at single-neuron resolution have generated image data easily exceeding the terabyte size. The image data generated are too large to be processed by usual computers. The common practice is to reformat the image dataset into lots of blocks and stored in a hierarchical structure with multi-resolution levels. So the computers could process small parts of dataset at a time. However, existing tools are time-consuming, or use too much computational memory when reformatting. Here, we present a fine-grained parallel reformatting (FPR) method to solve this critical problem in the processing of terabyte-sized image data. We tested its performance and demonstrated its applicability to various computing platforms. The results suggested that FPR was faster with low memory usage than existing tools during reformatting. It has ability to handle up to one petabyte of image data.

Disclosures: **Y. Li:** None. **A. Li:** None. **H. Gong:** None. **Q. Luo:** None.

Poster

096. Automation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 96.06/LLL6

Topic: I.06. Computation, Modeling, and Simulation

Title: Modulating the hippocampal functional connectivity using real-time optimization of distributed microstimulation

Authors: *B. MAHMOUDI[1], M. CONNOLLY[2], R. GROSS[2];
[1]Emory Univ., Alpharetta, GA; [2]Emory Univ., Atlanta, GA

Abstract: Introduction: Modifying the pattern of neural activities is at the core of neurostimulation therapies for neuropsychiatric disorders. One of the key problems to maximize the effectiveness of these therapies is optimization of neurostimulation parameters. The optimization of neuromodulation requires: (1) identifying biomarkers that can be effectively modulated by electrical stimulation, (2) developing algorithms for optimizing these biomarkers, and (3) verifying that optimizing these biomarkers has the desired effect. We found that the correlation coefficient (CC) between CA3 and CA1 in the rat hippocampus could be effectively modulated by distributed microelectrical stimulation. In this study we address the second requirement by developing an optimization algorithm to modulate this biomarker in real-time. Methods: A rat was implanted with a 16 channel microelectrode array in the hippocampus with 8

electrodes each recording and stimulating CA3 and CA1. A TDT RZ2 BioAmp processor and custom software platform was used to run the optimization. The target of this optimization was to identify the stimulation frequency that enhanced the functional connectivity between CA3 and CA1, in the form of the CC between the local field potential recordings between those two regions. Using the cross-entropy method we implemented a real-time closed-loop optimization algorithm that started with an initial Gaussian distribution over stimulation frequencies. 100 stimulation frequencies were randomly sampled from the distribution and applied sequentially. The CC after each stimulation was calculated. The mean and STD of the 10 frequencies with the greatest effect were used to define a new distribution. Each stimulation was a biphasic symmetric, 2V, 1ms width and 1s duration. The initial distribution had a mean of 100Hz, and a standard deviation of 70Hz
Results: The optimization performed 11 cycles (1100 stimulations). The average CA3-CA1 CC was 0.601 during the first cycle and 0.72 during the final cycle. Over the 11 cycles, the distribution changed from a mean of 100 to 193Hz and a standard deviation of 70 to 28Hz.
Discussion: This study demonstrates a novel approach to modulating neural state and investigating the way the brain responds to stimulation. Our initial study found that a stimulation frequency of 100Hz increased the CA3-CA1 CC more than 5, 20, 50, or 200Hz. However, this optimization converged on a frequency that was previously not tested in our parameter sweep.
Conclusion: Neural state biomarkers can be effectively modulated by electrical stimulation using real-time optimization, and may provide a new approach to improving neural modulation therapy.

Disclosures: **B. Mahmoudi:** None. **M. Connolly:** None. **R. Gross:** None.

Poster

096. Automation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 96.07/LLL7

Topic: I.06. Computation, Modeling, and Simulation

Support: Intel Corporation ISRA on on Neuromorphic Architectures for Mainstream Computing

National Science 1309 Foundation (NSF EFRI-1137279)

Office of Naval Research 1310 (ONR MURI 14-13-1-0205)

NSF Expedition on Computing (Visual Cortex on Silicon, award 1317470)

Title: Bio-inspired handwritten digit classification by a spiking neural network with synapse pruning

Authors: ***S. JOSHI**[1], S. KALYAN[2], S. SHEIK[1], E. NEFTCI[3], G. CAUWENBERGHS[1];
[1]Univ. of California San Diego, LA Jolla, CA; [2]UCSD, La Jolla, CA; [3]Univ. of California, Irvine, Irvine, CA

Abstract: Synaptic stochasticity methods inspired by neurobiology have been shown to improve performance in spiking artificial neural networks resulting in greater than 95% classification accuracy on a benchmark handwritten digit recognition task. Taking cues from neurobiological research we further analyze the effect of various methods of synaptic pruning on the performance of these spiking networks as well as showing links between biological data and modeled data.

Disclosures: **S. Joshi:** None. **S. Kalyan:** None. **S. Sheik:** None. **E. Neftci:** None. **G. Cauwenberghs:** None.

Poster

096. Automation

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Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 96.08/LLL8

Topic: I.06. Computation, Modeling, and Simulation

Support: EPSRC "Green Brain Project", grant EP/J019690/1

Senior Research Fellowship, The Royal Academy of Engineering and The Leverhulme Trust

Title: GeNN: Accelerated spiking neural network simulations on GPUs

Authors: ***T. NOWOTNY**, J. P. TURNER, E. YAVUZ;
Univ. of Sussex, Brighton, United Kingdom

Abstract: When simulating models of neural networks in the brain, the size of the simulated networks matters. The speed of neural network simulators and their capacity for simulating large networks therefore remains an important issue. Here we present the GPU enhanced neuronal networks (GeNN) framework [1,2] that we have created to gain the most from GPU accelerators. The use of GPU accelerators has increased explosively over the last 10 years, with many of the main actors in the area of machine learning now relying heavily on this technology (e.g. Amazon, Facebook, Google Deep Mind). One of the first applications for GPU accelerated computing were indeed neural networks. However, using GPU accelerators has become less

mainstream in computational neuroscience because of difficulties with optimizing computational neuroscience model code for the particular architecture of GPUs.
GeNN is offering an elegant solution to the most common problems, using an approach of automatic code generation from minimal descriptions provided by the user. The generated code will automatically be optimized for the detected GPU accelerator and the user-defined model. At the same time, GeNN remains very flexible and expert users can manipulate virtually every aspect of the simulation. GeNN supports all typical computational neuroscience models by allowing users to define their own equations for the model elements, such as neuron dynamics, synapse updates and learning rules. GeNN has been used for networks of Hodgkin-Huxley neurons, with Hebbian learning, STDP and 3-factor learning rules, as well as for more simple models such as networks of Izhikevich, integrate-and-fire and Rulkov map neurons.
For less expert users we have created additional interfaces to the SpineCreator graphical model definition interface [3] and to the popular Brian 2 simulator [4]. With the latter, it is as simple as issuing the command set_device('genn') to take advantage of GPU acceleration.
Acceleration compared to CPU-based solutions varies by model and GPU accelerator hardware and can be as high as 200X but also as low as none. GeNN is available as open source software under GPL v2 [2].
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2. GeNN, http://genn-team.github.io/genn/, accessed 2016-05-04
3. SpineCreator, http://bimpa.group.shef.ac.uk/SpineML/index.php/SpineCreator_-_A_Graphical_Tool, accessed 2016-05-04
4. Brian 2, https://github.com/brian-team/brian2, accessed 2016-05-04

Disclosures: **T. Nowotny:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NVIDIA Corporation. **J.P. Turner:** None. **E. Yavuz:** None.

Poster

096. Automation

Location: Halls B-H

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Program#/Poster#: 96.09/LLL9

Topic: I.06. Computation, Modeling, and Simulation

Support: BMBF / FKZ 01GQ1002

ERC No 633428

Title: Automatic detection of putative contacts between in-vivo labelled neurons

Authors: *M. M. SEETHARAMA;
Computat. Neuroanatomy, Max Planck Inst. For Biogical Cybernetics, Tuebingen, Germany

Abstract: Understanding the structural organization of the neural networks requires reconstruction of the underlying neural circuitry in anatomical detail and mapping of the synaptic contacts. A typical in-vivo labeled axon innervates a large volume and imaging such in-vivo labeled pairs of neurons at high resolution yields a large set of images, typically in the order of Tera Bytes. Manual mapping of synaptic contacts between such cell pairs would be labor intensive and error prone. Here, we present a software pipeline that automatically detects putative synaptic contacts between the Boutons and Spines of in-vivo labeled pairs of neurons. The pipeline has three phases. Firstly, the regions where the skeletons of axon and dendrites of different neurons come close to each other are detected. Secondly, in these proximity regions, the Boutons along the axons and spines along the dendrites are detected. Thirdly, the overlaps between Boutons and Spines along with their locations are detected. The resulting putative contacts are visualized on the original image stack using a visualization tool and can be verified by the user. This semi-automated approach reduces the number of sites to be manually inspected for putative contacts from tens of thousands to tens of putative contact sites. Hence achieving about three orders of magnitude reduction in the manual effort required.

Disclosures: M.M. Seetharama: None.

Poster

096. Automation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 96.10/LLL10

Topic: I.06. Computation, Modeling, and Simulation

Title: A neurobehavioral evaluation system using 3d depth tracking and computer vision: the case of stroke-kinect

Authors: *V. RAMESH, S. RICK, K. AGRAWAL, B. MEYERS, G. CAUWENBERGHS, N. WEIBEL;
UC San Diego, La Jolla, CA

Abstract: Due to the subtlety of their symptoms - slight tremors, blurred vision, and loss of mobility, for example - many neurological diseases are challenging to diagnose. As such, a computational tool that can identify and analyze these symptoms accurately will be of immense use to neurologists. We aim to characterize human motor and cognitive abilities through a multimodal approach that will lead to signatures for neurological disorders, based on patterns in

relevant identifiers. We focus here on stroke. Stroke is the 4th leading cause of death and the leading cause of disability in the United States. But Recombinant Tissue Plasminogen Activator (rt-PA), the only FDA-approved treatment currently available, is administered in less than 5% of acute stroke cases. The decision to prescribe rt-PA is based on the National Institute of Health Stroke Scale (NIHSS), a combination of multiple tests conducted by a neurologist to assess visual fields and motor and sensory impairments. Stroke evaluation with the NIHSS is inherently subjective. An inexperienced evaluator may miss key or almost imperceptible tells, misdiagnose the severity of a stroke, forego rt-PA prescriptions, and crudely predict long term outcomes. If this gap in objective and reliable stroke diagnosis is not addressed, stroke survivors will endure an arduous rehabilitation process. We are therefore developing Stroke-Kinect, a new system for automatic eye motion and body motion analysis to assist in the diagnosis of stroke. We obtain high-definition images and the spatial and temporal positions of 25 body joints in stroke and healthy control patients with the Microsoft Kinect v2. We employ machine learning classification algorithms and computer vision techniques to replicate the subjective NIHSS test computationally. Furthermore, we develop new tests for identifiers not captured by the NIHSS that are difficult to detect by the human eye: joint angles and thus body posture, velocity of gestures, twitches and jerks, and center of mass. Our analysis of depth data collected from stroke patients indicates accurate testing for the synchronicity of movements and reliable eye gaze tracking. The data also identifies posture as a key indicator of left side versus right side weakness. These results suggest that larger data sets will permit identification of only the vital indicators in stroke diagnosis, to simplify the NIHSS and mitigate the risk of false negatives and erroneous prescriptions of rt-PA. Stroke-Kinect also paves the way for the computational diagnosis of other neurological disorders, furthering the health sciences and ultimately aiding patients in their recovery.

Disclosures: **V. Ramesh:** None. **S. Rick:** None. **K. Agrawal:** None. **B. Meyers:** None. **G. Cauwenberghs:** None. **N. Weibel:** None.

Poster

096. Automation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 96.11/LLL11

Topic: I.06. Computation, Modeling, and Simulation

Support: ONR

Intel Corporation

IBM Corporation

Fujitsu

Title: Real-time reconfigurable event-driven emulation of integrate-and-fire neural networks with STDP

Authors: B. PEDRONI[1], *F. D. BROCCARD[2], S. SHEIK[1], G. CAUWENBERGHS[2,1]; [1]Bioengineering, Univ. of California San Diego, La Jolla, CA; [2]Inst. Neural Computation, UCSD, La Jolla, CA

Abstract: Simulations of spiking neural networks (SNNs) on conventional serial hardware suffer from a lack of scalability for embedded systems that must interact in real-time with complex and dynamic environments.
This limitation can be overcome with the implementation of SNNs on dedicated parallel hardware, making large-scale networks suitable for various real-time applications in autonomous robotics and neuroprosthetics.
However, developing custom application specific integrated circuit and neuromorphic devices for SNNs is both time consuming and expensive. Furthermore, some of these devices are limited in the types of spiking neuron model that can be implemented.
Here, we present a set of open-source software tools for the automation of spiking neural network models mapping on Field-Programmable Gate Arrays (FPGAs). FPGAs are compact, low-cost, reconfigurable integrated circuits that allows the parallel implementations of SNNs for low-power embedded applications.
We show a FPGA implementation of a SNN designed with the Brian simulator, using different neuron models. We also present preliminary results of the effect of coupling on the synchronization of two different SNNs implemented on the same FPGA.

Disclosures: B. Pedroni: None. **F.D. Broccard:** None. **S. Sheik:** None. **G. Cauwenberghs:** None.

Poster

096. Automation

Location: Halls B-H

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Program#/Poster#: 96.12/LLL12

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant 1R01EB019804

Title: Simultaneous state and parameter estimation with coupled data assimilation for tracking state of vigilance in rats

Authors: *F. BAHARI[1,2], M. W. BILLARD[3,2], C. R. TULYAGANOVA[3,2], K. D. ALLOWAY[4,2], B. J. GLUCKMAN[3,2,5];
[2]Ctr. for Neural Engin., [3]Engin. Sci. and Mechanics, [1]Pennsylvania State Univ., University Park, PA; [4]Neural and Behavioral Neurosciences, [5]Dept. of Neurosurg., Pennsylvania State Univ., Hershey, PA

Abstract: There is extensive clinical and experimental evidence that links state of vigilance to seizure generation (Langdon-Downs et al. 1929; Dinner 2002). Sleep-wake regulation is also altered in epileptic brain (Sedigh-Sarvestani et al. 2014). In order to understand this bi-directional coupling we need to further investigate the underlying neurophysiological interactions of brainstem sleep-wake regulatory system in normal and epileptic brain.
We have been using physiologically-based mathematical models of sleep-wake regulatory network synchronized with experimental measurements to reconstruct and predict the state of sleep-wake regulatory system in chronically implanted animals. Our objective is to assimilate sparse noisy measurements from the system - such as EEG and behaviorally measured state of vigilance as well as unit recordings from the represented cell groups- into these mathematical models (Sedigh-Sarvestani et al. 2012), and thereby both validate or improve the models and detect changes in physiology.
Critical to applying this technique to real biological systems is the need to estimate the underlying model parameters. Here, we present an estimation technique capable of simultaneously fitting and tracking multiple model parameters to optimize the reconstructed system state. Performance is gauged by reconstruction and forecasting of state from noisy observations of model-generated data, and compared to other conventional parameter tracking methods (Voss et al. 2004). In addition, we have extended our data assimilation methodology based on discretized observations of state of vigilance (Sedigh-Sarvestani et al. 2012) to assimilate these indirect observations into the optimized model dynamics to track and reconstruct the state of vigilance in normal and epileptic rats.
Langdon-Down, M., et al. (1929). The Lancet.
Dinner, D. S. (2002). J. Clinical Neurophys.
Sedigh-Sarvestani, M. et al. (2014). J. of Neuroscience.
Sedigh-Sarvestani, M.et al. (2012). PLoS Comp. Bio.
Voss, H.U., et al. (2004). IJBC.

Disclosures: F. Bahari: None. **M.W. Billard:** None. **C.R. Tulyaganova:** None. **K.D. Alloway:** None. **B.J. Gluckman:** None.

Poster

096. Automation

Location: Halls B-H

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Program#/Poster#: 96.13/LLL13

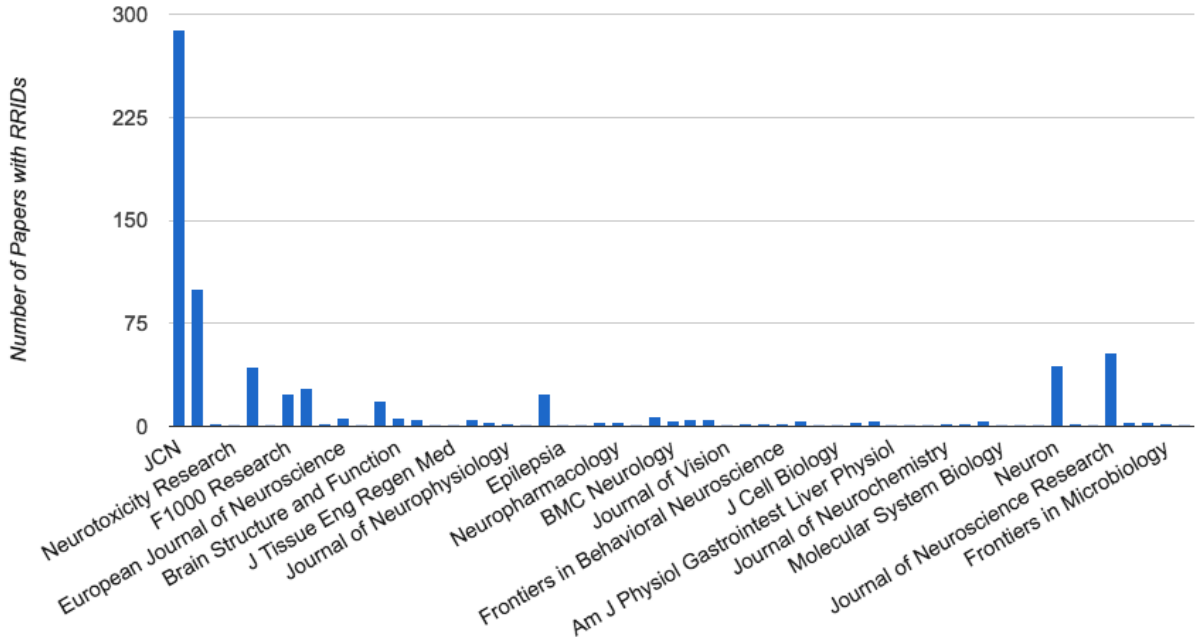
Topic: I.06. Computation, Modeling, and Simulation

Support: 1U24DA039832-01

Title: SciBot and Hypothes.is: a method for pulling properly cited key biological resources, RRIDs, from the literature

Authors: *A. E. BANDROWSKI, M. MARTONE, J. GRETHE, T. GILLESPIE, G. PINE; UCSD, La Jolla, CA

Abstract: Hypothes.is is an open web annotation tool, which allows users to annotate any web content they come in contact with within user defined groups, such as a class or a laboratory, or in public to leave a public note for others about a particular web page in the W3C open annotation format. SciBot is a text algorithm written in Python, which detects the presence of properly cited antibodies, cell lines, organisms and tools within scientific methods sections. SciBot uses the scicrunch resolver at scicrunch.org/resolver to gather information from the antibodyregistry, cellosaurus, scicrunch tool registry and ~25 model organism stock centers and databases bringing that information into the hypothes.is overlay of any article from any publisher, allowing any user to link back to the authoritative record about a biological resource from the article. Currently, curators have found 820 journal articles, from 112 different journals (http://bit.ly/1VLu0yd), that contain at least one RRID, Research Resource IDentifier, for an antibody, organism, or tool and we ran SciBot on all of the papers. SciBot detected 7068 total annotations and curators have checked 780 papers for their validity. Of these, 465 annotations are for organisms, predominantly mice, 2031 are for tools such as ImageJ, and 4572 are made for antibodies. Cell lines have just become available to use as RRIDs and as of this writing no paper cited a cell line via the RRID syntax yet. The accuracy rate for authors reporting research resources in the first 820 papers remains at 97%, though syntax errors account for about 30% of the total RRIDs, these are predominantly from publishers that do not typeset the RRID syntax.SciBot and Hypothes.is are useful tools for robot assisted curation of the scientific literature, with freely and openly available code bases that can be used for other purposes (https://github.com/SciCrunch/scibot).



Disclosures: **A.E. Bandrowski:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Scicrunch Inc. **M. Martone:** A. Employment/Salary (full or part-time): Hypothes.is. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Scicrunch Inc. **J. Grethe:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Scicrunch Inc. **T. Gillespie:** None. **G. Pine:** None.

Poster

096. Automation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 96.14/LLL14

Topic: I.06. Computation, Modeling, and Simulation

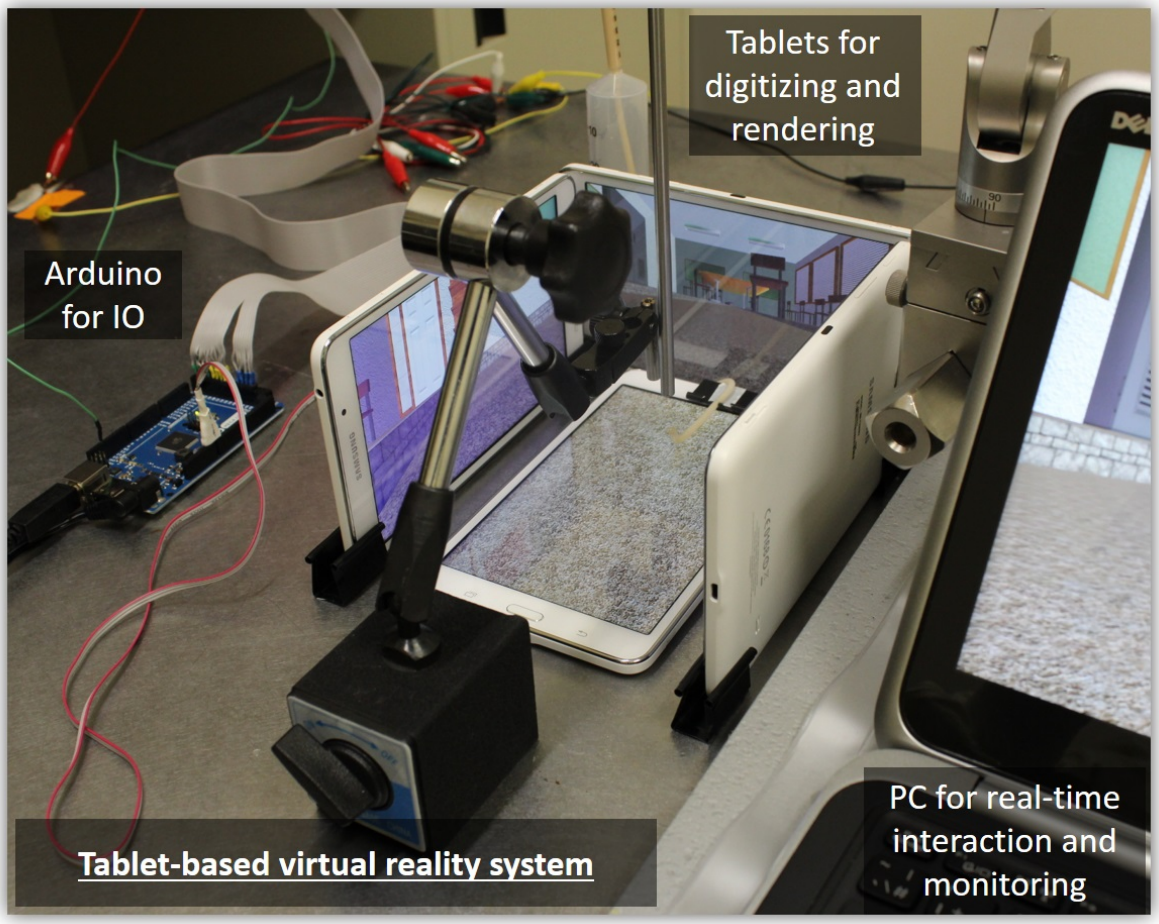
Support: AIHS Polaris Award MH46823-16

Title: Tablet based virtual reality system for research purposes

Authors: ***L. A. MOLINA**[1,2], M. A. NEEDHAM[1], M. H. MOHAJERANI[1], B. L. MCNAUGHTON[1];

[1]Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada; [2]Interphaser Ltd., Lethbridge, AB, Canada

Abstract: There is an increasing demand for virtual reality (VR) systems for research purposes, specifically in neuroscience. Implementing VR and neuroimaging or electrophysiology can be challenging due to limitations in the size or mobility of the data acquisition system, the behavioral apparatus or the subject. Here we describe a tablet-based VR system of easy implementation and maintenance, aimed at data collection, and allowing real-time interaction with the configuration of the virtual world and its state variables. Furthermore, we demonstrate its use in a goal oriented task in which mice learn to navigate towards cues associated with rewarding stimuli and avoid those associated to aversive stimuli. Lastly, we present a mechanism to synchronize this VR system with other data acquisition systems and use it to look at neuronal correlates of navigation.



Disclosures: **L.A. Molina:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); This method will be marketed by its author. **M.A. Needham:** None. **M.H. Mohajerani:** None. **B.L. McNaughton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual

property rights/patent holder, excluding diversified mutual funds); This method will be marketed by its author.

Poster

096. Automation

Location: Halls B-H

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Topic: I.06. Computation, Modeling, and Simulation

Support: This work was supported by DARPA Big Mechanism program under ARO contract 1130178-330433

NIH Grant GM109817

Title: Curating central injection studies from the literature using a general-purpose knowledge management strategy.

Authors: *G. A. BURNS[1], A. E. HERNANDEZ[2], A. M. KHAN[2];
[1]Intelligent Systems Div., Information Sci. Inst., Marina Dl Rey, CA; [2]Dept. of Biol. Sci., Univ. of Texas at El Paso, El Paso, TX

Abstract: The heterogeneity of neuroscience work and lack of consistent methods to model and curate experimentally-derived neuroscientific knowledge has led to a complex landscape of neuroinformatics data and knowledge repositories. We here describe a simple, well-defined knowledge modeling and curation methodology for experimental data and instantiate it with a systems-level neuroscience pilot study based on central (intracranial) injection studies. Experimental work involving central injections has involved injecting or infusing bioactive agents directly into the brain of awake behaving animals in order to observe behavioral effects of the infusion. These methods are now complemented by optogenetic approaches that provide more accurate methods of artificially stimulating or inhibiting genetically-defined neuronal populations. Importantly, we anticipate that for some neuroscience sub-domains, as the experimental focus of active researchers moves away from the central injection methodology, the details of the research findings from that body of work will likely no longer be actively incorporated into current research. We therefore seek to curate and preserve this referenced literature into an electronic resource rapidly and efficiently for future reference. We present several innovations to accelerate and ease the curation process: (A) the use of a PDF-based digital library with information retrieval methods to manage literature fragments supporting claims from the literature; (B) the application of general-purpose knowledge engineering methods (KEfED) that use descriptions of the protocols used in experiments templates to

generate spreadsheets for data entry; (C) the use of semantic web standard methods for storing, presenting and querying the data. We also investigate the use of machine reading techniques to further accelerate knowledge extraction from these papers and establish methods of practice to incorporate student training and involvement in this effort. All data and software gathered in this effort are provided as open source resources.

Disclosures: **G.A. Burns:** None. **A.E. Hernandez:** None. **A.M. Khan:** None.

Poster

096. Automation

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Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant NS095580

NIH Grant DA035913

NVIDIA Hardware Grant

Title: Neurogpu, a gpu framework for neuron-based simulation

Authors: R. BEN-SHALOM[1], N. S. ATHREYA[1], C. S. KI[1], H. SANGHVI[1], *K. J. BENDER[2,1];
[1]Neurol., UCSF, San Francisco, CA; [2]Sandler Neurosciences Building, San Francisco, CA

Abstract: Computational models, including compartmental models of individual neurons, are an important component of modern neuroscience. The NEURON simulation environment is one of the most common platforms for compartmental modeling, with an intuitive interface and broad user base. NEURON, like many simulation environments, relies on central processing unit (CPU) computation. Neuronal models can be computationally intensive, especially for large or complex simulations, and often models must be compromised in scale given available CPU-based resources. In our recent work, we developed a simulation environment, NeuroGPU, that relies on graphics processing units (GPUs) rather than CPUs. NeuroGPU is 150 times faster than traditional single core CPU processors, and offers a high level of computational power at low cost. Here, we developed new tools based on NeuroGPU processing that will allow for broad access to GPU-based modeling across the neuroscience community. First, we implemented NeuroGPU in the Python coding language, which allows one to run NEURON simulations via NeuroGPU. Second, previously NeuroGPU supported only Hodgin Huxley based ion channels mechanisms, recently we incorporated Markov ion channel models and point process such as

synapses and extracellular stimulation to NeuroGPU, allowing one to incorporate such models from ModelDB. These improvements to NeuroGPU will make it possible to support most of NEURON compartmental model capabilities and will make it possible for neuroscientists to take advantage of the computational power that low-cost GPU technology offers.

Disclosures: R. Ben-Shalom: None. **N.S. Athreya:** None. **C.S. Ki:** None. **H. Sanghvi:** None. **K.J. Bender:** None.

Poster

096. Automation

Location: Halls B-H

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Program#/Poster#: 96.17/LLL17

Topic: I.06. Computation, Modeling, and Simulation

Title: A neurally inspired spiking temporal processing unit computational architecture

Authors: *C. M. VINEYARD[1], J. B. AIMONE[1], M. R. SMITH[1], S. J. VERZI[1], J. DONALDSON[1], G. POPOOLA[1], F. WANG[1], D. R. FOLLETT[2], C. D. JAMES[1], J. H. NAEGLE[1];
[1]Sandia Natl. Labs., Albuquerque, NM; [2]Lewis Rhodes Labs, West Concord, MA

Abstract: Computational neural models are developed to help understand the brain and give insight into its functionality. Prior work modeling non-deterministic neuronal connectivity, brain plasticity, and spike timing have identified key differentiating operating principles from that of conventional von Neumann computation. Unlike general purpose computer architectures, which are based upon complex processor cores and sequential computation, the brain is innately parallel and comprised of simple computational units. Key to the architecture of the brain is a functionality encoded neurally by the combined effect of sparsity with unique variable efficacies and temporal latencies. Utilizing these neuroscience principles, we have developed a Spiking Temporal Processing Unit (STPU) architecture well suited for computational neural modeling and neural network instantiation by executing complexity and computation in the time domain. We implemented the neural inspired STPU architecture in a software simulator as well as on a field programmable gate array (FPGA). Here we show the results of implementing a variety of neural networks and algorithms including: circuits based upon early visual system and hippocampus, Liquid State Machine, SpikingSort, deep neural networks, and the SVM Game. The STPU architecture yields empirical evidence of the efficiency of cortically-inspired computation and provides a framework for a possible paradigm shift in problem solving from conventional computer architecture and processing.

Disclosures: **C.M. Vineyard:** None. **J.B. Aimone:** None. **M.R. Smith:** None. **S.J. Verzi:** None. **J. Donaldson:** None. **G. Popoola:** None. **F. Wang:** None. **D.R. Follett:** Other; Co-founder and owner of Lewis Rhodes Labs. **C.D. James:** None. **J.H. Naegle:** None.

Poster

096. Automation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 96.18/LLL18

Topic: I.06. Computation, Modeling, and Simulation

Title: Open science at the montreal neurological institute - LORIS & CBRAIN

Authors: *S. DAS;
McGill Ctr. for Integrative Neurosci., Montreal Neurolog. Inst., Montreal, QC, Canada

Abstract: As technologies mature and global research collaborations intensify, researchers are looking for practical solutions, not only to share large amounts of data, but also to manage, query and process datasets from complex multimodal studies, often well beyond the project's life cycle. In many cases, acquisition realities present a significant burden, therefore gaining access to public data allows for more robust analyses and greater exploratory data mining. To answer this demand, the Montreal Neurological Institute (MNI) has launched "Open Science", serving the public good by making both clinical and research data available to the world (Owens, 2016). The MNI ecosystem currently consists of two principal and complementary platforms, LORIS (www.loris.ca) and CBRAIN (www.cbrain.ca) (Das et al., 2015). Together, they will consolidate and be adapted for the following functionalities for a new implementation at an institutionallevel of public data sharing: 1) Comprehensive multimodal data linking: clinical, genomics, imaging, phenotypic, demographic. 2) Robust Quality Control mechanisms usable as covariates in analysis. 3) Long term storage (and web access) of collected data. 4) Enhanced, multimodal web visualization with realtime viewing and manipulation. 5) Secure, confidential encryption specifically designed for institutional data sharing. 6) Configurable pipelines and flags to facilitate acquisition and analysis 7) Access to High Performance Computing clusters for immediate processing. 8) Configurable modular functionality (filtering, summary statistics, personal dashboards). 9) Multimodal querying with full access control for all consented public data sharing. 10) Mobile access capabilities with responsive viewing. LORIS was first implemented at the MNI in 1999 to organize acquisition and analysis for the multisite MRI Study of Normal Brain Development (Evans and Brain Development Cooperative Group, 2006). It has since proliferated to more than 130 sites worldwide, with greater than 30,000 collection time points and 200,000 imaging scans. In complement, CBRAIN has been developed since

2009 as solution for reproducible distributed computing and tool sharing, serving over 420 users across 50 cities in 22 countries, with an allocation of approximately 6 million CPU hours/year from Compute Canada (www.computecanada.ca). Open Science at the MNI will be a concerted undertaking, leveraging years of experience from MNI platforms to facilitate data sharing on a global scale.

Disclosures: S. Das: None.

Poster

096. Automation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 96.19/LLL19

Topic: I.06. Computation, Modeling, and Simulation

Title: A high performance computing web service for the analysis of local field potentials in Neurodata Without Borders-fomatted datasets

Authors: *S. MACKESEY[1], P. PRABHAT[2], F. SOMMER[3];
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Abstract: Advances in recording technology driven by large-scale neuroscience projects (e.g. BRAIN initiative, Human Brain Project) promise to deliver unprecedented high-resolution recordings of brain activity. This flood of data is already straining both traditional methods of analysis and the existing neuroinformatics infrastructure. Analysis techniques requiring human intervention, such as manual editing of clusters in spike-sorting, do not scale to high-dimensional data. More promising are data mining techniques that reveal structure without human intervention. Unsupervised learning algorithms (e.g. sparse coding, independent component analysis) can extract features from both neural and behavioral signals. However, these methods are often computationally intensive and may require sophisticated implementations. The lack of any widely accepted common data format means that costly parallelized code is likely to be coupled to ad-hoc, idiosyncratic formats. This reduces the reuse potential of the code and disincentivizes development. In the past year, a consortium of large data producers operating under the name Neurodata Without Borders (NWB) have advanced an HDF5-based common data format. We are building a free web service on top of NWB that (1): exposes HDF5-based NWB datasets over an HTTP JSON API; (2): allows users to execute computationally intensive data analyses at scale; (3) provides job management and visualization capabilities. The service will leverage the co-location of data and high-performance computing resources at the National Energy Research Scientific Computing Center (NERSC). A library of analysis algorithms will be

made available for processing NWB-formatted datasets at CRCNS, visualizing the results, and sharing them with other users. At present, we are using convolutional sparse coding as a pilot algorithm.

Disclosures: **S. Mackesey:** None. **P. Prabhat:** None. **F. Sommer:** None.

Poster

096. Automation

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 96.20/LLL20

Topic: I.06. Computation, Modeling, and Simulation

Support: NSF 1458495

NSF 1458840

BBSRC Research Grant BB/N005236/1

Title: NSG-R: seamlessly integrating neuroscience tools with high performance computing

Authors: ***N. T. CARNEVALE**[1], P. GLEESON[2], R. A. SILVER[2], S. SIVAGNANAM[3], K. YOSHIMOTO[3], A. MAJUMDAR[3];
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Abstract: The recent trajectory of neuroscience research has been toward development and adoption of methods and tools that pose ever greater computational burdens. Examples include: powerful open-source neural simulators that enable creation of empirically based models of unprecedented complexity; neuroscience community projects (NCPs) that enable collaborative model development and promote data and model sharing; fMRI, high resolution light microscopy, and other imaging methods that generate large data sets that require numerically intensive processing and analysis. For growing numbers of neuroscientists, these advances have produced a critical need to use high performance computing (HPC) resources in their research and teaching--a need that the BRAIN Intitiative is likely to make more acute. That led us to develop the Neuroscience Gateway (NSG), the first and only science gateway that provdes neuroscientists convenient access to HPC resources. NSG's browser-based interface shields them from tedious technical and administrative details, enabling performance of tasks that exceed local hardware capabilities. However, users must first leave their familiar work environments and carry out numerous step-by-step actions that are potentially error prone. This motivated our next step in refining and extending NSG, which we present here: creation of a software

infrastructure that allows seamless access to HPC resources whether from the familiar environment of widely used NCPs or from the existing work environment on neuroscientists' own laptop or desktop computers. Called NSG-R, this infrastructure is implemented as a RESTful web services interface to the NSG. It will allow neuroscientists to access HPC resources in order to analyze data, run simulations, and retrieve results, all within the context of their familiar workflows. NSG-R will also allow running models directly on HPC from their own laptop or desktop computers. Open Source Brain is the first NCP that will integrate NSG-R, making HPC resources transparently available to its users. NSG-R will subsequently be integrated with other projects, e.g. ModelDB, the Neuroscience Information Framework, and OpenWorm, and made available to developers of neural simulators and neuroscience data analysis software. By enabling seamless, ubiquitous access to HPC, the NSG-R project is likely to have a transformative impact on research, catalyzing progress in neuroscience and widening opportunities for educational and career advancement regardless of the institutional affiliation of those who benefit from it. This project levels the playing field for all students and researchers by democratizing access to HPC.

Disclosures: **N.T. Carnevale:** None. **P. Gleeson:** None. **R.A. Silver:** None. **S. Sivagnanam:** None. **K. Yoshimoto:** None. **A. Majumdar:** None.

Poster

097. Data Analysis and Statistics: Software Tools I

Location: Halls B-H

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Program#/Poster#: 97.01/LLL21

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant NS057198

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International Headache Society in 2014 and TUBITAK in 2015

German Research Council (DFG TH 2031/1)

Title: Neurovascular Network Explorer 2.0: a database of 2-photon single-vessel diameter measurements in response to optogenetic stimulation of inhibitory neurons

Authors: P. A. SAISAN[1], P. TIAN[4,1], K. KıLıÇ[1], M. THUNEMANN[1], V. B. SRIDHAR[2], H. BARTSCH[3], A. M. DALE[1,3], A. DEVOR[1,3,5], *H. UHLIROVA[6,7,3];
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Abstract: Sharing of experimental data is of critical importance in neuroscience allowing a close inspection by the research community and facilitating the use of experimental data for modeling. However, with a few exceptions, data from individual studies conducted by regular size neuroscience labs are not shared. Previously, we provided an example of seamless and low-cost solution for sharing of such data. Specifically, we created a MATLAB® based Graphical User Interface (GUI) engine called Neurovascular Network Explorer 1.0 (NNE 1.0) to interact with a database of 2-photon measurements of sensory stimulus-induced diameter changes of rat cortical arterioles in vivo [1]. NNE 1.0 and the associated database can be downloaded from http://nil.ucsd.edu/index.php?menu=data and then runs either as a MATLAB script or as a standalone program on a Windows platform. The GUI allows browsing the database according to parameters specified by the user, simple manipulation and visualization of the retrieved records (such as averaging and peak-normalization), and export of the results. Further, we provided NNE 1.0 source code. With this source code, the user can database their own experimental results, given the appropriate data structure and naming conventions, and thus share their data in a user-friendly format with other investigators. Here, we present a second generation of this sharing engine called Neurovascular Network Explorer 2.0 (NNE 2.0). In addition to all previous functionalities, NNE 2.0 provides 3D structural vascular data and supports localization of individual measurements within the vascular network. NNE 2.0 operates on two independent databases from the mouse primary sensory cortex. The first one is analogous to that associated with NNE 1.0 and contains sensory stimulus-induced arteriolar diameter changes. The other one contains arteriolar diameter changes in response to selective optogenetic activation of cortical inhibitory neurons. The experimental data corresponding to these databases have been presented previously in the abstract form [2,3]. The new feature of the structural images may be utilized by the user for computational reconstruction of the microvascular network. Such reconstructions can provide a realistic foundation for bottom-up modeling of the vascular/hemodynamic responses, which are important for understanding cerebral blood flow regulation and physiological underpinning of functional Magnetic Resonance Imaging signals [4]. [1] Sridhar et al., Front. Neuroinform. 2014 May 20;8:56. [2] Uhlirova et al., SFN abstr 2014, 352.10 [3] Tian et al., SFN abstr 2014, 352.11 [4] Gagnon et al., J. Neurosci. 2015 Feb 25;35(8):3663-75.

Disclosures: **P.A. Saisan:** None. **P. Tian:** None. **K. Kılıç:** None. **M. Thunemann:** None. **V.B. Sridhar:** None. **H. Bartsch:** None. **A.M. Dale:** None. **A. Devor:** None. **H. Uhlirova:** None.

Poster

097. Data Analysis and Statistics: Software Tools I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 97.02/LLL22

Topic: I.07. Data Analysis and Statistics

Title: Development of mind monitoring system using call voice

Authors: *Y. OMIYA[1], N. HAGIWARA[1], S. SHINOHARA[2], M. NAKAMURA[2], S. MITSUYOSHI[2], S. TOKUNO[2];
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Abstract: Mental health disorder has become a problem in many developed countries and in order to cope with it, a screening technology that will help to check depression and stress is being sought. Self-administered psychological tests and biomarkers have been used in the past, but these are beset by problems such as reporting bias and inspection costs and they also impose a burden on those who conduct the tests. Previous research includes studies that estimate depression and stress state using voice data. The advantage with analysis using voice data is that, besides being non-invasive, it does not require special and exclusive equipment and therefore can be easily carried out remotely. In this research, we focus on the everyday speech sounds of the call voice over the phone and develop a system of monitoring the health condition using the smartphone voice. Additionally, we evaluate the algorithm implemented in this system. For voice evaluations we used the Disordered Voice Database and Program, Model4337 from KayPENTAX, a division of Pentax Medical Inc. This database contains recordings of voices from about 700 persons (healthy individuals and patients) of read speech of "Rainbow passage" (approximately the first 12 seconds). We compared the implemented method with other indices such as jitter, shimmer, and HNR, we have used the speech read of "Rainbow passage" to closely resemble natural free form speech. To evaluate the performance of each index in discriminating between patients and healthy individuals, we used area under the curve (AUC) in the receiver operating characteristic (ROC) plot, sensitivity, and specificity. With respect to jitter, the AUC was 0.827. With respect to shimmer, the AUC values was 0.706. With respect to HNR, the AUC values was 0.655. In contrast, implemented method, the AUC values was 0.852, demonstrating better discriminability between healthy individuals and patients compared to the conventional indices. Consequently, the system has been found to be consistent from speech input on the smartphone, through the analysis up to the output of result. Thereby, the mental health state of the user can now be easily measured on a daily basis.

Disclosures: **Y. Omiya:** None. **N. Hagiwara:** None. **S. Shinohara:** None. **M. Nakamura:** None. **S. Mitsuyoshi:** None. **S. Tokuno:** None.

Poster

097. Data Analysis and Statistics: Software Tools I

Location: Halls B-H

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Program#/Poster#: 97.03/LLL23

Topic: I.07. Data Analysis and Statistics

Support: JSPS KAKENHI Grant 15H03002

JST COI Program

Title: Psychological impact of Kumamoto earthquake by voice analysis using a smart phone application

Authors: *S. TOKUNO[1], Y. OMIYA[2], S. SHINOHARA[1], M. NAKAMURA[1], N. HAGIWARA[2], S. MITSUYOSHI[1];
[1]Verbal analysis of pathophysiology, The Univ. of Tokyo, Tokyo, Japan; [2]PST Corporation, Inc., Yokohama, Japan

Abstract: We developed an application that is measuring the health of the mind using the voice to call on smartphone which call MIMOSYS (mind monitoring system). This application determines the extent the depressive tendency by voice emotion recognition technology. The user does not need to be aware after once installed, can monitor the health of his/her mind by using the smartphone as a normal mobile phone.
We showed that this application has a sensitivity comparable to the self-administered questionnaire which is commonly used in past studies.
This application published on July 20, 2015 for research, to obtain about 1800 download until April 25th. We carried out a questionnaire about the user's attributes at the time of the first start-up of the application, and compared the results of questionnaire and the health score of mind by voice analysis. Additionally, there is a big earthquake in Kumamoto, the west part of Japan, thus we investigate the effect of the disaster on the mind of residence.
In comparison with the questionnaire, some interesting relationship between user's attributes and mind health score were observed. In the difference between men and women, women shows more depressive tendency compared with men. This is consistent with the male-to-female ratio of depression morbidity. In comparison with the body mass index (BMI), mild obesity is the most favorable, both of obesity and leanness showed depressive tendency. This also does not conflict with other health indicators. In addition, several results were obtain, such as the mind health correlates of income.
In the comparison of before and after earthquake, different reaction depending on the region has been observed.
The largest swing areas which close to the epicenter, initially, the mind health score is inclined to depressive tendency strongly, then it tends to be somewhat recovery was seen. In a little remote

areas from the epicenter, it was mutated to gradually depressive tendency from after the earthquake. In areas affected by the disaster in the Great East Japan Earthquake five years ago, tendency to gradually become high was observed after the earthquake. It might be affected by flashback of post-traumatic stress disorder (PTSD). In other areas, there was no particularly significant change.
As we show above, this application has big potential for health monitoring.

Disclosures: **S. Tokuno:** None. **Y. Omiya:** A. Employment/Salary (full or part-time): PST Corporation, Inc.. **S. Shinohara:** None. **M. Nakamura:** None. **N. Hagiwara:** A. Employment/Salary (full or part-time): PST Corporation, Inc.. **S. Mitsuyoshi:** None.

Poster

097. Data Analysis and Statistics: Software Tools I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 97.04/LLL24

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant MH093011

Title: Improvements to automatic detection and classification of dendritic spines

Authors: *S. TAPPAN, A. RODRIGUEZ, M. A. A. KARIM, D. HOPPES, P. J. ANGSTMAN, J. R. GLASER;
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Abstract: The role of dendritic spines is one of the most significant and important areas of neuroscience research. Plasticity of dendritic spine morphology plays a crucial role in development, aging, as well as in learning and memory. Also, many complex brain diseases, including autism spectrum disorders, schizophrenia, Alzheimer's disease, and Parkinson's disease are characterized by dendritic spine pathology including abnormal dendritic spine density and morphology, dendritic spine loss, and aberrant dendritic spine plasticity. In order to develop robust methods for quantifying three-dimensional (3D) dendritic spine morphology at repeated time points imaged *in vivo*, we improved upon existing methods to provide greater precision and accuracy.
Improvements were made to isolate spines in complex, high density regions. Elucidating individual dendritic spines in close proximity is difficult to do, particularly in the Z-dimension. Improved clustering utilizing whole clump branch assignment substantially improved correct branch assignment. Using morphological characteristics such as spine shape and size along with spatial constraints (e.g., their proximity to a dendrite), spines are more accurately identified and

assigned to correct dendritic branches that are in close proximity such as near bifurcations. Additionally, we developed a modified implementation of the Rayburst Sampling algorithm for diameter estimation for cases where spines are in direct contact. Within Neurolucida 360, spine detection can be restricted to individual branches and tools for splitting and merging spines, and manual reassignment to adjacent branches are provided. Preliminary results with manual validation demonstrate that these new methods are a substantial improvement as compared to prior implementations.
To improve dendritic spine shape classification, we compared our current method against a new method of length estimation rather than shortest path distance. By modeling a spine backbone that traces the length of the spine, and by positioning the head and neck of the spine along the backbone, we are able to more accurately characterize the spine shape. In cases where automatic determination is not sufficient, manual adjustment of the backbone points is permitted. The increased accuracy provides a more reliable means to classify the spines based on their morphology.
Together, these methods represent a substantial improvement over currently available automated methods. Furthermore, they open the possibility of improved automated comparison of dendritic spine morphology on 3D images acquired with *in vivo* multiphoton fluorescence microscopy at different time points.

Disclosures: **S. Tappan:** A. Employment/Salary (full or part-time): MBFBioscience. **A. Rodriguez:** A. Employment/Salary (full or part-time): MBF Bioscience. **M.A.A. Karim:** A. Employment/Salary (full or part-time): MBF Bioscience. **D. Hoppes:** A. Employment/Salary (full or part-time): MBF Bioscience. **P.J. Angstman:** A. Employment/Salary (full or part-time): MBF Bioscience. **J.R. Glaser:** A. Employment/Salary (full or part-time): MBF Bioscience.

Poster

097. Data Analysis and Statistics: Software Tools I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

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Topic: I.07. Data Analysis and Statistics

Support: NIH Grant R01MH1006674

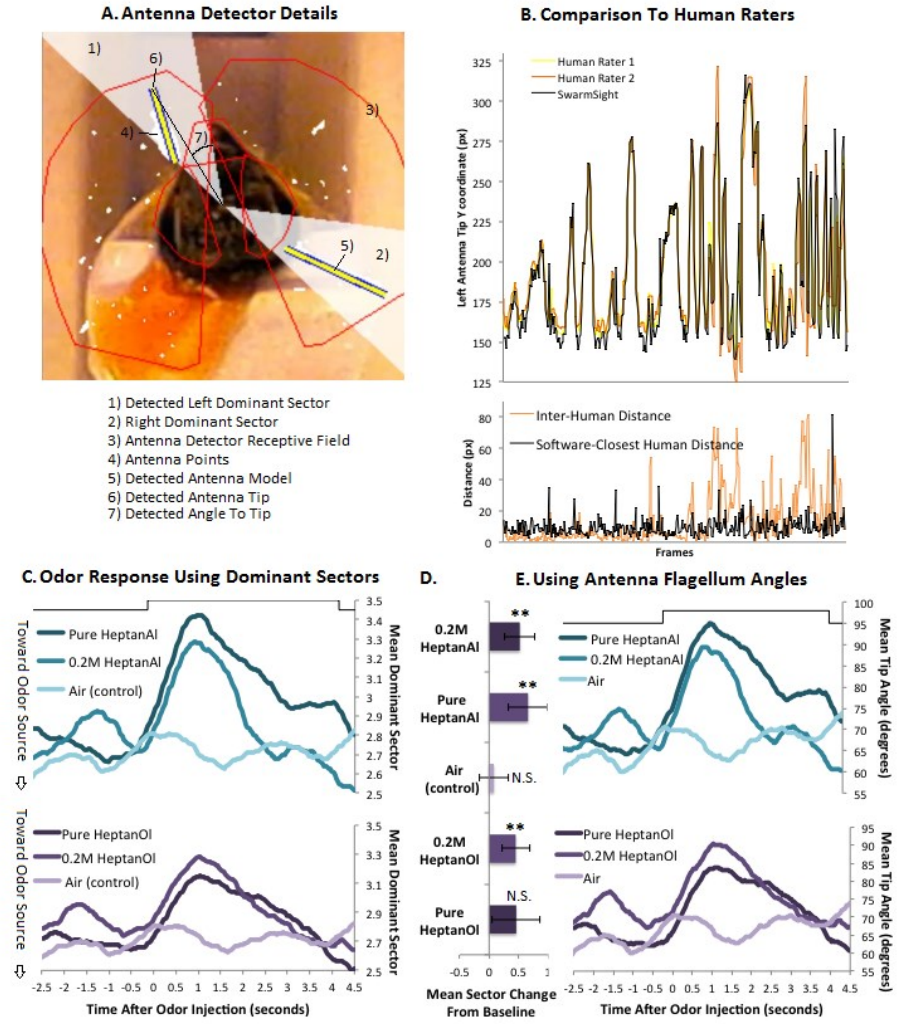
ASU School of Life Science Teaching Assistanship

NIH Grant NIH-NIDCD (DC007997)

Title: SwarmSight: Open-source software module for real-time, paint-free tracking of insect appendage movements using commodity hardware

Authors: *J. BIRGIOLAS[1], C. M. JERNIGAN[2], R. C. GERKIN[2], B. H. SMITH[3], S. M. CROOK[4];
[1]Neurosci., Arizona State Univ., Phoenix, AZ; [2]Sch. of Life Sci., Arizona State Univ., Tempe, AZ; [3]Sch. of Life Sci., Arizona State Univ., Phoenix, AZ; [4]Sch. of Mathematical and Statistical Sci., Arizona State Univ., Tempe, AZ

Abstract: Machine vision methods can greatly improve the efficiency of assessing animal behavior. In previous work, we described the open-source SwarmSight software (Github.com/JustasB/SwarmSight) for assessing animal group activity from videos [1]. Here we extend SwarmSight to analyze antenna movement from videos, with real-time speed processing (~30 fps). In the past, antenna movement analysis has required high-speed cameras and painting of the antenna tips [2], or lengthy (7.5 sec/frame) processing times [3].
We demonstrate the approach by obtaining the x, y and dominant angle sector locations of flagellar tips from conventional camera videos of restrained bees (Fig. 1A). We validate the software accuracy by comparing the tip locations found by two humans to the locations found by the software in several hundred video frames. Inter-human disagreement was 10.9 pixels (px) on average and within 30 px in 90.3% of the frames. The software was a mean of 8.5 px - and within 30 px in 99.2% of the frames - from the closest human location (Fig 1B).
We presented two odors to 23 restrained female bees (*Apis mellifera*) to assess the software's ability to measure temporal changes in innate antennal movement patterns. Videos of bees presented with pure and 35x diluted versions of heptanal and heptanol were analyzed with SwarmSight to track the dominant sectors (Fig 1C) and tip x, y locations (Fig 1E). For both odorants, dominant sector and angle means at both concentrations were significantly different (Holms adj. t-tests $p < 0.01$, Shapiro tests N.S.) from pre-odor-presentation means (Fig 1D).
Overall, SwarmSight enables efficient and accurate tracking of bee antenna tips without requiring special animal preparations or equipment. This approach can also be used to automate behavior assessment of appendage movements for bumblebees, crickets, ants, and other insects.
References [1] Birgiolas, Justas, et al. *Behavior Research Methods* (2016): 1-12. [2] Cholé, Hanna, et al. *Learn. Mem* 22 (2015): 604-616. [3] Shen, Minmin, et al. *Journal of Neuroscience Methods* 239 (2015): 194-205.



A. Video frame of a restrained bee and corresponding software antenna models and dominant angle sectors. Both sides are divided into five sectors, with Sector 1 enclosing angles 0º to 36º, and 144º to 180º for Sector 5. **B.** Comparison of human and software tip location detection. Top: Raw values of Y coordinate of left antenna tracked by two humans and software. Bottom: Inter-human rater per frame distance and software-closest human distance. **C.** Mean dominant sector per frame 2.5s before and 4.5s after odor presentation. Positive values are away from the odor **D.** Mean change in dominant sector from pre-puff baseline sector means. **E.** Mean tip angle per frame under same conditions as in C.

Disclosures: J. Birgiolas: None. **C.M. Jernigan:** None. **R.C. Gerkin:** None. **B.H. Smith:** None. **S.M. Crook:** None.

Poster

097. Data Analysis and Statistics: Software Tools I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 97.06/LLL26

Topic: I.07. Data Analysis and Statistics

Title: T-sne as a visualization step in the spike sorting pipeline

Authors: J. NETO, *G. DIMITRIADIS, A. KAMPFF;
Sainsbury Wellcome Ctr., London, United Kingdom

Abstract: Systems neuroscience is entering an era of 'Big Data' where recordings from multiple brain regions will be made with probes containing hundreds to thousands of individual electrodes. One of the problems introduced by these technologies is the transformation of the data into physiologically meaningful signals. Deriving the spiking information of neurons from such spatiotemporally dense sets is a problem that has attracted attention but is still far from resolved. Methodologies solely based on human input are becoming less appealing as the data sets increase in size and complexity. In tandem, as the feature space of the automated algorithms explodes, the previewing and manual correction of their results becomes impossible to achieve. Here we introduce the t-student stochastic neighbor embedding (t-sne) dimensionality reduction method as an extra step in the spike sorting pipeline. We position this step after the reduction of the signal with PCA of the detected spikes and before any further clustering. T-sne allows the embedding of the n-dimensional spike (n = Nchannels*Mpcs) into a low dimensional space. We show that this embedding creates obvious clusters of spikes that can be previewed and manually delineated with little effort and a high degree of confidence. We propose that these clusters represent single units and test the quality of this assertion by running our algorithm on labeled data sets both from hybrid and paired juxtacellular/extracellular recordings. Finally, we develop a GPU based extension to the algorithm that allows for fast embedding of millions of spikes. at <http://biorxiv.org/lookup/doi/10.1101/037937>

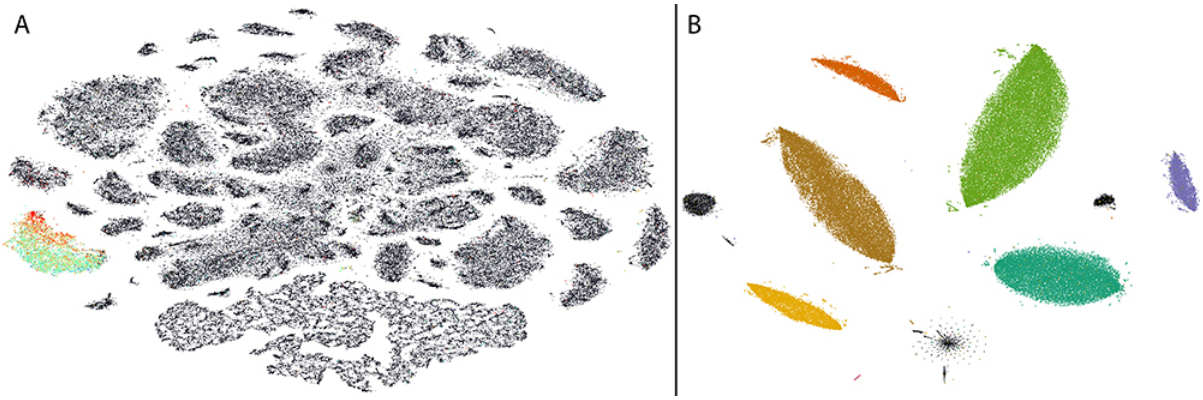


Figure 1. Spikes embedded in t-sne space. A) Paired recordings with one cell labeled by a parallel juxtacellular recording (color indicates size of juxtacellularly recorded spikes). B) Hybrid data with 7 units labelled (color indicates the units, black is unlabeled spikes).

Disclosures: J. Neto: None. **G. Dimitriadis:** None. **A. Kampff:** None.

Poster

097. Data Analysis and Statistics: Software Tools I

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NICHD Grant T32 HD040127

Simons Research Foundation

DARPA N66001-15-C-4032

IARPA MICrONS-D16PC00003

Title: Efficient and accurate extraction of *In vivo* calcium signals from microendoscopic video data

Authors: *P. ZHOU[1], S. RESENDEZ[2], G. STUBER[2], R. E. KASS[1], L. PANINSKI[3]; [1]Carnegie Mellon Univ., Pittsburgh, PA; [2]The Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [3]Columbia Univ., New York City, NY

Abstract: Monitoring the activity of large-scale neuronal ensembles during complex behavioral states is fundamental to neuroscience research. Continued advances in optical imaging technology are greatly expanding the number and depth of neuronal populations that can be visualized. Specifically, in vivo Ca2+ imaging through microendoscopic lenses and the development of miniaturized microscopes have enabled deep brain imaging of previously inaccessible neuronal populations within the brains of freely moving mice. While these techniques have been widely used in studies of brain-behavior relationships, automatic methods

for extracting cellular signals from this data are currently limited and suboptimal.
Constrained Nonnegative Matrix Factorization (CNMF) is a recently proposed method that has shown great advantages in simultaneously denoising, deconvolving, and demixing of calcium imaging data (Pnevmatikakis et al, Neuron 2016). This method was optimized for 2-photon and light sheet data, where the background is relatively weak and has a simple spatiotemporally separable structure. However, microendoscopic data suffer from high levels of background fluorescence from multiple sources as well as an increased potential for overlapping neuronal signals. The standard CNMF approach does not successfully isolate neural signals from the very strong fluctuating background in these experiments.
Here we extend the CNMF approach to better subtract strong background activity, using Singular Value Decomposition (SVD) together with low spatial frequency constraints. The spatial low-pass feature of the background is due to the large blurring effect of the out-of-focus fluorescence. We validate our method using 1-photon imaging data collected from freely behaving mice and find empirically that it is able to remove background signals that can be an order of magnitude larger than the individual neuronal signals of interest. Compared with the widely used PCA/ICA approach, the proposed method is much faster and extracts neurons with improved localized spatial structure and denoised temporal traces without negative events. Our analysis yielded fast, reliable, and high quality signal extraction under a wide variety of imaging parameters. We plan to release the code as open source software.

Disclosures: **P. Zhou:** None. **S. Resendez:** None. **G. Stuber:** None. **R.E. Kass:** None. **L. Paninski:** None.

Poster

097. Data Analysis and Statistics: Software Tools I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 97.08/LLL28

Topic: I.07. Data Analysis and Statistics

Title: Reliable identification of the same neurons across multiple days in calcium imaging data

Authors: *L. SHEINTUCH, A. RUBIN, N. GEVA, Y. ZIV;
Neurobio., Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Recent advances in optical imaging and genetically encoded Ca^{2+} indicators converged to enable chronic, cell-type specific recordings of neuronal activity from large neuronal populations in various brain structures of freely behaving rodents. The capability to repeatedly image populations of the same neurons over periods of time that range from days to months is important for longitudinal studies of neuronal dynamics, e.g., in learning and long-

term memory. Such longitudinal studies necessitate correct identification ("registration") of the same neurons across multiple imaging sessions. Accurate registration of the same neurons becomes challenging as the number of active cells in a session and the number of imaging sessions are increased. In previous work, cells were registered as the same if they were located within a certain distance threshold determined by the distribution of within-session nearest neighbor distances. Such an approach, however, does not always guarantee that the optimal registration threshold is being used and furthermore, it fails to take into account the morphology of the cells, which could provide critical additional information relevant to the registration decision. Previous work also lacked quantitative estimation of registration errors (i.e. false positives and false negatives), crucial for addressing coding stability and dynamics issues. Here we present a probabilistic method for registration of cells across multiple sessions based on the similarity in the spatial footprint of cellular activity. Our method inherently estimates the uncertainty associated with the registration of each cell-pair across sessions, allowing for the exclusion of uncertain registered cells from longitudinal studies. We have found morphology based cell registration to be superior to distance based registration, and that a high fraction (>85%) of the cell-pairs can be reliably registered ($p<0.05$) over periods of weeks. We validated the performance of our cell registration method using place cells in the CA1 of the hippocampus: over intervals of days–weeks, cells with high registration certainty levels had higher place field correlations compared to cells with lower certainty levels. Overall, our method allows for reliable cell registration to be performed across multiple sessions of Ca^{2+} imaging, facilitating longitudinal studies of neuronal dynamics.

Disclosures: **L. Sheintuch:** None. **A. Rubin:** None. **N. Geva:** None. **Y. Ziv:** None.

Poster

097. Data Analysis and Statistics: Software Tools I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 97.09/LLL29

Topic: I.07. Data Analysis and Statistics

Support: Brain/MINDS from AMED

Title: Detection of repetitive spike sequences in neural ensemble based on edit similarity

Authors: ***K. F. WATANABE**[1,2], T. HAGA[3], T. FUKAI[3];
[1]Riken BSI, Saitama, Japan; [2]Grad. Sch. of Frontier Sci., The Univ. of Tokyo, Kashiwa, Japan; [3]RIKEN BSI, Wako, Japan

Abstract: Recent technological advances enable us to record the activity of hundreds and thousands of neurons simultaneously in awake animals. However, mathematical methods available for analyzing large-scale neural data are still limited. Accumulating evidence suggests that sequences of neural firing play a role in various behavioral tasks such as spatial navigation, sensory discrimination and decision-making. In this study, we propose a novel method to detect the repetition of similar but noisy spatiotemporal patterns of firing in a large neural ensemble. Novel methods for sequence detection has to be developed since the conventional analysis methods, such as template matching, are neither efficient nor robust when applied to large-scale data in the presence of biological noise, which may induce strong fluctuations in the temporal order of neuronal firing, and eliminate some spikes from or add additional spikes to repeated sequences. To overcome the difficulties, our method extended a concept of edit similarity to determine similarity between two different temporal segments of neuronal activities. We will verify the proposed method by using artificial and experimentally obtained neural activity data.

Disclosures: **K.F. Watanabe:** A. Employment/Salary (full or part-time): Riken Brain Science Institution. **T. Haga:** A. Employment/Salary (full or part-time): Riken BSI. **T. Fukai:** A. Employment/Salary (full or part-time): Riken Brain Science Institution.

Poster

097. Data Analysis and Statistics: Software Tools I

Location: Halls B-H

Time: Saturday, November 12, 2016, 1:00 PM - 5:00 PM

Program#/Poster#: 97.10/LLL30

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant T32 AA007462

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NIH Grant AA4694763

Title: Improvements to information theory analysis techniques throughout neuroscience with matlab support

Authors: ***N. TIMME**, D. N. LINSENBARDT, M. MYROSHNYCHENKO, C. C. LAPISH; Psychology, IUPUI, Indianapolis, IN

Abstract: Understanding how neural systems integrate and encode information is central to understanding brain function. An explosion in the availability of approaches that can be used to examine interactions across varying levels of brain function brings with it new challenges and opportunities. Information theory is well suited to the wide array of experiments and the

challenging nature of data analysis typical to neuroscience. Frequently, data from neuroscience experiments are multivariate, the interactions between the variables are non-linear, and the landscape of hypothesized or possible interactions between variables is extremely broad. Information theory is well suited to address these types of data as it possesses multivariate analysis tools, it can capture non-linear interactions, and it is model independent (i.e. it does not require assumptions about the structure of interactions between variables). Methods currently exist to apply information theory analyses to many different types of data, including discrete data, continuous data, single trial data, trial based data, and aggregate data that result from dimensionality reduction techniques (e.g. principle component analysis). In total, information theory is a powerful tool for highlighting and detecting complex interactions in large systems with many types of variables. To reduce barriers to the use of information theory analyses, we have created a free MATLAB software package that can be applied to a wide variety of typical neuroscience data analysis scenarios. In addition to utilizing established analysis routines, this software package also includes several improvements to analyses of continuous data and trial based data. As demonstrations, we applied the software package to numerous model systems, including models of large Izhikevich networks, sensory habituation in Aplysia, location encoding in hippocampal place cells, movement direction encoding in primary motor cortex, and light stimulus encoding by center selective retinal ganglion cells. Among other things, analyses of these models showed time dependent information flow through networks, synergist and redundant encoding by neurons, and encoding schemes modulated by inhibition, background activity, and stimulation correlation.

Disclosures: **N. Timme:** None. **D.N. Linsenbardt:** None. **M. Myroshnychenko:** None. **C.C. Lapish:** None.

Poster

097. Data Analysis and Statistics: Software Tools I

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Topic: I.07. Data Analysis and Statistics

Support: Wellcome Trust 095668

Wellcome Trust 100154

Title: Alyx: an open-source database for efficient data organisation and collaboration in neurophysiology

Authors: *M. L. HUNTER, C. P. BURGESS, K. D. HARRIS;
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Abstract: Data management is a major and growing challenge in experimental neurophysiology. Many different types of information need to be stored, managed and shared, from animal husbandry, through many kinds of experimental data and metadata, to human-curated results and conclusions, as well as the relationships between these different data ontologies.
Current solutions are generally specific to individual experimenters or groups, and target only a subset of these data types. Not only does this take time in maintaining multiple systems and training users, but it also severely limits collaboration and sharing, and causes particular problems in experiments where many different data types need to be managed and linked together. Such an ad-hoc arrangement can also make it very difficult to reuse old datasets where crucial metadata is missing or unclear.
We developed 'Alyx', an open-source, extendable solution to the challenge of storing metadata and managing large neurophysiology datasets. An online, intuitive "electronic lab notebook" allows users to manage, view and share data with little to no training when performing an experiment or other procedure. The database links these records to the location of raw data files; multiple types of data acquired simultaneously can also be linked together. Software performing data acquisition or processing (such as automated weighing, spike sorting or two-photon ROI detection) uses a developer-friendly REST API, accessible from Python, MATLAB, and many other languages, to retrieve raw data files and store the associated results automatically. Sharing of data can be achieved by exposing the database API and relevant data files over the Internet, or exporting the files and metadata into a ZIP archive.
The system is based on mature, tested, and well-documented technologies. An SQL-backed data model, written in Python, is based on the Neurodata Without Borders specification. It currently incorporates standardized modules for managing experimental subjects and equipment, as well as data from electrophysiology, two-photon and widefield imaging experiments, behavioural data and arbitrary other files. Additionally, the data model allows for experiment-specific hierarchical data in the open JSON format with no need for a centrally agreed standard. Alyx can be hosted locally on a private server, cluster, or on commercial cloud hosting solutions. It integrates with network fileservers and can be easily adapted to use existing file storage structures. It efficiently deals with the problem of simultaneously storing data in multiple locations, including offline tape or disk archives.

Disclosures: M.L. Hunter: None. **C.P. Burgess:** None. **K.D. Harris:** None.

Poster

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Topic: I.07. Data Analysis and Statistics

Support: NIH NINS/NIMH 1R01NS092474 (TRA)

Title: NDIO: Neuroscience discovery input and output

Authors: *J. MATELSKY[1], S. BERG[5], A. EUSMAN[2], K. LILLANEY[2], J. T. VOGELSTEIN[3,4], G. D. HAGER[2], R. BURNS[2], W. R. GRAY RONCAL[2,6];
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Abstract: While neuroimagery datasets continue to grow, our methods for accessing them remain relatively disparate and inaccessible. Researchers have developed a rich set of tools to help reach neuroscientific conclusions. However, in order to store, retrieve and analyze data, it is often necessary to understand many different data specifications, and develop ad-hoc tools to standardize data formats and retrieve the data subvolumes of interest. This has the potential to impede scientific progress, especially across laboratories and datasets, and currently results in science that is difficult to reproduce or extend.

We present ndio, a toolkit that implements a neuroscience API in a simple and easily approachable software package. ndio is extensible and offers easy-to-use wrappers for RESTful calls to get and put image and annotation data using NeuroData, DVID, and S3 — enabling data access and sharing across many neuroscience research labs. ndio provides a common vocabulary for big data neuroscience and allows researchers to focus on scientific discovery, abstracting many of the problems associated with big data. We also implement RAMON (Reusable Annotation Markup for Open Neuroscience), an existing data standard providing common metadata for annotations across modalities, research groups and projects. An illustration of ndio operations from an end-user perspective is shown in Figure 1.

ndio is stable and is in active use by several teams across a variety of modalities, including Electron Microscopy, MRI, Array Tomography, and CLARITY. It is publicly developed under a permissive, open-source license and can be installed from PyPI using a single command line call. We demonstrate the utility of ndio through a series of claims that reproduce many of the scientific discoveries in the recent high-profile Kasthuri 2015 Cell Paper entitled: “A Saturated Volume of Neocortex.” Additional functionality and features are planned and the community is invited to contribute via our github repository at: github.com/neurodata/ndio.

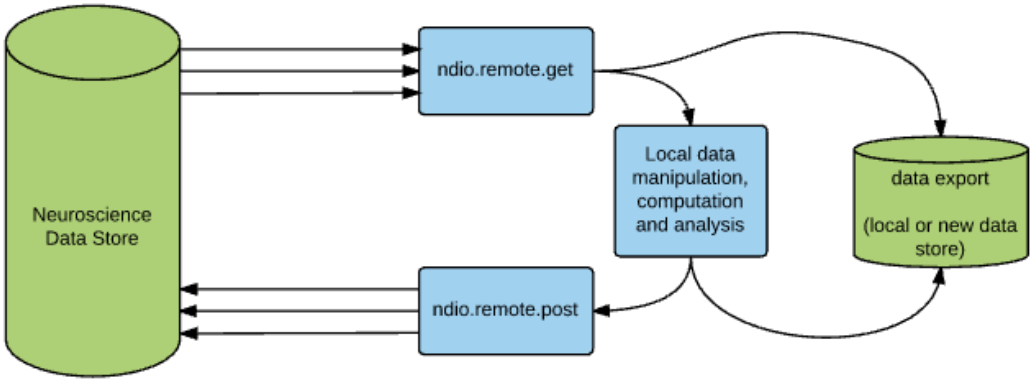

Figure 1: A high-level view of ndio being used for scientific discovery. Large data requests (gets and puts) are divided into smaller subvolumes in a process that is transparent to the end user. Data may be exported to common formats (e.g., numpy, hdf5, nifti) at any point using the ndio tools.

Disclosures: J. Matelsky: None. **S. Berg:** None. **A. Eusman:** None. **K. Lillaney:** None. **J.T. Vogelstein:** None. **G.D. Hager:** None. **R. Burns:** None. **W.R. Gray Roncal:** None.

Poster

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Topic: I.07. Data Analysis and Statistics

Support: University Of Manitoba

NSERC

Title: Quantitative tract integrity profile (q-tips) as a novel toolbox for assessing tract-based white matter integrity

Authors: *S. YOUNAS[1], S. M. COURTNEY[3], M. MARTIN[4], C. R. FIGLEY[2];
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Abstract: Quantitative approaches for diffusion tensor imaging (DTI) and other advanced white matter imaging methods have typically fallen into one of two categories: region-of-interest (ROI)

or voxel-wise analyses. ROI analyses extract data from relatively large, pre-determined regions, thereby facilitating the investigation of global white matter differences at the expense of sensitivity to small, localized changes. On the other hand, while voxel-wise analyses maintain high spatial resolution (i.e., by performing point-by-point analyses), sensitivity can suffer in group analyses if, for example, lesion sizes/locations vary across subjects. In order to address these limitations, a few recent studies have proposed tract-based analyses that extract quantitative imaging values along white matter tracts to combine the benefits of ROI and voxel-wise approaches.

However, although promising, existing tract-based analysis approaches have several limitations: 1) they currently use complicated, in-house software that requires multiple steps and command line inputs; 2) they are platform (Linux) dependent and therefore cannot be run on Windows-based workstations; 3) they require tractography to be performed in order to extract quantitative values, and are therefore exclusively for DTI data (i.e., cannot be used to create tract profiles from other quantitative white matter imaging modalities); and 4) most of them rely on visual examination of these curves and have not implemented more advanced methods to quantify the white matter integrity profiles (e.g., by comparing each subject's tract profile to a normative profile).

To address these issues, we have developed the *Quantitative Tract Integrity Profiles (Q-TIPs)* toolbox, which is purely MATLAB-based (making it compatible with any OS), has a flexible and user-friendly interface, and will be distributed as a standalone toolbox for the popular SPM software package. This toolbox extracts the orientation of any user-defined ROI mask by calculating its medial axis using Voronoi and Delaunay algorithms, and then cross-sectional segments along the medial axis are used to create profiles for any quantitative white matter imaging metric (e.g., DTI, myelin water imaging, magnetization transfer imaging, etc.). For final quantification, curve-fitting methods are then implemented to compare each individual tract to normative or group-averaged tract profiles.

This toolbox can be used to investigate structure-function or structure-behavior correlates as a function of neural development, healthy aging, or white matter disease, and is therefore expected to have broad research and clinical implications.

Disclosures: **S. Younas:** None. **S.M. Courtney:** None. **M. Martin:** None. **C.R. Figley:** None.

Poster

097. Data Analysis and Statistics: Software Tools I

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Topic: I.07. Data Analysis and Statistics

Support: NIMH grant R44-MH108053-01

Title: Automatic anatomic orientation from the atlas to histological sections and back

Authors: *N. J. O'CONNOR[1], B. S. EASTWOOD[1], S. J. TAPPAN[1], M. FAY[1], K. E. DAY[1], P. J. ANGSTMAN[1], C. GERFEN[2], J. R. GLASER[1];
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Abstract: Determining position in brain images can be a difficult task even for experienced neuroanatomists and usually involves comparing images in a microscope, or on a monitor, to structures in a paper or digital reference atlas. Here we present a novel software platform that facilitates whole brain analysis of anatomy and function by combining automated methods for reconstructing whole brain images from serial sections, aligning those sections to a reference atlas, and annotating both sections and 3D brain images with atlas delineations. The registration of brain images to a common reference atlas allows researchers to objectively make and compare measurements across brains and even across laboratories.
The components we developed for this platform include an automated alignment method for creating full-resolution 3D brain images from serial sections. This method corrects for translation, rotation, and flipped between sections as it compiles 3D images. The platform also includes alignment methods for matching individual experimental sections and 3D brain images with the Allen Institute for Brain Science's average mouse brain and its atlas (http://mouse.brain-map.org/). This matching process creates transforms that are used to maps the atlas's delineated brain structures onto a section or 3D brain. The transforms also enable the tabulation of cell counts obtained from experimental images into distinct brain regions.
To test these methods we analyzed fluorescent mouse brains comprised of over one-hundred coronal sections from mice injected with an AAV-Cre-dependent expression vector, counterstained with fluorescent Nissl stain and imaged by fluorescent whole slide imaging (Neurolucida). Sections from these images were automatically extracted, aligned, and compiled into full resolution 3D whole-brain images, and Cre-positive cells were marked using Laplacian of Gaussian automated detection methods (BrainMaker). The brain images were then matched to the Allen average brain and atlas to create annotation overlays, and mapped cell tabulated by brain region. The accuracy of mapping the atlas's delineations onto experimental sections was assessed by eye.
Here we introduce new technology to reconstruct whole brain images from serial sections, map annotations from a standard atlas onto experimental data, and determine anatomically partitioned cell counts in 3D brain images. The benefits of this new platform for neuroscience research include improved objectivity when identifying brain regions in images, the ability to analyzing more brain regions than is currently done, and the generation of data that can be compared across brains or laboratories.

Disclosures: **N.J. O'Connor:** A. Employment/Salary (full or part-time): MBF Bioscience. **B.S. Eastwood:** A. Employment/Salary (full or part-time): MBF Bioscience. **S.J. Tappan:** A. Employment/Salary (full or part-time): MBF Bioscience. **M. Fay:** A. Employment/Salary (full or part-time): MBF Bioscience. **K.E. Day:** A. Employment/Salary (full or part-time): MBF

Bioscience. **P.J. Angstman:** A. Employment/Salary (full or part-time): MBF Bioscience. **C. Gerfen:** None. **J.R. Glaser:** A. Employment/Salary (full or part-time): MBF Bioscience.

Poster

097. Data Analysis and Statistics: Software Tools I

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Topic: I.07. Data Analysis and Statistics

Support: NIH Grant DA036241

NIH Grant AA020960

NIH Grant AA021667

Title: Digital imaging library of brain CRF distribution in serial sections

Authors: *H. J. KARTEN[1], N. O'CONNOR[2], T. KAWAMURA[3], P. E. SAWCHENKO[4], P. SANNA[3];
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Abstract: Whole Slide Imaging (WSI) is a powerful tool for comprehensive viewing and mapping of complete sets of serial sections of histological material at a resolution of 0.5 um/pixel. WSI enabled us to generate detailed histological image databases that allow users to independently assess the distribution and density of molecular markers, using genetically encoded fluorescent markers, immunohistochemistry, *in situ* hybridization, and autoradiography in bright field and fluorescent material. These image databases will be freely shared over the Internet, thereby circumventing the subjective nature of most journal reports with regard to the localization and density of molecular markers. Corticotropin-releasing factor (CRF) is a neuropeptide that was isolated from the hypothalamus on the basis of its capacity to initiate the neuroendocrine stress cascade. In addition, its broad central distribution includes aspects of the limbic forebrain associated with affective behaviors, placing CRF in a position to participate in complementary modes of stress adaptation. Here, to provide a comprehensive resource of the brain CRF distribution, we used Crh-IRES-Cre transgenic mice (B6(Cg)-Crhtm1(cre)Zjh/J) that express Cre recombinase under the control of the endogenous CRF promoter crossed with a floxed td-Tomato reporter line (B6.Cg-Gt(ROSA)26Sortm14(CAG-TdTomato)Hze/J) in which a *loxP*-flanked STOP cassette prevents transcription of a CAG promoter-driven red fluorescent

protein variant (tdTomato). In these mice, TdTomato is expressed following Cre-mediated recombination in CRF neurons to visualize CRF-expressing cells with high fidelity (Chen Y. et al 2015). To generate a database of histological images of CRF-expressing neurons, we developed software to automatically detect, order, and align serial sections from whole slide images. Anatomic regions in aligned sections, scored for the density of CRF-expressing cells and hyperlinked to a list of their anatomical structures within section images, are compiled into a web-based, interactive anatomical structure hierarchy. The sections, scores and hierarchy published to an image server can be made available for public viewing and searching.

Disclosures: **H.J. Karten:** None. **N. O'Connor:** None. **T. Kawamura:** None. **P.E. Sawchenko:** None. **P. Sanna:** None.

Poster

097. Data Analysis and Statistics: Software Tools I

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Support: National Science Foundation China under grant 61402279

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Open Project Program of the State Key Lab of CAD&CG under grant A1502

Title: Disseminating Vaa3D and its high-performance, open-source, cross-platform neuron image toolbox

Authors: ***Y. WANG**[1,2], Z. ZHOU[2], X. LIU[2], A. BRIA[3], Y. GUO[4], H. PENG[2];
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Abstract: Vaa3D (http://vaa3d.org) is a widely used image visualization and analysis software system in recent years. Vaa3D has a number of powerful functions such as visualization, interaction, computation, analysis, and management of multidimensional terabyte-level image data [1,2,3].
However, whereas Vaa3D is a cross-platform (Windows, Mac OS, and Linux) software package and is quickly becoming one of the most advanced software systems for neuroscience applications, building Vaa3D from source code is sometimes a cumbersome process. For instance, while 80% of the downloaders of Vaa3D are Windows users, it is not trivial to

successfully build a Windows Vaa3D distribution from source code due to various recent upgrades in Windows operating system, the underlying Qt libraries, and the C/C++ compilers. In this study, we improve the cross-platform compatibility of Vaa3D. We have updated the GitHub documentation for the Vaa3D Windows building procedures for several often-used building tools, which can be easily followed and reproduced. In our latest release of Vaa3D, we have produced more than 100 plugins on top of the core Vaa3D platform (version 3.2) for various neuroscience applications including displaying, filtering, comparison, segmentation, stitching, fusion, 3D geometry representation generation, quantitative calculation and analysis, heterogeneous image and surface data processing, automatic and interactive neuron morphology tracing and analysis, terabyte image processing and annotation, and so on.

[1] Peng, Hanchuan, et al. "V3D enables real-time 3D visualization and quantitative analysis of large-scale biological image data sets." *Nature biotechnology* 28.4 (2010): 348-353.
[2] Peng, Hanchuan, et al. "Extensible visualization and analysis for multidimensional images using Vaa3D." *Nature protocols* 9.1 (2014): 193-208.
[3] Bria, Alessandro, et al. "TeraFly: real-time three-dimensional visualization and annotation of terabytes of multidimensional volumetric images." *Nature methods* 13.3 (2016): 192-194.

YW, ZZ, XL are of equal contribution.

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Disclosures: **Y. Wang:** None. **Z. Zhou:** None. **X. Liu:** None. **A. Bria:** None. **Y. Guo:** None. **H. Peng:** None.

Poster

097. Data Analysis and Statistics: Software Tools I

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Topic: I.07. Data Analysis and Statistics

Support: NIH 1U01NS090455-01 Award

Title: Baseline drift detrending techniques for fast scan cyclic voltammetry

Authors: *M. DEWAELE[1], Y. OH[1], C. PARK[1], Y. KANG[1], H. SHIN[1], I. KIM[1], K. BENNET[2], K. LEE[3], D. P. JANG[1];
[1]Grad. Sch. of Biomed. Engin., Hanyang Univ., Seoul, Korea, Republic of; [2]Engineering, Neurosurg., [3]Neurosurg., Mayo Clin., Rochester, MN

Abstract: Removing baseline drift from fast scan cyclic voltammetry (FSCV) is essential for accurate data analysis. While FSCV is useful because of its ability to track short term changes in

metabolite concentration, accurate analysis of concentration changes in vivo is difficult because of the large changes in background signals over extended time. The drifting background signals can obscure the much smaller signals from metabolites such as dopamine. During brain surgery, while using FSCV to track metabolites such as dopamine, which can take hours, the background must be stabilized, so that it can be subtracted, which can take valuable time. Current limitations of FSCV analysis include needing to move the background reference point often because of changes over long times. In this paper, we will investigate three techniques to attempt to remove the baseline drift and accurately display phasic changes in dopamine concentration both in vitro and in vivo. Using time polynomial regression, shape component regression, and a high pass filter, we will compare and contrast these methods to detrend the data series. When comparing the three detrending techniques, high pass filtering significantly showed the best performance. Both the time polynomial regression and shape component regression techniques failed to produce a stable baseline, resulting in significant variance from the desired baseline of zero. Using a high pass filter, the low frequency baseline drift was removed, and only the signals due to phasic changes in dopamine concentration remained. Thus we conclude that this filtering technique is preferable to the aforementioned alternatives. Keywords: Fast-scan cyclic voltammetry (FSCV); Detrending; Dopamine; Baseline drift. **Acknowledgement:** This research was supported by the NIH 1U01NS090455-01 award.

Disclosures: **M. Dewaele:** None. **Y. Oh:** None. **C. Park:** None. **Y. Kang:** None. **H. Shin:** None. **I. Kim:** None. **K. Bennet:** None. **K. Lee:** None. **D.P. Jang:** None.

Poster

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Topic: I.07. Data Analysis and Statistics

Support: Whitehall 2014-5-18

NSF BCS143221

NEI R01EY026924

Title: Optimizing cage-based training for non-human primates

Authors: ***M. D. CURRY**[1], M. PARSA[2], A. CILKER[3], L. VIOLETTI[3], M.-R. A. DEHAQANI[4], B. NOUDOOST[1];

[1]Cell Biol. and Neurosci., [2]Computer Sci., [3]Montana State Univ., Bozeman, MT; [4]Sch. of Cognitive Sci., Inst. for Res. in Fundamental Sci., Tehran, Iran, Islamic Republic of

Abstract: Non-human primates (NHPs) are widely used experimental models in neurophysiological studies. Training on cognitive tasks prior to collecting neurophysiological data is an inseparable part of much of the research conducted using NHPs. Any improvement in the training method that reduces stress to the animal, increases the speed of training or improves performance on the task is of great potential value. Nevertheless, training procedures vary greatly from lab to lab and are likely far from being optimized. We have designed, built and successfully utilized a fully portable cage-mountable system to train NHPs. Although this system has been developed using Rhesus monkeys (*Macaca mulatta*), it could be used to train a variety of monkey species. Here we describe the technical specifications, construction, and operation of the system. The flexibility and portability of both the animal interface (AI) and the control unit (CU) of this system would allow it to be used for a large variety of behavioral paradigms. This method has been used to train rhesus monkeys on two different behavioral paradigms including the delayed match-to-sample (DMS) task and a change detection task. Utilizing the in-cage training system allows the animal greater control over when and how long it chooses to work, rather than imposing a training schedule based on the availability of the experimenter. Using this method the animal learned to perform both behavioral tasks in a short amount of time; based on our experience, learning of the DMS task was faster with in-cage training than with traditional methods. In some cases the animal would use the training system without the need for any water restriction. In addition to allowing voluntary, self-paced engagement with the task, this method has the advantage of being less disruptive to the monkey's social interactions, and presumably eliminating some of the stress occasioned by relocating for chair training. This system has the potential to ease and expedite the behavioral training of non-human primates on a variety of tasks.

Disclosures: **M.D. Curry:** None. **M. Parsa:** None. **A. Cilker:** None. **L. Violetti:** None. **M.A. Dehaqani:** None. **B. Noudoost:** None.

Poster

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Topic: I.07. Data Analysis and Statistics

Support: NIH Intramural Research Program

Title: Feeding Experimentation Device Pus (fed+): an open-source behavior device for monitoring rodent home cage feeding in real-time

Authors: *J. A. LICHOLAI[1,2], K. P. NGUYEN[1], A. V. KRAVITZ[1,3];
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Abstract: To study obesity and related disorders, researchers need to accurately measure food intake. The most common method for monitoring food intake involves manual periodic weighing of food, which is time consuming, labor intensive, and produces data with a low temporal resolution. Several excellent commercial systems exist that can capture high-resolution information about food intake, but these cost thousands of dollars. Previously, our lab made the Feeding Experimentation Device (FED) that (1) is low-cost, (2) is home cage compatible, and (3) can measure food intake and feeding patterns over multiple days (Nguyen et al., J Neurosci Methods, 2016). FED is packaged within a 3D printed case and equipped with technology to detect and record the removal of food pellets. With this project, we upgraded FED to provide more flexibility in conducting food intake and operant experiments, with the ease and security of online data management. The new modifications includes an output connector allowing integration of FED+ with other equipment, such as data acquisition systems for electrophysiology or photometry, by synchronizing time points from the two systems via TTL signals. We also added an ability to stream data to an open-source phant server, where the data can be accessed and graphed in real-time. Lastly, FED+ has two additional sensors for training rodents on operant tasks. This permits operant tasks to be learned in a home-cage environment and with extended training sessions. FED+ continues to be made from easily accessible hardware components and all of its code and hardware are open-source. Altogether, FED+ monitors feeding behavior and operant learning in real-time, with easy synchronization with other scientific equipment.

Disclosures: J.A. Licholai: None. **K.P. Nguyen:** None. **A.V. Kravitz:** None.

Poster

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Topic: I.07. Data Analysis and Statistics

Support: NIH Grant 1U24DA039832-01

Title: The neuroscience lexicon: return of the STD

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Abstract: Scientific communities communicate using shared language. Communication between subfields of neuroscience, which all have their own partially overlapping technical vocabularies, is especially challenging due to the fantastic diversity of their methodologies and subjects of research. Given this diversity of perspectives on the nervous system, formal vocabularies, terminologies, lexicons, and ontologies are vital for enabling effective communication between experts in different subfields of neuroscience. To this end, the Neuroscience Information Framework (NIF) has built and maintains the NIF standard ontology (NIFSTD) and NeuroLex, a community lexicon for neuroscience. Since the start of the NIF ontology in 2008, tooling for working with ontologies has improved enormously. In addition, a suite of new tools for online collaboration has achieved wide adoption and provides better ways for individuals to contribute to formal ontologies. We have migrated the NIF ontology to GitHub at https://github.com/SciCrunch/NIF-Ontology to make it easier for individuals and organizations to contribute, and have participated in the development of SciGraph, a new tool for deploying ontologies at scale which vastly simplifies integration of ontologies into other information systems. We have restructured the layout of the NIF ontology and converted all internal ontology files to Turtle format (ttl, terse triple language) to improve human readability and simplify change tracking. We are curating the community contributed information contained in NeuroLex and integrating it into the NIF ontology so that the knowledge can be used to tag and search neuroscience data sources via the NIF data federation. Finally, we have updated and streamlined the process by which community contributions are reviewed and incorporated into the formal NIF ontology and built a new tool, InterLex, for managing community terminologies. Here we will present a detailed overview of the new architecture for the community and formal ontologies, as well as details on how to contribute to and make use of them.

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Poster

097. Data Analysis and Statistics: Software Tools I

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Topic: I.07. Data Analysis and Statistics

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Title: EEG as an imaging tool: which inverse method can successfully disentangle sources in proximity?

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Abstract: The accuracy of EEG source localization depends on the choice of the inverse method, the resolution of the forward model, and the signal to noise ratio (SNR) of the recordings. Since we are interested in disentangling sources in proximity, the goal of our study is to examine the sensitivity of spatial resolution of EEG source reconstruction to a wide variety of factors like reconstruction method, SNR, orientation, inter-dipole distance and depth of the simulated dipoles, etc.
We simulated time series to resemble waveforms of somatosensory evoked potentials. Inter-dipole distances and different dipole orientations were investigated as well as the effect of (realistic) noise. We employed both spherical and realistic head models. Source reconstruction was realized using a conventional stationary dipole model, MUSIC, self-consistent MUSIC (SC-MUSIC) algorithm, and e-LORETA. In addition to the above mentioned methods, a new approach is tested building upon the e-LORETA solution: the topography of the maximum of the e-LORETA distribution is projected out of the data before calculating the next e-LORETA inverse solution in a iterative process.
The quality of fit (or localization) was defined as the distance between the simulated point-sources and either the estimated point-sources or the activity distributions by means of the Euclidean distance or of the Earth Mover''s Distance, respectively.
As expected, inter-dipole distances played an important role in the ability of every method to disentangle the simulated sources. Overall, SC-MUSIC appeared best suited for disentangling the two simulated sources even at high-noise simulations.

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Poster

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Title: Label free capillary velocimetry in mouse cerebral cortex using dynamic laser speckle

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Abstract: Many brain disorders including Alzheimer's disease or stroke are known to be correlated with blood flow. Label free assessment of blood flow in capillaries under normal and diseased conditions could be helpful for diagnosis and therapeutic treatments. Laser speckle contrast imaging (LSCI) is a simple wide-field imaging tool and has been widely used for flow analysis. However, quantitative information from LSCI has been restricted due to the presence of static scattering from tissue without *a priori* knowledge of the flow behaviour. Here we suggest a method for blood flow estimation in capillaries using full field time varying speckle. We use temporal demodulation to distinguish the dynamic speckle from its stationary counterpart, based on which we perform temporal autocorrelation to estimate the blood flow in capillary vessels. The results obtained from flow phantom experiment are in good agreement with the calibrated values. We also showed our method in case of *in vivo* experiment on mouse brain. Our results show dynamic laser speckle is an indicative of dynamic red blood cell (dRBC) content and our proposed method combined with qualitative perfusion map may prove to be a powerful tool to investigate the heterogeneities of capillary velocities.

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Poster

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Topic: I.07. Data Analysis and Statistics

Title: A novel method for automated cytoarchitectonic parcellation of the rhesus monkey neocortex

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Abstract: INTRODUCTION
Validation of commonly used MRI techniques for in-vivo cytoarchitectonic analysis with respect to histology is crucial for assessing the reliability and accuracy of cytoarchitectural differences seen with the progression of the disease. In this work, we present an algorithm for the semi-automated parcellation of cytoarchitectonic areas from a high-resolution histological image of one thionin-stained coronal section of the Rhesus monkey (macaca mulatta) brain through the level of the hippocampus and pulvinar nucleus from Brainmaps.org. The algorithm first creates a binary mask of neurons from histological sections, and then measures the density, size, and number of neurons in a specific radial distance in the binary masked image. After that, a map including all the aforementioned features is constructed. The algorithm finally segments the map into cytoarchitectonic areas.
METHODS
The slice number 0721 from the dataset number RH04 from Brainmaps.org was acquired at the resolution 0.05 mm. An ROI over the left side of the monkey brain was defined. Partitioning the cortex into homogeneous sub-regions involved a multi-step, semi-automated process of 1) making a binary mask of the neurons based on Otsu's thresholding, 2) acquiring neuron centroids from the binary masked image, 3) defining a radial distance over each neuron centroid, 4) calculating the *density*, *size*, and *number* of all the neurons with a centroid inside the radial distance, and 4) dividing the cortex into sub-regions based the similarity of texture and content extracted from the features. The reference map for this histological slice has also provided at Brainmaps.org. An expert neuroanatomist evaluated the determined sub-regions in comparison with visually inspected sub-regions from the same ROI.
RESULTS: Our semi-automated method agrees with that of the expert visualization, but also differentiates slight changes in the cytoarchitecture. For example, in area TEO, neither anatomists from BrainMaps.org, nor our group found changes in the cytoarchitecture. However, our algorithm demarcated TEO into three different areas. The algorithm finds more parcellations

that are difficult to see by eye alone.
CONCLUSION
The evaluation of the algorithm by our expert neuroanatomist based on visual inspection indicates that the semi-automated technique is identifying what the neuroanatomist would consider sub-regions, in addition to correctly identifying major cytoarchitectonic areas.

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Poster

097. Data Analysis and Statistics: Software Tools I

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Topic: I.07. Data Analysis and Statistics

Support: National Cancer Institute Grant NIH R01 CA189665-01A1

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Title: Accurate detection of muscle activation onset based on surface EMG using a maximum profile likelihood approach

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Abstract: Background: Myoelectric signals recorded with surface electromyography (sEMG) electrodes provide valuable information for understanding muscle function and neuromuscular diseases especially under human movement conditions. However, investigators working with sEMG for this goal often face a challenge of precisely estimating the onset of muscle activation as this requires detection of the smallest muscle activity at the earliest time based on the sEMG recordings that typically vary from trial to trial and subject to subject. The current study attempted to address this challenge by employing a robust statistical approach to treat the sEMG data for accurate detection of muscle activation onset (MAO) in an unsupervised fashion. **Objective:** To design and evaluate the performance of an algorithm relying on the maximum profile likelihood (MPL) [Zhu and Ghodsi, 2006] for predicting the MAO of a handgrip contraction. **Methods:** The sEMG data were collected from the Flexor Digitorum Superficialis (FDS) and Extensor Digitorum (ED) muscles of a healthy individual (male, 24 years) while performing 100 intermittent handgrip contractions at 20% maximum voluntary contraction (MVC). The acquired sEMG signals were first preconditioned with the Teager-Kaiser energy

operator [Kaiser, 1990], and then supplied to the MPL and wavelet change detection (WCD) [Lavielle, 1999] algorithms. The performance of the algorithms was evaluated by mean errors between the MAO time (in ms) estimated by each of the algorithms and MAO time derived from manual detection based on independent demarcation of the MAOs on the raw sEMG signals by five motor control/sEMG experts. The MAO detection accuracy of the MPL algorithm was then compared to that of the WCD algorithm, a widely accepted and used approach.
Results: The MPL-based approach detected the FDS and ED activation onset with a mean accuracy of ±20 and ±32 ms, respectively, whereas, the conventional WCD algorithm produced a mean accuracy of ±30 and ±76 ms, respectively.
Conclusions: The statistical approach based on the MPL is capable of more accurately detecting the MAO from the FDS and ED muscles in a voluntary handgrip. This unsupervised approach obviates the need for parameter tuning, which is a unique advantage over other popular methods such as the WCD and those based on threshold detection. Research is underway to further validate this technique subject to various experimental conditions.

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Poster

097. Data Analysis and Statistics: Software Tools I

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Support: JSPS Postdoctoral Fellowship for Foreign Researchers

Title: Minimizing ROI crosstalk in neuronal activity data from miniature microscope calcium imaging.

Authors: *A. LUCHETTI, A. BOTA, Y. HAYASHI;
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Abstract: One of the most exciting neuroscience innovations in the last decade has been the development of techniques for imaging neuronal activity in the living, freely behaving animal. These techniques potentially allow the recording of several hundred neurons simultaneously by using a single-photon epifluorescence mini microscope and a GRIN lens implanted in the animal's brain. The next challenge is finding optimal ways to analyze this data by identifying individual neurons in the recording and transforming image data into neuronal activity data. Due to light scattering generated in single-photon epifluorescence, the crosstalk between nearby neurons becomes a significant issue when imaging tissue expressing densely expressed calcium

indicator. Here, we propose a novel approach to solving this difficulty, by using an algorithm that evaluates how bright-pixels are distributed within the Region of Interest (ROI) area, rather than the average brightness of the ROI itself. The technique first determines a local area around each ROI, then evaluates the distribution of the brightest pixels within this local area and assigns a score to the ROI based on how the brightest pixels are clustered within it. We test this technique on experimental data from both sparsely and densely expressed CA1 in vivo samples and measure the number of errors by comparing automatically detected spikes against manual analysis. We find that this technique can keep the number of false positives low without sacrificing the sensitivity of detection, thus also preventing accumulation of false negatives.

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